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Contains No CDI

ciba

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ORIGINAL

September 2, 1994



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RE.: TSCA Section 8(e) Notice; R&D Pesticidal Chemical, Monocrotophos

Dear Section 8(e) Coordinator:

This letter and the enclosed toxicity study contains no Confidential Business Information.

Ciba-Geigy Corporation (Ciba) previously submitted an 8(e) notice for this same chemical substance on ~~May 17, 1994~~ in which results from 15 studies were reported. **We have not yet received an acknowledgement or Document Control Number for this previous submission.**

In accordance with EPA's March 16, 1978 policy on Section 8(e) reporting under the Toxic Substances Control Act and EPA's June 1991 TSCA Section 8(e) Reporting Guide, Ciba wishes to bring to your attention certain information from an acute oral range-finding toxicity study in rats conducted at Ciba's Environmental Health Center in Farmington, CT, with the compound monocrotophos technical. Chemically, monocrotophos is (E)-dimethyl 1-methyl-3-(methylamino)-3-oxo-1-propenyl phosphate, CAS Registry Number 6923-22-4. It is also known internally under the designation C-1414.

Monocrotophos technical was administered to rats by gavage in three separate study phases. In Phase 1, groups of 5 rats/sex were administered monocrotophos as a single dose of 0.3, 3, or 5 mg/kg. Clinical signs generally associated with cholinesterase inhibition were seen at 3 and 5 mg/kg. In Phase 2, groups of 5 female rats were administered 3 mg monocrotophos/kg, and groups were sacrificed at 2, 4, 6, and 24 hours after dosing. Clinical signs were similar to those seen in Phase 1. Statistically significant depression of serum, RBC, and brain cholinesterase activity occurred from 2-24 hours. The depression was highest at 2 hours (serum 80%, RBC 72%, and brain 87% reduction from control). In Phase 3, groups of 5 rats/sex were administered monocrotophos at 0, 0.01, 0.03, 0.1, 0.3 or 1 mg/kg. The animals were sacrificed 2 hours post-dosing. Serum cholinesterase activity was significantly lower

11/18/94

than controls for males at doses  $\geq 0.1$  mg/kg and for females at  $\geq 0.3$  mg/kg. RBC cholinesterase activity was significantly reduced for both males and females at doses  $\geq 0.3$  mg/kg, and brain cholinesterase activity was significantly reduced in both sexes dosed with  $\geq 0.1$  mg monocrotophos/kg body weight. These findings are corroborative of existing information on the cholinesterase-inhibiting effects of monocrotophos.

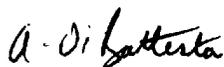
A copy of the final report, entitled "Acute Oral Toxicity Study of Monocrotophos Technical in Rats" (Study Number F-00189), is enclosed.

Ciba recently imported a 100 gm sample of monocrotophos, for toxicity testing purposes only, in connection with an import tolerance application on behalf of our parent company, Ciba-Geigy Limited in Basel, Switzerland. Ciba has never held a U. S. FIFRA registration for monocrotophos. It is possible that monocrotophos may no longer be marketed in the U. S. as a registered pesticide by any company.

In response to these findings, Ciba has examined the Material Safety Data Sheet for monocrotophos and determined that the existing sheet adequately reflects these findings, and that no revision is necessary.

Please contact the undersigned if you need any additional information.

Very truly yours,



Anthony Di Battista

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Encl.

**Contains No CBI**

VOLUME \_\_ OF \_\_ OF SUBMISSION

MONOCROTOPHOS TECHNICAL

FINAL REPORT

ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

AUTHOR - ROBERT F. POTREPKA

STUDY COMPLETED ON 8/9/94

CONDUCTED BY CIBA-GEIGY CORPORATION  
CROP PROTECTION DIVISION  
ENVIRONMENTAL HEALTH CENTER  
400 FARMINGTON AVENUE  
FARMINGTON, CT 06032

LABORATORY STUDY NUMBER F-00189

VOLUME 1 OF 1

PAGE 1 OF 74

CIBA-GEIGY CORPORATION  
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F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA section 10 (d) (1) (A), (B) or (C).

Company: Ciba-Geigy Corporation, Crop Protection Division

Company Agent:

Carolyn Bussey

Carolyn Bussey  
Signature

Regulatory Manager

Title

8-10-94

Date

These data are the property of the Crop Protection Division of Ciba-Geigy Corporation, and as such, are considered to be confidential for all purposes other than compliance with FIFRA Section 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality which may exist under any other statute or in any other country.

CIBA-GEIGY CORPORATION -3-

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

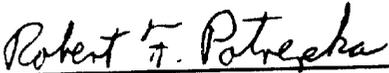
CERTIFICATION OF GOOD LABORATORY PRACTICES

This study, "F-00189: Acute Oral Toxicity Study of Monocrotophos Technical in Rats", was conducted to be in compliance with EPA-FIFRA GLP Standards (40 CFR, part 160), the OECD Principles of Good Laboratory Practice (Annex 2, C(81)30) and the Good Laboratory Practice Standards of Japan (MAFF, 59 NohSan, No. 3850, August 10, 1984). The Environmental Health Center is certified as a GLP Compliance Facility by the Ministry of Agriculture, Forestry and Fisheries of Japan.

Signatures:

  
Christine C. Jacobs, B.S.  
Quality Assurance Unit

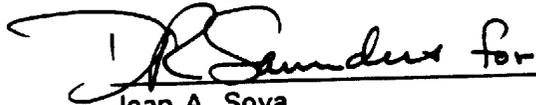
8-9-94  
Date

  
Robert F. Potrepka, Ph.D.  
Study Director

8-9-94  
Date

  
Donald R. Saunders, Ph.D., D.A.B.T.  
Director, Toxicology, GSO  
Sponsor/Applicant

8/9/94  
Date

  
Joan A. Sova  
Agent of Submitter

8/9/94  
Date

Submitter: Ciba-Geigy Corporation  
Crop Protection Division  
410 Swing Road  
Post Office Box 18300  
Greensboro, NC 27419

CIBA-GEIGY CORPORATION -4-

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

EPA FLAGGING CRITERIA STATEMENT

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria.

  
\_\_\_\_\_  
Signature of Agent of Submitter

8/9/94  
Date

*for* Joan A. Sova  
Typed Name

Submitter: Ciba-Geigy Corporation  
Crop Protection Division  
410 Swing Road  
Post Office Box 18300  
Greensboro, North Carolina 27419

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

PREFACE

This study was conducted at the Ciba Environmental Health Center (EHC), 400 Farmington Avenue, Farmington, Connecticut 06032. This study was initiated on March 29, 1994, the in-life phase was started on March 30, 1994 and was completed on May 12, 1994.

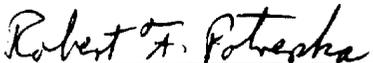
Personnel responsible for the conduct of this study were as follows:

Study Director:	Robert F. Potrepka, Ph.D.
Alternate Study Director:	Edward Chow, Ph.D., D.A.B.T
Clinical Veterinarian:	Daniel R. Schwartz, D.V.M.
Study Pathologist:	John C. Turnier, V.M.D., D.A.C.V.P.
Toxicology Support:	Sharon L. Flowers, B.S., L.A.T. Tammy H. Gaghan, B.S., L.A.T.G.
Necropsy/Histology Laboratories:	Judith B. Galley, B.S.

Supervisory personnel at the time of the study were as follows:

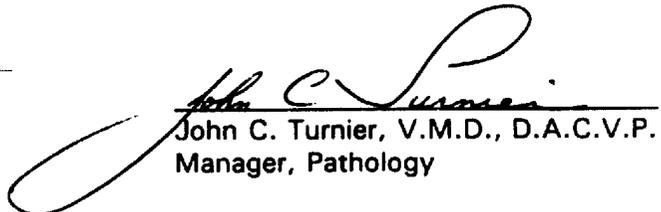
Toxicology Support:	Joseph L. Polino, B.S. Holly G. Such, A.A.S., L.A.T.G.
Clinical Laboratory:	Karen L. Norton, B.A., MT, ASCP
Necropsy/Histology Laboratories:	Brenda R. Duprey, HT, ASCP Margaret M. Bill, M.S., HT, ASCP Tina L. Lapointe, B.S., HT, ASCP
Pharmacy:	James P. Tarca, B.S.
Analytical Chemistry:	David R. Holschlag, M.S.

Report written by:

  
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Study Director      Date  
Senior Group Leader, Toxicology

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Manager, Pathology

  
Joan A. Sova  
Product Manager  
Toxicology - Greensboro, NC

Report reviewed and approved by:

  
Donald R. Saunders, Ph.D., D.A.B.T.  
Director, Toxicology - Greensboro, NC

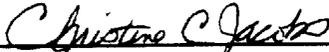
F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

QUALITY ASSURANCE STATEMENT

A Quality Assurance review of "F-00189: Acute Oral Toxicity Study of Monocrotophos Technical in Rats", completed on 8/9/94, confirmed that the reported results accurately reflect the data for this study.

Inspections of F-00189 were conducted on the days listed below. All findings were reported to the Study Director and to management.

<u>Dates of Inspections</u>	<u>Dates Reported to Study Director</u>	<u>Dates Inspections Reported to Management</u>
3/30/94	4/5/94	4/5/94
5/13/94	5/17/94	5/17/94

  
\_\_\_\_\_  
Christine C. Jacobs, B.S.  
Quality Assurance Auditor

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE OF CONTENTS

Volume 1

	<u>Page</u>
TITLE PAGE .....	1
STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS .....	2
CERTIFICATION OF GOOD LABORATORY PRACTICES .....	3
EPA FLAGGING CRITERIA STATEMENT .....	4
PREFACE .....	5
QUALITY ASSURANCE STATEMENT .....	6
TABLE OF CONTENTS .....	7
1. SUMMARY .....	10
2. INTRODUCTION .....	11
3. MATERIALS AND METHODS .....	12
3.1 Study Schedule .....	12
3.2 Test Substance .....	12
3.3 Test Animals .....	12
3.3.1 Quarantine/Acclimation .....	12
3.3.2 Animal Housing and Maintenance .....	13
3.3.3 Method of Identification .....	13
3.4 Dose Preparation and Administration .....	14
3.4.1 Phase 1 .....	14
3.4.2 Phase 2 .....	14
3.4.3 Phase 3 .....	15
3.5 In-Life Observations .....	16
3.5.1 Clinical Signs and Mortality .....	16
3.5.2 Body Weights .....	16
3.5.3 Cholinesterase Activity Measurements .....	16
3.6 Postmortem Examination .....	17
3.6.1 Euthanasia .....	17
3.6.2 Necropsy .....	17

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## TABLE OF CONTENTS (Cont'd.)

## Volume 1 (Cont'd.)

	<u>Page</u>
<b>3. MATERIALS AND METHODS (Cont'd.)</b>	
3.7 Statistical Analysis .....	17
3.8 Data Retention .....	17
<b>4. RESULTS .....</b>	<b>18</b>
4.1 Analyses of the Test Substance in the Vehicle .....	18
4.2 Phase 1 .....	18
4.2.1 Survival .....	18
4.2.2 Body Weights .....	18
4.2.3 Clinical Observations .....	18
4.2.4 Postmortem Examination .....	18
4.3 Phase 2 .....	18
4.3.1 Survival .....	18
4.3.2 Body Weights .....	19
4.3.3 Clinical Observations .....	19
4.3.4 Postmortem Examination .....	19
4.3.5 Cholinesterase Activity Measurements .....	19
4.4 Phase 3 .....	19
4.4.1 Body Weights .....	19
4.4.2 Clinical Observations .....	20
4.4.3 Cholinesterase Activity Measurements .....	20
<b>5. DISCUSSION .....</b>	<b>21</b>
<b>6. CONCLUSIONS .....</b>	<b>22</b>
<b>7. REFERENCES .....</b>	<b>23</b>
<b>8. TABLES</b>	
8.1 Analyses of the Test Substance in the Vehicle for Concentration and Homogeneity .....	24
8.2 Phase 1 - Individual and Mean Body Weights and Body Weight Gains (Grams) - Males .....	25
8.3 Phase 1 - Individual and Mean Body Weights and Body Weight Gains (Grams) - Females .....	26
8.4 Phase 1 - Clinical Signs - Males .....	27
8.5 Phase 1 - Clinical Signs - Females .....	32

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## TABLE OF CONTENTS (Cont'd.)

## Volume 1 (Cont'd.)

	<u>Page</u>
8. TABLES (Cont'd.)	
8.6 Phase 1 - Individual Necropsy Comments - Males . . . . .	36
8.7 Phase 1 - Individual Necropsy Comments - Females . . . . .	37
8.8 Phase 2 - Individual and Mean Body Weights and Body Weight Gains (Grams) - Females . . . . .	38
8.9 Phase 2 - Clinical Signs - Females . . . . .	40
8.10 Phase 2 - Individual Necropsy Comments - Females . . . . .	44
8.11 Summary of Phase 2 Cholinesterase Data - Females . . . . .	46
8.12 Phase 3 - Individual and Mean Body Weights (Grams) - Males . . . . .	47
8.13 Phase 3 - Individual and Mean Body Weights (Grams) - Females . . . . .	49
8.14 Phase 3 - Clinical Signs - Males . . . . .	51
8.15 Phase 3 - Clinical Signs - Females . . . . .	52
8.16 Summary of Phase 3 Cholinesterase Data . . . . .	53
9. APPENDICES	
9.1 Study Protocol & Amendments . . . . .	54
9.1.1 Study Protocol . . . . .	55
9.1.2 List of Protocol Amendments . . . . .	67
9.2 Cholinesterase Activity - Individual Animal . . . . .	69
9.2.1 Individual Animal Cholinesterase Activity - Phase 2 . . . . .	70
9.2.2 Individual Animal Cholinesterase Activity - Phase 3 . . . . .	71
9.2.3 Analytical Methods for Cholinesterase Activity . . . . .	72

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

1. SUMMARY

The purpose of this study was to determine the acute oral toxicity of the organophosphate insecticide monocrotophos technical (FL-940574, EHC Code No. 0173-19, an amber crystalline solid with 77.6% purity) when prepared in distilled deionized water and administered to Charles River CD rats (8-9 weeks old). This study was conducted in three phases. Phase 1 determined the no-observable-effect level (NOEL) for clinical/behavioral/body weight effects, Phase 2 the peak inhibition time(s) for serum, red blood cell (RBC) and brain cholinesterase (ChE) activity levels and Phase 3 the NOEL for inhibition of serum, RBC and brain ChE activity levels.

In Phase 1, groups of five rats/sex were administered monocrotophos at 0.3, 3 or 5 mg/kg. Clinical signs were recorded at 0, 1, 2, 4 and 6 hours after dosing and once daily thereafter for 7 days. Body weights were recorded before dosing and prior to necropsy at termination.

Males and females receiving doses of 3 or 5 mg/kg exhibited clinical signs which included flattened posture, muscle fasciculations (general or local, limited to the limbs), staining of the eyes, mouth and nose, partially formed feces or diarrhea, miosis, lacrimation, and salivation. No deaths or effects on body weight gain were observed, nor were there any treatment-related necropsy observations. The NOEL for clinical/behavioral/body weight effects was 0.3 mg/kg.

In Phase 2, eight groups of five female rats/group were administered monocrotophos at 0 or 3 mg/kg. Clinical signs were recorded before treatment and at 1, 2, 4, 6 and 24 hours after dosing. Body weights were recorded before treatment and prior to sacrifice at 2, 4, 6 and 24 hours. Serum, RBC and brain ChE activity levels were determined at 2, 4, 6 or 24 hours after dosing.

Clinical signs were similar to those seen in Phase 1. The severity of these signs peaked at 1-2 hours post-dosing and complete recovery was apparent by 24 hours. No deaths or gross necropsy lesions were noted. Statistically significant depression of serum, RBC and brain ChE activity levels occurred from 2-24 hours; the depression appeared to peak at 2 hours (serum 80%, RBC 72% and brain 87% reduction from control) but a 30 percent reduction relative to control was still demonstrable at 24 hours.

In Phase 3, groups of five rats/sex were administered monocrotophos at 0, 0.01, 0.03, 0.1, 0.3 or 1 mg/kg. Body weights were recorded before treatment. Clinical signs were recorded before treatment and at 1 and 2 hours post-dose. Serum, RBC and brain ChE activity levels were determined at 2 hours (which was determined to be the time for maximal inhibition of ChE activity in the previous phase).

Serum ChE activity was significantly lower for males at  $\geq 0.1$  mg/kg and for females at  $\geq 0.3$  mg/kg relative to controls. Both sexes showed a lower RBC ChE activity at  $\geq 0.3$  mg/kg. Brain ChE activity was significantly lower for males and females dosed at  $\geq 0.1$  mg/kg. Clinical signs were noted at 1 mg/kg in male rats (muscle fasciculations, miosis, staining of the nose and slight salivation) and in female rats (miosis and staining of the nose).

In summary, a single oral dose of monocrotophos resulted in a NOEL for clinical signs/behavioral effects of 0.3 mg/kg and peak ChE activity inhibition in serum, RBC or brain samples at 2 hours. The NOEL for inhibition of ChE activity at 2 hours post-dose was 0.03 mg/kg.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

2. INTRODUCTION

This study was conducted to determine 1) the no-observable-effect level (NOEL) for clinical/behavioral/body weight effects (Phase 1), 2) the peak inhibition time(s) for serum, RBC, and brain ChE activity (Phase 2), and 3) the NOEL for inhibition of serum, RBC and brain ChE activity (Phase 3) following administration of a single oral gavage dose of monocrotophos technical to rats.

This study was conducted at the Ciba Environmental Health Center (EHC) according to a predesigned protocol (Appendix 9.1). The exposure was initiated on March 30, 1994 and the in-life phase was completed on May 12, 1994. Unless otherwise specified, all procedures and tests were performed in accordance with EHC Standard Operating Procedures, the EPA-FIFRA GLP Standards (40 CFR, Part 160), the OECD Principles of Good Laboratory Practices (Annex 2, C(81)30) and the Good Laboratory Practice Standards of Japan (MAFF, 59 NohSan, No. 3850, August 10, 1984). The Environmental Health Center is certified as a GLP Compliance Facility by the Ministry of Agriculture, Forestry and Fisheries of Japan.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

3. MATERIALS AND METHODS

3.1 Study Schedule

Animals were received on March 23, 1994, April 5, 1994 and April 26, 1994 for Phases 1, 2 and 3, respectively. Animals were weighed and assigned to the study groups on March 29, 1994, April 18, 1994 and May 9 (males) or 11 (females), 1994 for Phases 1, 2, and 3, respectively. Animals were sacrificed on April 6, 11 or 13 for Phase 1, April 19-20, 1994 for Phase 2, and May 10, 1994 (males) or May 12, 1994 (females) for Phase 3.

3.2 Test Substance

The test substance, monocrotophos technical (C1414, Lot Number FL-940574, Batch No. OP 107001, EHC Code No. 0173-19), an organophosphate insecticide, was received from the Ciba-Geigy Crop Protection Division in Greensboro, NC on March 28, 1994. The expiration date was December 6, 1996. The test substance, an amber crystalline solid, with a purity of 77.6%, was stored at room temperature and was protected from light sources and flammable materials.

3.3 Test Animals

Male and female CrI:CD<sup>®</sup>(SD)BR VAF/Plus<sup>™</sup> rats were received from Charles River Laboratories, Inc., Portage, Michigan. The CD rat is a standard laboratory animal for toxicity evaluation. Considerable background data are available for this strain in this laboratory.

Five male and five female (nulliparous and nonpregnant) rats were required for each dose level in Phases 1 and 3, and 20 females for each dose level in Phase 2. Animals determined to be healthy and suitable for study were ranked by body weight and assigned to the study groups such that all study groups of the same sex had similar mean body weights. All rats were approximately 6-7 weeks old at receipt and approximately 8-9 weeks of age at the time of dosing.

3.3.1 Quarantine/Acclimation

Upon arrival at the EHC, the animals were randomly distributed from the shipping cartons and housed 2 per same sex per cage. The sex of each animal was confirmed during this transfer procedure. During the quarantine/acclimation period, rats were examined by a veterinarian (general physical examinations) with respect to their state of health and suitability as test animals. Only healthy animals were included in the study. The quarantine/acclimation period was at least 7 days.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

3. MATERIALS AND METHODS (Cont'd.)

3.3. Test Animals (Cont'd.)

3.3.2 Animal Housing and Maintenance

The rats were housed and maintained in compliance with the Animal Welfare Act, 1985. They were housed in rooms 307 (Phase 1), 305 (Phase 2) and 317 (Phase 3) during the quarantine/acclimation and study periods. During the study, rats were housed individually in suspended polycarbonate cages measuring approximately 19 x 21 x 20 cm each. Hardwood chips were used as bedding material. Cages, racks and feeders were changed weekly. Conventional disease control was practiced and only authorized personnel were permitted in the study room. The facility's Heating-Ventilation and Air Conditioning (HVAC) system provided at least 15 room air changes per hour and maintained the temperature between 19-24°C and the relative humidity between 40-60%. Fluorescent lighting was set to provide a 12-hour light/dark cycle (light on approximately 6 a.m. EST). Room temperature and humidity were monitored continuously, recorded daily and documents archived weekly.

The rats were provided ad libitum (except during the fasting periods prior to and up to four hours after dosing) with PMI® Feeds' Certified Rodent Diet #5002 ground meal diet in rat feeders. The feed was analyzed by the manufacturer for nutrients and contaminants. Concentrations of contaminants listed in the analysis profile were not sufficient to have affected the conduct or purpose of the study.

Water from the municipal water supply was provided ad libitum by an automatic watering system and in water bottles when necessary. The water supplied to the facility is analyzed (periodically) for contaminants. Concentrations of the potential contaminants tested for were below detection levels or below the maximum allowable concentrations published by the State of Connecticut. Concentrations of contaminants found in drinking water were not sufficient to interfere with the conduct or purpose of the study.

3.3.3 Method of Identification

Each rat was individually identified with a permanent animal number on a Monel® ear tag attached to the right ear. Any animal that lost an ear tag was re-tagged on the right ear whenever possible. Otherwise, the left ear was tagged. Color-coded transfer labels for the cages were issued bearing the study number, the permanent animal number, sex and dose group number (Appendix 9.1.1, Study Protocol).

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## 3. MATERIALS AND METHODS (Cont'd.)

## 3.4 Dose Preparation and Administration

An aliquot of the test substance was melted in a waterbath set at approximately 55°C for 30-60 minutes and the appropriate amount was mixed with distilled deionized water at concentrations to allow a dosing volume of 5 ml/kg. Depending on the phase of the study, the concentration of monocrotophos was 0.002, 0.006, 0.02, 0.06, 0.2, 0.6 or 1.0 mg/ml for the 0.01, 0.03, 0.1, 0.3, 1, 3 and 5 mg/kg dose levels. The oral route is a route of administration specified by regulatory test agencies. Dosing preparations were prepared by the EHC Pharmacy Laboratory according to a procedure approved by the Study Director. Six samples from each dosing preparation and one sample from the control (0 mg/kg) vehicle were obtained and analyzed by the EHC Analytical Chemistry Laboratory. Monocrotophos was analyzed by supercritical fluid chromatography at  $\geq 0.06$  mg/ml according to EHC Method No. 94-1 and by HPLC at  $\leq 0.02$  mg/ml according to EHC Method No. 94-3.

The animals were fasted for 17 to 20 hours before test substance administration (between 6:00 and 9:00 a.m. EST). Food was restored to the Phase 1 and group c and d Phase 2 animals approximately 4 hours post-dose. The dosing preparations were utilized immediately after mixing. After administration, any remaining dosing preparations were discarded appropriately.

## 3.4.1 Phase 1

Initially, 3 mg/kg was administered to five males and five females by oral gavage. Based on these results, dose levels of 0.3 and 5 mg/kg were added in order to determine the NOEL and better characterize the behavioral and clinical signs for each sex. Animal identification and dose levels for Phase 1 were as follows:

Dose Level (mg/kg)	Males		Females	
	Group No.	ID No.	Group No.	ID No.
3	10	101 - 105	15	1101 - 1105
5	20	201 - 205	25	1201 - 1205
0.3	30	301 - 305	35	1301 - 1305

## 3.4.2 Phase 2

Five female rats per group were administered vehicle (groups 65a, 65b, 65c and 65d) or 3 mg/kg monocrotophos (groups 75a, 75b, 75c, 75d). The 3 mg/kg dose level was selected following evaluation of the Phase 1 results. In this phase, the peak inhibition time(s) for serum, RBC and brain ChE activity levels were determined by sacrificing five monocrotophos and five

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## 3. MATERIALS AND METHODS (Cont'd.)

## 3.4 Dose Preparation and Administration (Cont'd.)

## 3.4.2 Phase 2 (Cont'd.)

control animals at 2, 4, 6 and 24 hours post-dose. Animal identification and dose levels for Phase 2 were as follows:

Sacrifice Interval (Hours Post-Dose)	Dose Level - 0 mg/kg		Dose Level - 3 mg/kg	
	Group No.	ID No.	Group No.	ID No.
2	65a	1106 - 1110	75a	1206 - 1210
4	65b	1111 - 1115	75b	1211 - 1215
6	65c	1116 - 1120	75c	1216 - 1220
24	65d	1121 - 1125	75d	1221 - 1225

Groups 65 a, b, c and d received only distilled deionized water at a volume of 5.0 ml/kg. The first animal of each 65 group was dosed before the first animal of each 75 group. For each time interval, sacrifice was alternated by animal between the two dose levels (i.e., 1 control, 1 test).

## 3.4.3 Phase 3

Five rats/sex were administered 0, 0.01, 0.03, 0.1, 0.3 or 1 mg/kg monocrotophos. All animals were sacrificed 2 hours post-dose. Dose levels and the 2-hour sacrifice interval were selected on the basis of the Phase 1 and 2 results. The order of dosing and sacrifice of each animal was across each dose group for each sex (i.e., 1 animal/group). Animal identification and dose levels for Phase 3 were as follows:

Dose Level (mg/kg)	Males		Females	
	Group No.	ID No.	Group No.	ID No.
0	80	106 - 110	85	1126 - 1130
0.01	90	206 - 210	95	1226 - 1230
0.03	100	306 - 310	105	1326 - 1330
0.1	110	406 - 410	115	1426 - 1430
0.3	120	506 - 510	125	1526 - 1530
1	130	606 - 610	135	1626 - 1630

Groups 80 and 85 received only distilled/deionized water at a volume of 5.0 ml/kg.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

3. MATERIALS AND METHODS (Cont'd.)

3.5 In-Life Observations

3.5.1 Clinical Signs and Mortality

General physical examinations were conducted as described in the study protocol (see form in Appendix 9.1.1) which was based on the abbreviated Functional Observation Battery which is used by the EHC Neurotoxicity Testing Program. The observations selected from this battery were based on prior testing experience with organophosphates.

In Phase 1, general physical examinations were performed to record clinical signs immediately after dosing, at approximately 1, 2, 4 and 6 hours after test substance administration, and daily (a.m.) thereafter for 7 days. Throughout the study, all animals were observed at least twice daily (a.m. and p.m.) for mortality.

In Phase 2, general physical examinations were performed to record clinical signs just before dosing and at approximately 1, 2, 4, 6 and 24 hours after test substance administration (when applicable).

In Phase 3, general physical examinations were performed to record clinical signs just before dosing and at approximately 1 and 2 hours post-dose.

3.5.2 Body Weights

In Phase 1, individual body weights were recorded before dose administration and prior to termination (Day 7).

In Phase 2, body weights were recorded before dose administration and prior to termination (2, 4, 6 or 24 hours).

In Phase 3, body weights were only recorded before dose administration. No additional body weight measurements were taken at termination.

3.5.3 Cholinesterase Activity Measurements

In Phases 2 and 3, all animals were anesthetized with isoflurane (AErrane®) and blood samples were collected via the retro-orbital plexus for determination of serum and RBC ChE activity levels using modified procedures of Ellman et al. (1961) (See Appendix 9.2.3).

The right half of the brain was analyzed for ChE activity using a modified procedure of Ellman et al. (1961) (See Appendix 9.2.3). The other half of the brain (which was not required) was kept frozen at -70° to -90°C for possible further analyses.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

3. MATERIALS AND METHODS (Cont'd.)

3.6 Postmortem Examination

3.6.1 Euthanasia

The animals were anesthetized by sodium pentobarbital injected i.p. and exsanguinated via the abdominal aorta.

3.6.2 Necropsy

All animals in Phases 1 and 2 that were euthanatized during the study received an abbreviated gross necropsy examination and were then discarded. Abnormalities were recorded and all tissues with lesions were collected and preserved in 10% neutral buffered formalin. No histopathological evaluation was performed.

For Phases 2 and 3, a midline sagittal cut of the brain was made and each half was collected, weighed and placed on ice until transferred to a freezer set to maintain a temperature of  $-70^{\circ}\text{C}$  to  $-90^{\circ}\text{C}$  until analyzed for brain ChE activity.

3.7 Statistical Analysis

Statistical analyses of body weight and ChE parameters were performed using a one-way analysis of variance (ANOVA) (Steel and Torrie, 1960) and Dunnett's (1955, 1964) procedure to compare all treatments to the control. When the overall F-statistic from ANOVA was significant ( $p \leq 0.05$ ), Dunnett's t-test (two-tailed) was used to detect treatment differences from control, otherwise no treatment comparisons were performed. The probability of Type I error (alpha) was set at 0.05. Statistical test results that reached the 0.01 level of significance were also noted.

3.8 Data Retention

The original final report and all raw data, records, protocol and specimens are archived at the EHC.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

4. RESULTS

4.1 Analyses of the Test Substance in the Vehicle

Analyses of the monocrotophos preparations in distilled deionized water for concentration and homogeneity indicated that the preparations administered to test animals were within acceptable limits (Table 8.1).

4.2 Phase 1

4.2.1 Survival

All animals survived to termination.

4.2.2 Body Weights

Individual and mean body weights and body weight gains are shown in Tables 8.2 (males) and 8.3 (females). There were no treatment-related effects for either sex.

4.2.3 Clinical Observations

A summary of clinical signs is presented in Tables 8.4 (males) and 8.5 (females). Male and female animals treated at 0.3 mg/kg appeared normal throughout the study. Clinical signs of toxicity at 3 and 5 mg/kg included flattened posture; muscle fasciculations, generally (over the body) or local (limited to the limbs); staining of the eyes, mouth, nose; partially formed feces or diarrhea; miosis; lacrimation; and salivation. The clinical signs were observed at 1 hour, peaked at 2 to 4 hours and recovered by 24 hours.

4.2.4 Postmortem Examination

Individual necropsy comments are listed in Tables 8.6 (males) and 8.7 (females). There were no treatment-related gross necropsy findings. The observations in 1 male at 5 mg/kg (an ulcer and enlarged lymph node) and in 1 female each at 0.3 mg/kg (a malformed/misshaped eye) and 5 mg/kg (a kidney cyst, focus on the cornea and malformed/misshaped pupil) were consistent with occasional findings and not treatment-related. Histopathological evaluation was not required.

4.3 Phase 2

4.3.1 Survival

All animals survived to termination.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

4. RESULTS (Cont'd.)

4.3 Phase 2 (Cont'd.)

4.3.2 Body Weights

Individual and mean body weights and body weight gains for female rats at 2, 4, 6, and 24 hours post-dose are shown in Table 8.8. Statistically significant decreases in body weight gain were noted at 2 and 4 hours post-dose when compared with controls. There were no statistically significant effects on body weight or body weight gain at 6 or 24 hours post dose.

4.3.3 Clinical Observations

A summary of clinical signs for female rats at predose and 1, 2, 4, 6 and 24 hours post-dose is presented in Table 8.9. Clinical signs generally associated with cholinesterase inhibition included flattened posture; muscle fasciculations, general (over the body) or local (limited to the limbs); partially formed feces; miosis; lacrimation; staining of the eyes, mouth, nose; and salivation. Clinical signs were apparent at 1 to 2 hours post-dose, decreased at later observation periods and complete recovery occurred by 24 hours after treatment.

4.3.4 Postmortem Examination

Individual necropsy comments for female rats sacrificed at 2, 4, 6 and 24 hours post-dose are listed in Table 8.10. There were no significant gross lesions at 3 mg/kg for female rats sacrificed at 2, 4, 6, or 24 hours post-dose. Since there were no necropsy findings, histopathological evaluation was not required.

4.3.5 Cholinesterase Activity Measurements

The group means are presented in Table 8.11 and the individual animal data are presented in Appendix 9.2.1. A statistically significant depression of serum, RBC and brain ChE activity levels was observed at all the measured time intervals of 2, 4, 6, and 24 hours post-dose. Maximal inhibition occurred at 2 hours (expressed as percent of control: serum 20%, RBC 28% and brain 13%). By 24 hours, the level of inhibition of ChE activity indicated recovery of activity and was approximately 70 percent of the control values for each parameter.

4.4 Phase 3

4.4.1 Body Weights

Individual and mean pretest and fasted (Day 0) body weights are shown in Tables 8.12 (males) and 8.13 (females). No additional body weight measurements were taken at termination, 2 hours post-dose. Body weights were not statistically different for either sex.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## 4. RESULTS (Cont'd.)

## 4.4 Phase 3 (Cont'd.)

## 4.4.2 Clinical Observations

A summary of clinical signs is presented in Tables 8.14 (males) and 8.15 (females). Clinical signs in male rats were muscle fasciculations of the limbs (1 mg/kg), miosis ( $\geq 0.1$  mg/kg), staining of the nose (0.01, 0.03, 0.1 and 1 mg/kg) and slight salivation (1 mg/kg). Results from six reported studies (F-00129, F-00133, F-00166, F-00167, F-00175 and F-00178) for control male rats showed the percentage of animals with miosis during any of the measured time points could vary from 0 to 80 percent. The percentages of male rats with miosis at 0.1 and 0.3 mg/kg for this phase were not dose-related and therefore not regarded as treatment-related. Staining of the nose may also be of dubious value as a clinical sign at these low dose levels with only one animal at 0.01, 0.03 and 0.1 mg/kg and no animals at 0.3 mg/kg. Clinical signs in female rats were miosis (dose levels of 0.01 and 1 mg/kg) and staining of the nose (dose levels 0.01, 0.3 and 1 mg/kg). The clinical signs of miosis and staining of the nose were not considered treatment-related at 0.01 mg/kg as there were no findings at dose levels of 0.03 or 0.1 mg/kg. All other animals in other dose groups appeared normal throughout the study.

## 4.4.3 Cholinesterase Activity Measurements

The group means are presented in Table 8.16 and the individual animal data are presented in Appendix 9.2.2. The table below expresses ChE activity as a percent of control. Serum ChE activity levels were significantly lower for males at  $\geq 0.1$  mg/kg and for females at  $\geq 0.3$  mg/kg. Both sexes showed a lower RBC ChE activity level at  $\geq 0.3$  mg/kg. Brain ChE activity levels were significantly lower for males and females dosed at  $\geq 0.1$  mg/kg.

Parameter	Males					Females				
	Dose Level (mg/kg)									
	0.01	0.03	0.1	0.3	1	0.01	0.03	0.1	0.3	1
Serum	90 <sup>a</sup>	79	66**	44**	17**	96	110	109	71*	43**
RBC	115	94	87	56**	34**	98	93	93	35**	25**
Brain	99	100	83**	56**	28**	97	93	85**	74**	32**

\* Significantly different from control using Dunnett's "t" test,  $p \leq 0.05$

\*\* Significantly different from control using Dunnett's "t" test,  $p \leq 0.01$

<sup>a</sup> Values represent percent of control.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

5. DISCUSSION

No deaths were observed for either sex within 7 days after a single oral gavage dose of 5 mg/kg monocrotophos. In Phase 1, clinical signs of toxicity were observed at 3 and 5 mg/kg with a NOEL of 0.3 mg/kg.

A dose of 3 mg/kg was chosen for Phase 2 based upon the clinical signs observed in Phase 1. Female rats were chosen as the preferred sex for determining peak ChE inhibition time since for many organophosphates, females are the more sensitive sex. Clinical signs consistent with cholinergic poisoning were observed at one to two hours post-dose and abated by 24 hours. Inhibition of serum, RBC and brain ChE activity levels was observed at all measured time points of 2, 4, 6 and 24 hours. The time of peak ChE inhibition for all three parameters was 2 hours. By 24 hours, ChE activity levels were recovering but were still depressed to approximately two-thirds the control values for each parameter.

For Phase 3, serum ChE activity was significantly lower for males at  $\geq 0.1$  mg/kg with a NOEL of 0.03 mg/kg and for females at  $\geq 0.3$  mg/kg with a NOEL of 0.1 mg/kg. Both sexes showed lower RBC ChE activity levels at  $\geq 0.3$  mg/kg and a NOEL of 0.1 mg/kg. Brain ChE activity was significantly lower for males and females dosed at  $\geq 0.1$  mg/kg with a NOEL of 0.03 mg/kg. The levels of inhibition of RBC and brain ChE values were in general agreement between both sexes. The level of inhibition of serum ChE activity was greater in male than female rats. Treatment-related clinical signs were noted at 1 mg/kg in male rats (muscle fasciculations, miosis, staining of the nose and slight salivation) and in female rats (miosis and staining of the nose).

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

6. CONCLUSIONS

Administration of a single dose of monocrotophos technical in distilled deionized water to Charles River (Sprague Dawley derived) rats at 0.01, 0.03, 0.1, 0.3, 1, 3 or 5 mg/kg resulted in:

- No mortality for either sex;
- NOEL for clinical signs/behavioral effects in both sexes at 0.3 mg/kg;
- NOEL for inhibition of serum cholinesterase activity in male rats at 0.03 mg/kg and in female rats at 0.1 mg/kg;
- NOEL for inhibition of RBC cholinesterase activity in both sexes at 0.1 mg/kg;
- NOEL for inhibition of brain cholinesterase activity in both sexes at 0.03 mg/kg;
- Overall NOEL for inhibition of cholinesterase activity was 0.03 mg/kg.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

7. REFERENCES

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## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.1 ANALYSES OF THE TEST SUBSTANCE IN THE VEHICLE FOR CONCENTRATION AND HOMOGENEITY

Sample Blend Date	Concentration (mg/ml) <sup>a</sup>		Percent Difference <sup>b</sup> (%)	Homogeneity <sup>c</sup> (Relative Standard Deviation, %)
	Nominal	Actual		
3/30/94	0.6	0.616	2.7	1.9
4/4/94	1.0	0.963	-3.7	7.6
4/6/94	0.06	0.061	1.7	4.9
4/19/94	0.6	0.612	2.0	2.8
5/10/94	0.2	0.1985	-0.8	2.6
	0.06	0.0603	0.5	2.2
	0.02	0.01944	-2.8	1.2
	0.006	0.005992	-0.1	0.8
	0.002	0.001983	-0.8	1.1
5/12/94	0.2	0.1874	-6.3	3.5
	0.06	0.0573	-4.5	4.4
	0.02	0.018374	-8.1	0.4
	0.006	0.005580	-7.0	0.5
	0.002	0.001850	-7.5	1.1

<sup>a</sup> Data expressed as mean for six samples. Test substance was not detected in the control sample (0 mg/ml).

<sup>b</sup> % Difference =  $\frac{(\text{Actual Concentration} - \text{Nominal Concentration}) \times 100}{\text{Nominal Concentration}}$

The acceptable concentration limits (based on % difference) are:

- ± 10% for concentrations > 0.1 mg/ml
- ± 15% for concentrations > 0.01 but ≤ 0.1 mg/ml
- ± 20% for concentrations > 0.001 but ≤ 0.01 mg/ml

<sup>c</sup> The acceptable homogeneity limits based on relative standard deviation (standard deviation/average concentration) X 100) are:

- ≤ 10% for concentrations > 0.1 mg/ml
- ≤ 15% for concentrations > 0.01 but ≤ 0.1 mg/ml
- ≤ 20% for concentrations > 0.001 but ≤ 0.01 mg/ml

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.2 PHASE 1 - INDIVIDUAL AND MEAN BODY WEIGHTS  
AND BODY WEIGHT GAINS (GRAMS) - MALES

Animal Number	Body Weight			Weight Gain
	Pretest	Day 0	Day 7	
Dose Level: 0.3 mg/kg				
301	253.6	281.4	340.9	59.5
302	245.8	292.2	349.7	57.5
303	260.1	304.2	394.2	90.0
304	250.3	297.1	366.5	69.4
305	235.6	271.6	344.8	73.2
Mean	249.08	289.30	359.22	69.92
S.D.	9.16	12.91	21.86	13.01
Dose Level: 3 mg/kg				
101	237.9	217.6	294.7	77.1
102	250.2	226.6	319.6	93.0
103	258.9	230.1	313.9	83.8
104	245.8	220.0	272.5	52.5
105	253.5	225.0	304.8	79.8
Mean	249.26	223.86	301.10	77.24
S.D.	7.95	5.05	18.57	15.08
Dose Level: 5 mg/kg				
201	249.8	277.5	337.8	60.3
202	252.2	288.7	356.0	67.3
203	234.4	254.9	317.4	62.5
204	255.6	281.3	368.5	87.2
205	241.1	267.3	331.5	64.2
Mean	246.62	273.94	342.24	68.30
S.D.	8.68	13.15	20.19	10.87

Gain = Body Weight at Day 7 - Body Weight at Day 0

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.3 PHASE 1 - INDIVIDUAL AND MEAN BODY WEIGHTS  
AND BODY WEIGHT GAINS (GRAMS) - FEMALES

Animal Number	Body Weight			Weight Gain
	Pretest	Day 0	Day 7	
Dose Level: 0.3 mg/kg				
1301	202.2	208.9	238.3	29.4
1302	214.2	208.3	244.2	35.9
1303	198.9	196.2	233.4	37.2
1304	210.3	214.0	256.8	42.8
1305	194.4	187.9	222.2	34.3
Mean	204.00	203.06	238.98	35.92
S.D.	8.14	10.70	12.83	4.85
Dose Level: 3 mg/kg				
1101	196.0	180.2	197.7	17.5
1102	213.0	192.9	244.4	51.5
1103	200.3	180.0	217.3	37.3
1104	199.9	177.3	245.6	68.3
1105	210.2	189.5	233.6	44.1
Mean	203.88	183.98	227.72	43.74
S.D.	7.31	6.80	20.26	18.67
Dose Level: 5 mg/kg				
1201	200.0	205.9	245.5	39.6
1202	206.2	207.7	240.5	32.8
1203	188.6	187.7	220.9	33.2
1204	210.9	209.6	236.0	26.4
1205	197.1	203.7	246.7	43.0
Mean	200.56	202.92	237.92	35.00
S.D.	8.57	8.78	10.42	6.47

Gain = Body Weight at Day 7 - Body Weight at Day 0

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.4 PHASE 1 - CLINICAL SIGNS - MALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hour</u>					<u>Day</u>						
	<u>PD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
<u>Group 30 (0.3 mg/kg)</u>												
<u>Posture</u>												
Resting	0	1	1	2	4	3	2	1	2	2	1	2
Sitting or Standing	3	4	4	3	1	2	3	4	3	3	4	3
Rearing	2	0	0	0	0	0	0	0	0	0	0	0
Flattened	0	0	0	0	0	0	0	0	0	0	0	0
<u>Fecal Consistency</u>												
Normal pellets	5	4	5	5	5	5	5	5	5	5	5	5
Partially formed	0	1	0	0	0	0	0	0	0	0	0	0
<u>Gait</u>												
Normal	4	0	1	0	0	0	0	0	0	0	0	1
Not Mobile	1	5	4	5	5	5	5	5	5	5	5	4
<u>Ease of Removal from Cage</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Pupillary Size</u>												
Miosis	2	2	2	2	2	2	1	2	2	2	2	2
<u>Ease of Handling</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5

PD - Immediately post-dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.4 PHASE 1 - CLINICAL SIGNS - MALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hour</u>				<u>Day</u>							
	<u>PD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
<u>Group 10 (3 mg/kg)</u>												
<u>Posture</u>												
Resting	0	1	3	0	4	4	3	3	4	2	2	1
Sitting or Standing	3	2	0	3	1	1	2	2	1	2	1	3
Rearing	2	0	0	2	0	0	0	0	0	1	2	1
Flattened	0	2	2	0	0	0	0	0	0	0	0	0
<u>Stereotypy</u>												
Compulsive Licking	0	0	3	0	0	0	0	0	0	0	0	0
None	5	5	2	5	5	5	5	5	5	5	5	5
<u>Bizarre Behavior</u>												
Muscle Fasciculation												
-General	0	5	2	0	0	0	0	0	0	0	0	0
-Limbs	0	0	3	1	0	0	0	0	0	0	0	0
None	5	0	0	4	5	5	5	5	5	5	5	5
<u>Fecal Color</u>												
Normal	4	5	5	5	5	5	5	5	5	5	5	5
None present	1	0	0	0	0	0	0	0	0	0	0	0
<u>Fecal Consistency</u>												
Normal pellets	4	5	2	5	5	5	5	5	5	5	5	5
Partially formed	0	0	3	0	0	0	0	0	0	0	0	0
None present	1	0	0	0	0	0	0	0	0	0	0	0
<u>Gait</u>												
Normal	2	0	0	2	0	0	5	5	5	0	1	1
Not Mobile	3	5	5	3	5	5	0	0	0	5	4	4
<u>Ease of Removal from Cage</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Hindlimb Position</u>												
Abnormal	0	0	2	0	0	0	0	0	0	0	0	0
Normal	5	5	3	5	5	5	5	5	5	5	5	5
<u>Pupillary Size</u>												
Miosis	0	5	5	4	2	1	0	0	0	0	0	0
<u>Lacrimation</u>												
0	0	0	1	0	0	0	0	0	0	0	0	0
<u>Palpebral closure</u>												
Ptosis	0	0	1	0	0	0	0	0	0	0	0	0
Wide open	5	5	4	5	5	5	5	5	5	5	5	5
<u>Staining Eyes</u>												
Absent	5	4	4	4	5	5	5	5	5	5	5	5
Present	0	1	1	1	0	0	0	0	0	0	0	0
<u>Staining Mouth</u>												
Absent	5	4	4	4	5	5	5	5	5	5	5	5
Present	0	1	1	1	0	0	0	0	0	0	0	0

PD - Immediately post-dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.4 PHASE 1 - CLINICAL SIGNS - MALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hour</u>					<u>Day</u>						
	<u>PD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
<u>Group 10 (3 mg/kg)</u>												
<u>Staining Nose</u>												
Absent	5	3	0	4	5	5	5	5	5	5	5	5
Present	0	2	5	1	0	0	0	0	0	0	0	0
<u>Ease of Handling</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5

PD - Immediately post-dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.4 PHASE 1 - CLINICAL SIGNS - MALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hour</u>					<u>Day</u>						
	<u>PD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
<u>Group 20 (5 mg/kg)</u>												
<u>Posture</u>												
Resting	0	0	5	4	1	2	1	0	2	1	1	0
Sitting or Standing	4	1	0	1	3	2	2	4	2	4	4	4
Rearing	1	0	0	0	1	1	2	1	1	0	0	1
Flattened	0	4	0	0	0	0	0	0	0	0	0	0
<u>Stereotypy</u>												
Compulsive Licking	0	1	1	0	0	0	0	0	0	0	0	0
None	5	4	4	5	5	5	5	5	5	5	5	5
<u>Bizarre Behavior</u>												
Muscle Fasciculation												
-General	0	5	5	0	0	0	0	0	0	0	0	0
-Limbs	0	0	0	5	0	0	0	0	0	0	0	0
None	5	0	0	0	5	5	5	5	5	5	5	5
<u>Fecal Color</u>												
Normal	5	5	5	5	5	5	5	5	5	5	5	5
None present	0	0	0	0	0	0	0	0	0	0	0	0
<u>Fecal Consistency</u>												
Normal pellets	5	0	3	4	5	5	5	5	5	5	5	5
Partially formed	0	4	2	1	0	0	0	0	0	0	0	0
Unformed, diarrhea	0	1	0	0	0	0	0	0	0	0	0	0
None present	0	0	0	0	0	0	0	0	0	0	0	0
<u>Gait</u>												
Normal	1	0	0	0	1	2	2	1	1	0	0	0
Not Mobile	4	5	5	5	4	3	3	4	4	5	5	5
<u>Ease of Removal from Cage</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Hindlimb Position</u>												
Abnormal	0	3	1	0	0	0	0	0	0	0	0	0
Normal	5	2	4	5	5	5	5	5	5	5	5	5
<u>Pupillary Size</u>												
Miosis	1	5	4	3	3	1	1	1	1	2	2	1
<u>Lacrimation</u>												
Absent	5	4	4	5	5	5	5	5	5	5	5	5
Slight	0	1	1	0	0	0	0	0	0	0	0	0
Severe	0	0	0	0	0	0	0	0	0	0	0	0
<u>Palpebral closure</u>												
Ptosis	0	0	0	0	0	0	0	0	0	0	0	0
Wide open	5	5	5	5	5	5	5	5	5	5	5	5

PD - Immediately post-dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.4 PHASE 1 - CLINICAL SIGNS - MALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hour</u>					<u>Day</u>						
	<u>PD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
<u>Group 20 (5 mg/kg)</u>												
<u>Staining Eyes</u>												
Absent	5	3	3	1	3	5	5	5	5	5	5	5
Present	0	2	2	4	2	0	0	0	0	0	0	0
<u>Staining Mouth</u>												
Absent	5	4	4	4	4	5	5	5	5	5	5	5
Present	0	1	1	1	1	0	0	0	0	0	0	0
<u>Staining Nose</u>												
Absent	5	3	2	1	2	5	5	5	5	5	5	5
Present	0	2	3	4	3	0	0	0	0	0	0	0
<u>Fur Appearance</u>												
Normal	5	5	4	4	5	5	5	5	5	5	5	5
Slightly soiled	0	0	1	1	0	0	0	0	0	0	0	0
<u>Salivation</u>												
None	5	1	4	5	5	5	5	5	5	5	5	5
Slight	0	3	1	0	0	0	0	0	0	0	0	0
Profuse	0	1	0	0	0	0	0	0	0	0	0	0
<u>Vocalization</u>												
Normal/None	5	4	5	5	5	5	5	5	5	5	5	5
Unusual	0	1	0	0	0	0	0	0	0	0	0	0
<u>Ease of Handling</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Other</u>												
Abrasion Scapula	0	0	0	0	0	0	0	0	1	1	1	1

PD - Immediately post-dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.5 PHASE 1 - CLINICAL SIGNS - FEMALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hour</u>					<u>Day</u>						
	<u>PD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
<u>Group 35 (0.3 mg/kg)</u>												
<u>Posture</u>												
Resting	0	0	1	4	3	3	2	0	1	0	1	0
Sitting or Standing	3	3	1	1	2	1	2	5	4	2	2	2
Rearing	2	2	3	0	0	1	1	0	0	3	2	3
Flattened	0	0	0	0	0	0	0	0	0	0	0	0
<u>Gait</u>												
Normal	2	2	2	0	0	1	1	1	0	2	1	2
Not Mobile	3	3	3	5	5	4	4	4	5	3	4	3
<u>Ease of Removal from Cage</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Pupillary Size</u>												
Miosis	2	2	2	2	2	2	2	2	2	2	2	2
<u>Ease of Handling</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5

PD - Immediately post-dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.5 PHASE 1 - CLINICAL SIGNS - FEMALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hour</u>					<u>Day</u>						
	<u>PD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
<u>Group 15 (3 mg/kg)</u>												
<u>Posture</u>												
Resting	0	2	4	1	5	5	4	3	3	4	3	1
Sitting or Standing	4	1	0	3	0	0	1	1	2	1	1	2
Rearing	1	0	0	1	0	0	0	1	0	0	1	2
Flattened	0	2	1	0	0	0	0	0	0	0	0	0
<u>Stereotypy</u>												
Compulsive Licking	0	1	0	0	0	0	0	0	0	0	0	0
None	5	4	5	5	5	5	5	5	5	5	5	5
<u>Bizarre Behavior</u>												
Muscle Fasciculation												
-General	0	5	2	0	0	0	0	0	0	0	0	0
-Limbs	0	0	3	0	0	0	0	0	0	0	0	0
None	5	0	0	5	5	5	5	5	5	5	5	5
<u>Fecal Color</u>												
Normal	5	5	5	5	5	5	5	5	5	5	5	5
None present	0	0	0	0	0	0	0	0	0	0	0	0
<u>Fecal Consistency</u>												
Normal pellets	5	5	3	5	5	5	5	5	5	5	5	5
Partially formed	0	0	2	0	0	0	0	0	0	0	0	0
None present	0	0	0	0	0	0	0	0	0	0	0	0
<u>Gait</u>												
Normal	1	0	0	1	0	0	5	5	5	0	1	2
Not Mobile	4	5	5	4	5	5	0	0	0	5	4	3
<u>Ease of Removal from Cage</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Pupillary Size</u>												
Miosis	0	5	5	4	3	1	0	0	0	1	1	1
<u>Lacrimation</u>												
0	0	0	2	0	0	0	0	0	0	0	0	0
<u>Staining Eyes</u>												
Absent	5	5	3	5	5	5	5	5	5	5	5	5
Present	0	0	2	0	0	0	0	0	0	0	0	0
<u>Staining Nose</u>												
Absent	5	5	3	4	5	5	5	5	5	5	5	5
Present	0	0	2	1	0	0	0	0	0	0	0	0
<u>Ease of Handling</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5

PD - Immediately post-dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.5 PHASE 1 - CLINICAL SIGNS - FEMALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hour</u>				<u>Day</u>							
	<u>PD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
<u>Group 25 (5 mg/kg)</u>												
<u>Posture</u>												
Resting	0	0	2	4	4	2	2	1	1	0	1	1
Sitting or Standing	2	1	2	1	1	2	2	1	2	5	4	1
Rearing	3	0	0	0	0	1	1	3	2	0	0	3
Flattened	0	4	1	0	0	0	0	0	0	0	0	0
<u>Stereotypy</u>												
Compulsive Licking	0	2	2	0	0	0	0	0	0	0	0	0
None	5	3	3	5	5	5	5	5	5	5	5	5
<u>Bizarre Behavior</u>												
Muscle Fasciculation												
-General	0	5	4	0	0	0	0	0	0	0	0	0
-Limbs	0	0	1	5	0	0	0	0	0	0	0	0
None	5	0	0	0	5	5	5	5	5	5	5	5
<u>Fecal Color</u>												
Normal	5	5	5	5	5	5	5	5	5	5	5	5
None present	0	0	0	0	0	0	0	0	0	0	0	0
<u>Fecal Consistency</u>												
Normal pellets	5	1	3	5	5	5	5	5	5	5	5	5
Partially formed	0	4	2	0	0	0	0	0	0	0	0	0
None present	0	0	0	0	0	0	0	0	0	0	0	0
<u>Gait</u>												
Normal	4	0	0	0	0	1	1	3	2	0	0	3
Not Mobile	1	5	5	5	5	4	4	2	3	5	5	2
<u>Ease of Removal from Cage</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Hindlimb Position</u>												
Abnormal	0	3	0	0	0	0	0	0	0	0	0	0
Normal	5	2	5	5	5	5	5	5	5	5	5	5
<u>Pupillary Size</u>												
Miosis	0	5	5	5	3	0	0	1	1	1	2	1
<u>Lacrimation</u>												
Absent	5	4	3	5	5	5	5	5	5	5	5	5
Slight	0	1	1	0	0	0	0	0	0	0	0	0
Severe	0	0	1	0	0	0	0	0	0	0	0	0
<u>Staining Eyes</u>												
Absent	5	4	4	2	4	5	5	5	5	5	5	5
Present	0	1	1	3	1	0	0	0	0	0	0	0
<u>Staining Mouth</u>												
Absent	5	4	5	4	5	5	5	5	5	5	5	5
Present	0	1	0	1	0	0	0	0	0	0	0	0

PD - Immediately post-dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.5 PHASE 1 - CLINICAL SIGNS - FEMALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hour</u>					<u>Day</u>						
	<u>PD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>
<u>Group 25 (5 mg/kg)</u>												
<u>Staining Nose</u>												
Absent	5	4	5	1	4	5	5	5	5	5	5	5
Present	0	1	0	4	1	0	0	0	0	0	0	0
<u>Fur Appearance</u>												
Normal	5	4	4	3	4	5	5	5	5	5	5	5
Slightly soiled	0	1	1	2	1	0	0	0	0	0	0	0
<u>Salivation</u>												
None	5	1	3	5	5	5	5	5	5	5	5	5
Slight	0	3	2	0	0	0	0	0	0	0	0	0
Profuse	0	1	0	0	0	0	0	0	0	0	0	0
<u>Vocalization</u>												
Normal/None	5	3	5	5	5	5	5	5	5	5	5	5
Unusual	0	2	0	0	0	0	0	0	0	0	0	0
<u>Ease of Handling</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Other</u>												
Scab mouth left	1	1	1	1	1	0	0	0	0	0	0	0

PD - Immediately post-dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.6 PHASE 1 - INDIVIDUAL NECROPSY COMMENTS - MALES

<u>Animal Number</u>	<u>Sex</u>	<u>Died</u>	<u>Day Sacrificed</u>	<u>Necropsy Observation</u>
<u>0.3 mg/kg</u>				
301	M	-	7	No significant gross lesions.
302	M	-	7	No significant gross lesions.
303	M	-	7	No significant gross lesions.
304	M	-	7	No significant gross lesions.
305	M	-	7	No significant gross lesions.
<u>3 mg/kg</u>				
101	M	-	7	No significant gross lesions.
102	M	-	7	No significant gross lesions.
103	M	-	7	No significant gross lesions.
104	M	-	7	No significant gross lesions.
105	M	-	7	No significant gross lesions.
<u>5 mg/kg</u>				
201	M	-	7	No significant gross lesions.
202	M	-	7	No significant gross lesions.
203	M	-	7	No significant gross lesions.
204	M	-	7	No significant gross lesions.
205	M	-	7	Ulcer, Moist Scapular Region: Right, 10mm, Round Enlarged Axillary Lymph Node: Right Moderate

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.7 PHASE 1 - INDIVIDUAL NECROPSY COMMENTS - FEMALES

<u>Animal Number</u>	<u>Sex</u>	<u>Died</u>	<u>Day Sacrificed</u>	<u>Necropsy Observation</u>
<u>0.3 mg/kg</u>				
1301	F	-	7	No significant gross lesions.
1302	F	-	7	No significant gross lesions.
1303	F	-	7	No significant gross lesions.
1304	F	-	7	No significant gross lesions.
1305	F	-	7	Malformed/Misshaped Eye,Pupil:Right
<u>3 mg/kg</u>				
1101	F	-	7	No significant gross lesions.
1102	F	-	7	No significant gross lesions.
1103	F	-	7	No significant gross lesions.
1104	F	-	7	No significant gross lesions.
1105	F	-	7	No significant gross lesions.
<u>5 mg/kg</u>				
1201	F	-	7	No significant gross lesions.
1202	F	-	7	No significant gross lesions.
1203	F	-	7	Focus, Red Eye, Cornea:Left, 1mm Malformed/Misshaped Eye,Pupil:Right Cyst,Clear Kidney,Cortex:Right,One, 1mm
1204	F	-	7	No significant gross lesions.
1205	F	-	7	No significant gross lesions.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.8 PHASE 2 - INDIVIDUAL AND MEAN BODY WEIGHTS AND BODY WEIGHT GAINS (GRAMS) - FEMALES

Animal Number	Body Weight		Weight at Sacrifice	
	Pretest	Day 0	Weight	Gain
Sacrifice Interval: 2 Hours			<u>Group 65a (0 mg/kg)</u>	
1106	228.8	214.7	210.2	-4.5
1107	213.6	196.3	188.2	-8.1
1108	242.4	223.0	219.4	-3.6
1109	222.1	201.9	198.8	-3.1
1110	233.7	211.5	208.2	-3.3
Mean	228.12	209.48	204.96	-4.52
S.D.	10.98	10.55	11.89	2.07
Sacrifice Interval: 2 Hours			<u>Group 75a (3 mg/kg)</u>	
1206	229.4	213.1	204.9	-8.2
1207	234.6	208.7	200.9	-7.8
1208	211.3	192.0	186.0	-6.0
1209	243.9	224.8	216.4	-8.4
1210	221.6	202.6	195.9	-6.7
Mean	228.16	208.24	200.82	-7.42*
S.D.	12.43	12.18	11.22	1.03
Sacrifice Interval: 4 Hours			<u>Group 65b (0 mg/kg)</u>	
1111	230.2	207.8	205.0	-2.8
1112	221.5	199.4	196.8	-2.6
1113	244.8	225.5	223.7	-1.8
1114	209.1	185.7	183.8	-1.9
1115	235.4	214.1	210.5	-3.6
Mean	228.2	206.50	203.96	-2.54
S.D.	13.61	15.03	14.93	0.73
Sacrifice Interval: 4 Hours			<u>Group 75b (3 mg/kg)</u>	
1211	247.5	223.5	214.6	-8.9
1212	220.9	200.4	188.9	-11.5
1213	237.1	213.9	203.5	-10.4
1214	230.7	206.5	198.8	-7.7
1215	206.5	189.4	180.7	-8.7
Mean	228.54	206.74	197.30	-9.44**
S.D.	15.66	12.97	13.10	1.50

Gain = Body Weight at Time of Sacrifice - Body Weight at Day 0

\* Significantly different from control,  $p \leq 0.05$  by Dunnett's "t" test (two-tailed).\*\* Significantly different from control,  $p \leq 0.01$  by Dunnett's "t" test (two-tailed).

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.8 PHASE 2 - INDIVIDUAL AND MEAN BODY WEIGHTS AND BODY WEIGHT GAINS (GRAMS) - FEMALES

Animal Number	Body Weight		Weight at Sacrifice	
	Pretest	Day 0	Weight	Gain
Sacrifice Interval: 6 Hours			<u>Group 65c (0 mg/kg)</u>	
1116	239.7	215.5	213.3	-2.2
1117	220.6	197.9	203.5	5.6
1118	227.4	204.4	202.2	-2.2
1119	231.3	210.4	212.9	2.5
1120	204.6	186.0	190.3	4.3
Mean	224.72	202.84	204.44	1.60
S.D.	13.20	11.49	9.43	3.64
Sacrifice Interval: 6 Hours			<u>Group 75c (3 mg/kg)</u>	
1216	231.7	204.7	205.6	0.9
1217	216.6	201.0	197.6	-3.4
1218	240.1	213.1	205.4	-7.7
1219	204.6	186.4	180.5	-5.9
1220	226.5	208.6	207.9	-0.7
Mean	223.9	202.76	199.40	-3.36
S.D.	13.75	10.19	11.26	3.55
Sacrifice Interval: 24 Hours			<u>Group 65d (0 mg/kg)</u>	
1121	225.3	201.1	220.3	19.2
1122	240.8	219.0	244.3	25.3
1123	203.2	183.3	203.4	20.1
1124	231.8	210.7	229.3	18.6
1125	216.5	196.4	210.4	14.0
Mean	223.52	202.10	221.54	19.44
S.D.	14.43	13.66	16.07	4.04
Sacrifice Interval: 24 Hours			<u>Group 75d (3 mg/kg)</u>	
1221	200.2	184.8	200.2	15.4
1222	223.7	201.3	187.9	-13.4
1223	242.2	222.0	234.4	12.4
1224	215.5	194.3	211.8	17.5
1225	232.3	213.1	226.0	12.9
Mean	222.78	203.10	212.06	8.96
S.D.	16.06	14.77	18.83	12.67

Gain = Body Weight at Time of Sacrifice - Body Weight at Day 0

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.9 PHASE 2 - CLINICAL SIGNS - FEMALES  
(Number of Animals with Observation)

Observation	Hours			Hours		
	PreD	1	2	PreD	1	2
	<u>Group 65a (0 mg/kg)</u>			<u>Group 75a (3 mg/kg)</u>		
<u>Posture</u>						
Resting	1	1	4	2	0	3
Sitting or Standing	4	2	1	3	2	2
Rearing	0	2	0	0	0	0
Flattened	0	0	0	0	3	0
<u>Stereotypy</u>						
Compulsive Licking	0	0	0	0	1	0
None	5	5	5	5	4	5
<u>Bizarre Behavior</u>						
Muscle Fasciculation						
-General	0	0	0	0	5	2
-Limbs	0	0	0	0	0	3
None	5	5	5	5	0	0
<u>Fecal Color</u>						
Normal	5	5	5	5	5	5
None present	0	0	0	0	0	0
<u>Fecal Consistency</u>						
Normal pellets	5	5	5	5	5	5
Partially formed	0	0	0	0	0	0
None present	0	0	0	0	0	0
<u>Gait</u>						
Normal	2	1	0	1	0	0
Not Mobile	3	4	5	4	5	5
<u>Ease of Removal from Cage</u>						
Easy	5	5	5	5	5	5
<u>Hindlimb Position</u>						
Normal	5	5	5	5	3	5
Abnormal	0	0	0	0	2	0
<u>Pupillary Size</u>						
Miosis	0	0	0	1	5	4
<u>Lacrimation</u>						
	0	0	0	0	1	1
<u>Staining Eyes</u>						
Absent	5	5	5	5	4	3
Present	0	0	0	0	1	2
<u>Staining Mouth</u>						
Absent	5	5	5	5	4	5
Present	0	0	0	0	1	0
<u>Staining Nose</u>						
Absent	5	5	5	5	4	3
Present	0	0	0	0	1	2
<u>Ease of Handling</u>						
Easy	5	5	5	5	5	5

PreD - Pre-Dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.9 PHASE 2 - CLINICAL SIGNS - FEMALES  
(Number of Animals with Observation)

Observation	Hours				Hours			
	PreD	1	2	4	PreD	1	2	4
	<u>Group 65b (0 mg/kg)</u>				<u>Group 75b (3 mg/kg)</u>			
<u>Posture</u>								
Resting	2	2	3	5	1	0	2	2
Sitting or Standing	3	3	2	0	3	1	1	3
Rearing	0	0	0	0	1	0	0	0
Flattened	0	0	0	0	0	4	2	0
<u>Stereotypy</u>								
Compulsive Licking	0	0	0	0	0	0	0	0
None	5	5	5	5	5	5	5	5
<u>Bizarre Behavior</u>								
Muscle Fasciculation								
-General	0	0	0	0	0	5	0	0
-Limbs	0	0	0	0	0	0	5	2
None	5	5	5	5	5	0	0	3
<u>Fecal Color</u>								
Normal	5	5	5	5	5	5	5	5
None present	0	0	0	0	0	0	0	0
<u>Fecal Consistency</u>								
Normal pellets	5	5	5	5	5	0	5	5
Partially formed	0	0	0	0	0	5	0	0
None present	0	0	0	0	0	0	0	0
<u>Gait</u>								
Normal	0	0	0	0	1	0	0	0
Not Mobile	5	5	5	5	4	5	5	5
<u>Ease of Removal from Cage</u>								
Easy	5	5	5	5	5	5	5	5
<u>Hindlimb Position</u>								
Normal	5	5	5	5	5	4	5	5
Abnormal	0	0	0	0	0	1	0	0
<u>Pupillary Size</u>								
Miosis	0	0	0	0	0	5	5	3
<u>Lacrimation</u>								
	0	0	0	0	0	2	1	0
<u>Staining Eyes</u>								
Absent	5	5	5	5	5	5	4	2
Present	0	0	0	0	0	0	1	3
<u>Staining Mouth</u>								
Absent	5	5	5	5	5	3	3	5
Present	0	0	0	0	0	2	2	0
<u>Staining Nose</u>								
Absent	5	5	4	3	5	3	3	2
Present	0	0	1	2	0	2	2	3
<u>Salivation</u>								
	0	0	0	0	0	1	0	0
<u>Ease of Handling</u>								
Easy	5	5	5	5	5	5	5	5
<u>Other</u>								
Alopecia Forelimb	0	0	0	0	1	1	1	1

PreD - Pre-Dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.9 PHASE 2 - CLINICAL SIGNS - FEMALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hours</u>					<u>Hours</u>				
	<u>PreD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>	<u>PreD</u>	<u>1</u>	<u>2</u>	<u>4</u>	<u>6</u>
	<u>Group 65c (0 mg/kg)</u>					<u>Group 75c (3 mg/kg)</u>				
<u>Posture</u>										
Resting	1	3	4	3	1	1	0	3	4	4
Sitting or Standing	3	1	1	2	4	3	1	2	1	1
Rearing	1	1	0	0	0	1	0	0	0	0
Flattened	0	0	0	0	0	0	4	0	0	0
<u>Stereotypy</u>										
Compulsive Licking	0	0	0	0	0	0	4	1	0	0
None	5	5	5	5	5	5	1	4	5	5
<u>Bizarre Behavior</u>										
Muscle Fasciculation										
-General	0	0	0	0	0	0	5	0	0	0
-Limbs	0	0	0	0	0	0	0	5	0	0
None	5	5	5	5	5	5	0	0	5	5
<u>Fecal Color</u>										
Normal	5	5	5	5	5	5	5	5	5	5
None present	0	0	0	0	0	0	0	0	0	0
<u>Fecal Consistency</u>										
Normal pellets	5	5	5	5	5	5	5	5	5	5
Partially formed	0	0	0	0	0	0	0	0	0	0
None present	0	0	0	0	0	0	0	0	0	0
<u>Gait</u>										
Normal	0	1	0	1	0	1	0	0	1	0
Not Mobile	5	4	5	4	5	4	5	5	4	5
<u>Ease of Removal from Cage</u>										
Easy	5	5	5	5	5	5	5	5	5	5
<u>Pupillary Size</u>										
Miosis	1	1	1	1	1	0	5	5	3	3
<u>Lacrimation</u>										
0	0	0	0	0	0	0	1	0	0	0
<u>Staining Eyes</u>										
Absent	5	5	5	5	5	5	5	3	2	5
Present	0	0	0	0	0	0	0	2	3	0
<u>Staining Mouth</u>										
Absent	5	5	5	5	5	5	5	4	5	5
Present	0	0	0	0	0	0	0	1	0	0
<u>Staining Nose</u>										
Absent	5	3	3	5	5	5	3	2	3	5
Present	0	2	2	0	0	0	2	3	2	0
<u>Ease of Handling</u>										
Easy	5	5	5	5	5	5	5	5	5	5

PreD - Pre-Dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.9 PHASE 2 - CLINICAL SIGNS - FEMALES  
(Number of Animals with Observation)

Observation	Hours						Hours					
	PreD	1	2	4	6	24	PreD	1	2	4	6	24
	Group 65d (0 mg/kg)						Group 75d (3 mg/kg)					
<u>Posture</u>												
Resting	2	0	3	4	3	2	2	0	4	3	3	2
Sitting or Standing	3	4	1	1	1	2	2	0	0	2	1	2
Rearing	0	1	1	0	1	1	1	0	1	0	1	1
Flattened	0	0	0	0	0	0	0	5	0	0	0	0
<u>Stereotypy</u>												
Compulsive Licking	0	0	0	0	0	0	0	0	0	0	0	0
None	5	5	5	5	5	5	5	5	5	5	5	5
<u>Bizarre Behavior</u>												
Muscle Fasciculation												
-General	0	0	0	0	0	0	0	5	3	0	0	0
-Limbs	0	0	0	0	0	0	0	0	2	1	0	0
None	5	5	5	5	5	5	5	0	0	4	5	5
<u>Fecal Color</u>												
Normal	5	5	5	5	5	5	5	5	5	5	5	5
None present	0	0	0	0	0	0	0	0	0	0	0	0
<u>Fecal Consistency</u>												
Normal pellets	5	5	5	5	5	5	5	3	5	5	5	5
Partially formed	0	0	0	0	0	0	0	2	0	0	0	0
None present	0	0	0	0	0	0	0	0	0	0	0	0
<u>Gait</u>												
Normal	1	1	1	0	1	0	1	0	0	0	0	1
Not Mobile	4	4	4	5	4	5	4	5	5	5	5	4
<u>Ease of Removal from Cage</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Pupillary Size</u>												
Miosis	1	1	2	1	1	1	0	5	5	3	2	0
<u>Lacrimation</u>												
0	0	0	0	0	0	0	0	3	1	0	0	0
<u>Staining Eyes</u>												
Absent	5	5	5	5	5	5	5	5	4	4	5	5
Present	0	0	0	0	0	0	0	0	1	1	0	0
<u>Staining Nose</u>												
Absent	5	5	5	5	5	5	5	4	3	3	4	5
Present	0	0	0	0	0	0	0	1	2	2	1	0
<u>Salivation</u>												
None	5	5	5	5	5	5	5	2	5	5	5	5
Slight	0	0	0	0	0	0	0	2	0	0	0	0
Profuse	0	0	0	0	0	0	0	1	0	0	0	0
<u>Ease of Handling</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5

PreD - Pre-Dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.10 PHASE 2 - INDIVIDUAL NECROPSY COMMENTS - FÉMALES

<u>Animal Number</u>	<u>Sex</u>	<u>Died</u>	<u>Hour Sacrificed</u>	<u>Necropsy Observation</u>
<u>0 mg/kg</u>				
1106	F	-	2	No significant gross lesions.
1107	F	-	2	No significant gross lesions.
1108	F	-	2	No significant gross lesions.
1109	F	-	2	No significant gross lesions.
1110	F	-	2	No significant gross lesions.
<u>0 mg/kg</u>				
1111	F	-	4	No significant gross lesions.
1112	F	-	4	No significant gross lesions.
1113	F	-	4	No significant gross lesions.
1114	F	-	4	No significant gross lesions.
1115	F	-	4	No significant gross lesions.
<u>0 mg/kg</u>				
1116	F	-	6	No significant gross lesions.
1117	F	-	6	No significant gross lesions.
1118	F	-	6	No significant gross lesions.
1119	F	-	6	No significant gross lesions.
1120	F	-	6	No significant gross lesions.
<u>0 mg/kg</u>				
1121	F	-	24	No significant gross lesions.
1122	F	-	24	No significant gross lesions.
1123	F	-	24	No significant gross lesions.
1124	F	-	24	No significant gross lesions.
1125	F	-	24	No significant gross lesions.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.10 PHASE 2 - INDIVIDUAL NECROPSY COMMENTS - FÉMALES

<u>Animal Number</u>	<u>Sex</u>	<u>Died</u>	<u>Hour Sacrificed</u>	<u>Necropsy Observation</u>
<u>3 mg/kg</u>				
1206	F	-	2	No significant gross lesions.
1207	F	-	2	No significant gross lesions.
1208	F	-	2	No significant gross lesions.
1209	F	-	2	No significant gross lesions.
1210	F	-	2	No significant gross lesions.
<u>3 mg/kg</u>				
1211	F	-	4	No significant gross lesions.
1212	F	-	4	No significant gross lesions.
1213	F	-	4	No significant gross lesions.
1214	F	-	4	No significant gross lesions.
1215	F	-	4	No significant gross lesions.
<u>3 mg/kg</u>				
1216	F	-	6	No significant gross lesions.
1217	F	-	6	No significant gross lesions.
1218	F	-	6	No significant gross lesions.
1219	F	-	6	No significant gross lesions.
1220	F	-	6	No significant gross lesions.
<u>3 mg/kg</u>				
1221	F	-	24	No significant gross lesions.
1222	F	-	24	No significant gross lesions.
1223	F	-	24	No significant gross lesions.
1224	F	-	24	No significant gross lesions.
1225	F	-	24	No significant gross lesions.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.11 SUMMARY OF PHASE 2 CHOLINESTERASE DATA - FEMALES

TEST NAME AND UNITS	INT.	DOSE LEVELS (MG/KG)		PERCENT OF CONTROL	
		0	3		
		TEST RESULTS			
SERUM CHOLINESTERASE (U/L)	02	MEAN	919	182**	19.8
		S.D.	136	49	
		N	5	5	
	04	MEAN	820	258**	31.5
		S.D.	120	34	
		N	5	5	
	06	MEAN	793	290**	36.6
		S.D.	179	52	
		N	5	5	
	24	MEAN	835	564**	67.5
		S.D.	147	31	
		N	5	5	
RBC CHOLINESTERASE (U/L PRBC)	02	MEAN	1576	448**	28.4
		S.D.	264	78	
		N	5	5	
	04	MEAN	1336	388**	29.0
		S.D.	220	160	
		N	5	5	
	06	MEAN	1576	676**	42.9
		S.D.	178	89	
		N	5	5	
	24	MEAN	1356	896**	66.1
		S.D.	229	38	
		N	5	5	
BRAIN CHOLINESTERASE (U/G TISSUE)	02	MEAN	8.89	1.12**	12.6
		S.D.	1.78	0.21	
		N	5	5	
	04	MEAN	9.71	2.02**	20.8
		S.D.	0.73	0.31	
		N	5	5	
	06	MEAN	8.98	3.78**	42.1
		S.D.	0.89	0.46	
		N	5	5	
	24	MEAN	8.29	6.04**	72.9
		S.D.	0.42	1.17	
		N	5	5	

\*\* Significantly different from control,  $p \leq 0.01$  by Dunnett's "t" test (two-tailed).

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.12 PHASE 3 - INDIVIDUAL AND MEAN BODY WEIGHTS (GRAMS) - MALES

Animal Number	Body Weight	
	Pretest	Day 0

Dose Level: (0 mg/kg)

106	290.3	264.7
107	312.6	282.5
108	299.0	270.8
109	295.3	266.0
110	305.5	274.4
Mean	300.54	271.68
S.D.	8.73	7.18

Dose Level: (0.01 mg/kg)

206	299.0	273.5
207	301.2	273.0
208	286.5	264.0
209	309.7	279.8
210	292.3	265.3
Mean	297.74	271.12
S.D.	8.84	6.50

Dose Level: (0.03 mg/kg)

306	306.3	278.2
307	295.2	268.4
308	299.1	262.4
309	314.5	280.3
310	287.9	260.7
Mean	300.60	270.00
S.D.	10.23	8.95

Dose Level: (0.1 mg/kg)

406	304.0	275.5
407	284.3	254.1
408	310.2	282.8
409	297.9	270.2
410	292.2	265.5
Mean	297.72	269.62
S.D.	10.07	10.80

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.12 PHASE 3 - INDIVIDUAL AND MEAN BODY WEIGHTS (GRAMS) - MALES

Animal Number	Body Weight	
	Pretest	Day 0
Dose Level: <u>(0.3 mg/kg)</u>		
506	308.7	276.5
507	293.3	269.4
508	314.7	282.9
509	287.1	261.5
510	299.9	269.1
Mean	300.74	271.88
S.D.	11.18	8.13
Dose Level: <u>(1 mg/kg)</u>		
606	283.0	259.1
607	297.1	270.5
608	304.4	273.8
609	291.2	263.1
610	312.4	283.5
Mean	297.62	270.00
S.D.	11.40	9.53

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.13 PHASE 3 - INDIVIDUAL AND MEAN BODY WEIGHTS (GRAMS) - FEMALES

Animal Number	Body Weight	
	Pretest	Day 0

Dose Level: (0 mg/kg)

1126	197.2	176.0
1127	215.8	194.0
1128	210.4	188.5
1129	208.4	182.1
1130	218.0	192.3
Mean	209.96	186.58
S.D.	8.13	7.47

Dose Level: (0.01 mg/kg)

1226	202.3	179.8
1227	214.2	187.3
1228	196.3	174.9
1229	216.4	200.1
1230	210.1	188.3
Mean	207.86	186.08
S.D.	8.41	9.58

Dose Level: (0.03 mg/kg)

1326	218.5	200.5
1327	207.7	188.7
1328	216.0	194.5
1329	211.0	189.5
1330	196.5	180.6
Mean	209.94	190.76
S.D.	8.61	7.38

Dose Level: (0.1 mg/kg)

1426	215.1	197.2
1427	196.0	181.8
1428	210.0	192.6
1429	216.4	191.4
1430	200.8	179.7
Mean	207.66	188.54
S.D.	8.95	7.47

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.13 PHASE 3 - INDIVIDUAL AND MEAN BODY WEIGHTS (GRAMS) - FEMALES

<u>Animal Number</u>	<u>Body Weight</u>	
	<u>Pretest</u>	<u>Day 0</u>
Dose Level: <u>(0.3 mg/kg)</u>		
1526	202.9	180.2
1527	219.5	192.1
1528	213.0	194.8
1529	196.3	178.9
1530	216.3	196.3
Mean	209.60	188.46
S.D.	9.70	8.28
Dose Level: <u>(1 mg/kg)</u>		
1626	195.4	171.0
1627	215.8	191.8
1628	208.9	183.0
1629	200.8	180.0
1630	216.7	192.6
Mean	207.52	183.68
S.D.	9.31	8.95

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.14 PHASE 3 - CLINICAL SIGNS - MALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hours</u>											
	<u>PreD</u>	<u>1</u>	<u>2</u>	<u>PreD</u>	<u>1</u>	<u>2</u>	<u>PreD</u>	<u>1</u>	<u>2</u>	<u>PreD</u>	<u>1</u>	<u>2</u>
	<u>(0 mg/kg)</u>			<u>(0.01 mg/kg)</u>			<u>(0.03 mg/kg)</u>			<u>(0.1 mg/kg)</u>		
<u>Posture</u>												
Resting	2	4	5	2	4	4	3	4	4	2	3	3
Sitting or Standing	3	0	0	3	1	1	2	0	1	3	1	0
Rearing	0	1	0	0	0	0	0	1	0	0	1	2
<u>Gait</u>												
Normal	0	1	0	0	0	0	0	0	0	0	0	0
Not Mobile	5	4	5	5	5	5	5	5	5	5	5	5
<u>Ease of Removal from Cage</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Pupillary Size</u>												
Miosis	0	0	0	0	0	0	1	1	1	0	2	2
<u>Staining Nose</u>												
Absent	5	5	5	5	5	4	4	4	3	5	4	4
Present	0	0	0	0	0	1	1	1	2	0	1	1
<u>Ease of Handling</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5

<u>Observation</u>	<u>Hours</u>					
	<u>PreD</u>	<u>1</u>	<u>2</u>	<u>PreD</u>	<u>1</u>	<u>2</u>
	<u>(0.3 mg/kg)</u>			<u>(1 mg/kg)</u>		
<u>Posture</u>						
Resting	0	2	2	0	0	1
Sitting or Standing	4	3	2	4	5	4
Rearing	1	0	1	1	0	0
<u>Bizarre Behavior</u>						
Muscle Fasciculation						
-Limbs	0	0	0	0	1	1
None	5	5	5	5	4	4
<u>Gait</u>						
Normal	1	0	0	0	0	0
Not Mobile	4	5	5	5	5	5
<u>Ease of Removal from Cage</u>						
Easy	5	5	5	5	5	5
<u>Pupillary Size</u>						
Miosis	1	2	2	0	4	3
<u>Staining Nose</u>						
Absent	5	5	5	5	4	5
Present	0	0	0	0	1	0
<u>Salivation - Slight</u>	0	0	0	0	1	0
<u>Ease of Handling</u>						
Easy	5	5	5	5	5	5

PreD - Pre-Dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.15 PHASE 3 - CLINICAL SIGNS - FEMALES  
(Number of Animals with Observation)

<u>Observation</u>	<u>Hours</u>											
	<u>PreD</u>	<u>1</u>	<u>2</u>	<u>PreD</u>	<u>1</u>	<u>2</u>	<u>PreD</u>	<u>1</u>	<u>2</u>	<u>PreD</u>	<u>1</u>	<u>2</u>
	<u>(0 mg/kg)</u>			<u>(0.01 mg/kg)</u>			<u>(0.03 mg/kg)</u>			<u>(0.1 mg/kg)</u>		
<u>Posture</u>												
Resting	3	3	2	2	5	1	1	4	2	3	3	3
Sitting or Standing	2	1	2	3	0	3	3	0	3	1	2	0
Rearing	0	1	1	0	0	1	1	1	0	1	0	2
<u>Gait</u>												
Normal	0	1	0	0	0	0	1	0	0	0	0	1
Not Mobile	5	4	5	5	5	5	4	5	5	5	5	4
<u>Ease of Removal from Cage</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5
<u>Pupillary Size</u>												
Miosis	0	0	0	0	1	1	1	1	1	0	0	0
<u>Staining Nose</u>												
Absent	5	5	5	5	4	4	5	5	5	5	5	5
Present	0	0	0	0	1	1	0	0	0	0	0	0
<u>Ease of Handling</u>												
Easy	5	5	5	5	5	5	5	5	5	5	5	5

<u>Observation</u>	<u>Hours</u>					
	<u>PreD</u>	<u>1</u>	<u>2</u>	<u>PreD</u>	<u>1</u>	<u>2</u>
	<u>(0.3 mg/kg)</u>			<u>(1 mg/kg)</u>		
<u>Posture</u>						
Resting	3	3	1	1	0	2
Sitting or Standing	2	1	2	2	4	2
Rearing	0	1	2	2	1	1
<u>Gait</u>						
Normal	0	1	1	1	1	1
Not Mobile	5	4	4	4	4	4
<u>Ease of Removal from Cage</u>						
Easy	5	5	5	5	5	5
<u>Pupillary Size</u>						
Miosis	0	0	0	0	4	3
<u>Staining Nose</u>						
Absent	5	5	4	5	3	5
Present	0	0	1	0	2	0
<u>Ease of Handling</u>						
Easy	5	5	5	5	5	5

PreD - Pre-Dose.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

TABLE 8.16 SUMMARY OF PHASE 3 CHOLINESTERASE DATA

SEX: MALE

TEST NAME AND UNITS	INT.	----- DOSE LEVELS (MG/KG) -----						
		0	0.01	0.03	0.1	0.3	1	
SERUM CHOLINESTERASE (U/L)	02	MEAN	377	340	296	250**	167**	63**
		S.D.	97	43	55	64	35	11
		N	5	5	5	5	5	5
RBC CHOLINESTERASE (U/L PRBC)	02	MEAN	1884	2164	1780	1648	1064**	648**
		S.D.	144	344	229	213	245	92
		N	5	5	5	5	5	5
BRAIN CHOLINESTERASE (U/G TISSUE)	02	MEAN	9.33	9.21	9.36	7.78**	5.22**	2.58**
		S.D.	0.70	0.36	0.38	1.13	0.65	0.43
		N	5	5	5	5	5	5

SEX: FEMALE

TEST NAME AND UNITS	INT.	----- DOSE LEVELS (MG/KG) -----						
		0	0.01	0.03	0.1	0.3	1	
SERUM CHOLINESTERASE (U/L)	02	MEAN	791	757	874	865	565*	337**
		S.D.	89	130	182	136	126	82
		N	5	4	5	5	5	5
RBC CHOLINESTERASE (U/L PRBC)	02	MEAN	2140	2100	1988	1980	748**	544**
		S.D.	211	377	246	163	135	194
		N	5	5	5	5	5	5
BRAIN CHOLINESTERASE (U/G TISSUE)	02	MEAN	9.18	8.93	8.51	7.78**	6.77**	2.94**
		S.D.	0.36	0.48	0.51	0.71	0.51	0.46
		N	5	5	5	5	5	5

\* Significantly different from control,  $p \leq 0.05$  by Dunnett's "t" test (two-tailed).\*\* Significantly different from control,  $p \leq 0.01$  by Dunnett's "t" test (two-tailed).

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1 STUDY PROTOCOL & AMENDMENTS

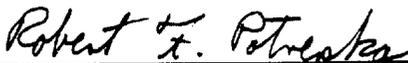
- Appendix 9.1.1 Study Protocol
- Appendix 9.1.2 List of Protocol Amendments

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1.1 STUDY PROTOCOL

STUDY NO: F-00189

Study Director:  
Senior Group Leader,  
Toxicology

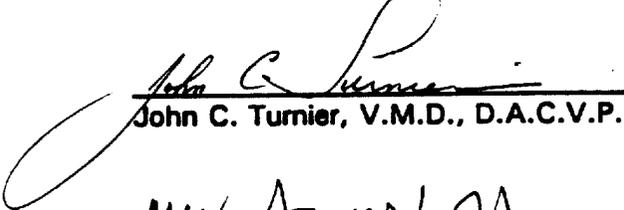
  
Robert F. Potrepka, Ph.D.

Date: 3-29-94

Alternate Study Director:  
Senior Toxicologist,  
Toxicology

  
Edward Chow, Ph.D., D.A.B.T.

Study Pathologist:  
Manager, Pathology

  
John C. Turnier, V.M.D., D.A.C.V.P.

Chairperson:  
Institutional Animal Care  
and Use Committee

  
Mitchell W. Sauerhoff, Ph.D., D.A.B.T.

Manager:  
Toxicological Sciences

  
Dale W. Matheson, Ph.D.

Product Manager:  
Toxicology  
Greensboro, NC

  
Jean A. Sova

Director:  
Toxicology  
Greensboro, NC

  
Donald R. Saunders, Ph.D., D.A.B.T.

Laboratory:

Ciba-Geigy Corporation  
Environmental Health Center  
400 Farmington Avenue  
Farmington, CT 06032-1959

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1.1 STUDY PROTOCOL

1. Purpose

This study is designed to determine 1) the no observable effect level for clinical/behavioral/body weight effects (Phase 1), 2) the peak inhibition time(s) for red blood cell, serum and brain cholinesterase activity (Phase 2), and 3) the no observable effect level for inhibition of red blood cell, serum and brain cholinesterase activity (Phase 3) when the test substance is administered by the oral route (gavage) to rats. This study will be conducted according to current EHC Standard Operating Procedures, EPA-FIFRA GLP Standards (40 CFR, Part 160), the OECD Principles of Good Laboratory Practices (Annex 2, C(81)30) and the Good Laboratory Practice Standards of Japan (MAFF, 59 NohSan, No. 3850, August 10, 1984).

2. Alternatives to the Study

There are currently no acceptable alternative methods to acute toxicity testing in animals as required by the regulatory agencies for the registration of pesticides (EPA, OECD and MAFF). As rats are used in this study, the Institutional Animal Care and Use Committee SOP (Z-01-002) will be followed. This study does not unnecessarily duplicate a previous experiment.

3. Sponsor

Ciba-Geigy Corporation  
Plant Protection Division

Testing Facility:  
Environmental Health Center (EHC)  
400 Farmington Avenue  
Farmington, CT 06032-1959

Headquarters:  
410 Swing Road  
Post Office Box 18300  
Greensboro, NC 27419-8300

4. Project Number

F00189

5. Responsible Personnel

Study Director:  
Alternate Study Director:  
Toxicology Support

Study Pathologist:  
Product Manager:

R. F. Potrepka, Ph.D.  
E. Chow, Ph.D., D.A.B.T.  
S. L. Flowers, B.S.  
T. H. Gaghan, B.S.  
J. C. Turnier, V.M.D., D.A.C.V.P.  
J. A. Sova

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1.1 STUDY PROTOCOL

6. Proposed Schedule

Starting Date of Acclimation:	March 23, 1994 (Phase 1) April 5, 1994 (Phase 2) April 26, 1994 (Phase 3)
Starting Date of Administration:	March 30, 1994 (Phase 1) April 19, 1994 (Phase 2) May 10, 1994 (Phase 3)
Experimental Completion Date:	May 17, 1994
Draft Report Date:	June 15, 1994
Final Report Date:	To be determined

7. Test Substance Data

- 7.1 Identification: Monocrotophos (Technical), (C1414)
- 7.2 Lot No.: FL-940574, Batch No. OP 107001, EHC Code No.: 0173-19
- 7.3 Description: Brown liquid
- 7.4 Purity/Stability: 77.6% Samples of the test substance and control materials will be taken before administration and at the end of the study and frozen for possible purity and stability determinations. Samples of test substance/vehicle will also be taken and frozen for possible determination of concentration and homogeneity.
- 7.5 Source: Ciba-Geigy Corporation  
Plant Protection Division  
Greensboro, NC
- 7.6 Stability and Storage Conditions: The test substance will be stored at room temperature in tightly sealed containers and protected from light and away from flammable materials, sources of heat and flame, and food stuffs.
- 7.7 Safety Precautions: A separate Safety Supplement (issued with the final protocol) outlines the safety procedures to be followed when working with the test substance, its mixtures and waste.

8. Vehicle Control

- 8.1 Identification: Distilled water
- 8.2 Physical Description: Clear, colorless liquid
- 8.3 Storage: Room temperature

9. Test Animals

- 9.1 Species: Rat
- 9.2 Stock/Strain: Crl:CD®(SD)BR VAF/Plus™

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1.1 STUDY PROTOCOL

9. Test Animals - (Cont'd.)

9.3 Reason for Selection

The Sprague Dawley rat is a standard laboratory animal for evaluation of chemical toxicity. Rats are preferred by various regulatory agencies as a representative of rodent species.

9.4 Source: Charles River Laboratories, Inc., Portage, Michigan

9.5 Age at Start of Study: The animals received for each phase of the study will be approximately 6-7 weeks old at receipt and will be approximately 8-9 weeks of age at the initiation of dosing. The quarantine-acclimation period will be at least 7 days.

9.6 Number on Study: 5 males and 5 females (nulliparous and nonpregnant) for each dose level in Phases 1 and 3 and 20 females for each dose level in Phase 2.

9.7 Animal Housing and Maintenance

Rats will be housed in rooms 307 (Phase 1), 305 (Phase 2) and 317 (Phase 3) during the quarantine/acclimation and study periods. Rats will be housed no more than two per same sex per cage during acclimation, but will be housed individually in suspended polycarbonate cages (19 x 21 x 20 cm) during the study. Hardwood chips will be used for bedding. Racks, cages and feeders will be changed at least weekly. Conventional disease control will be practiced and only authorized personnel will be permitted in the study rooms.

The animals will receive PMI® Feeds' Certified Rodent Chow® #5002 ground meal diet in glass jar feeders. Food is provided ad libitum but will be withheld at least 17 hours before dosing. The identity of each lot used will be recorded. Certified diets are analyzed by the manufacturer, prior to delivery, for nutrients and potential contaminants. The manufacturer's analysis was evaluated by the Study Director and it was determined that the maximum concentration of the contaminants listed in the analysis profile would not be likely to affect the conduct or purpose of the study.

Water will be provided ad libitum by an automatic watering system, and if necessary, in water bottles. Drinking water from the local municipal system will be provided to the animals ad libitum by an automatic watering system. If necessary, water bottles which contain the same source of water as the automatic watering system will be provided. The water supplied to the facility is analyzed periodically for contaminants. The most recent water analysis (reported on July 26, 1993) was evaluated by the Study Director and it was determined that the level of contaminants listed in the analysis would not be likely to affect the conduct or purpose of the study. Concentrations of the potential contaminants tested for were below detection levels or below the maximum allowable concentrations for drinking water published by the State of Connecticut.

The facility's Heating-Ventilation and Air Conditioning (HVAC) system is designed to provide at least 15 room air changes per hour while maintaining the temperature between 19-24°C and the relative humidity between 40-60%. Room lighting will be on a timer-controlled 12 hour light-dark cycle (light from approximately 6 a.m. to

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1.1 STUDY PROTOCOL

9. Test Animals - (Cont'd.)

9.7 Animal Housing and Maintenance - (Cont'd.)

6 p.m. EST). Temperature and humidity will be monitored continuously, recorded daily and the documentation will be placed in the EHC archives weekly.

9.8 Quarantine/Acclimation

Upon arrival at the EHC, the rats will be removed from the shipping crates and housed at no more than 2 per same sex per cage. The correct identification and separation of the sexes will be confirmed during distribution. Prior to or during the pretest physical examination, the rats in each cage will be assigned sequential quarantine numbers and given a tail mark so that cagemates can be distinguished. The quarantine cage cards will be marked accordingly. During the quarantine/acclimation period, the animals will be examined by a veterinarian with respect to their state of health and suitability as test animals.

9.9 Group Assignment

The animals for each phase of the study will be ranked by body weight and assigned to the study groups such that all study groups of the same sex will have similar mean body weights at the time of assignment (weight variation of animals used should not exceed  $\pm 20\%$  of the mean weight). After assignment, the animals will be housed individually and non-assigned animals will be removed from the study room as soon as possible.

9.10 Method of Identification

Each animal will be individually identified with an animal ID number by an ear tag. Color-coded transfer labels for the cages will be issued bearing the study number, the animal ID number, sex, dose group number and/or dose level.

10. Dose Administration

10.1 Dose Preparation and Administration

The test substance will be mixed with distilled water at appropriate concentrations to allow oral gavage delivery of the desired dose to the animals at 5 ml/kg based on the animal's body weight taken just before dosing. Dosing preparations will be prepared by Pharmacy according to a procedure approved by the Study Director. The animals will be fasted for 17 to 20 hours before test substance administration (between 6:00 and 9:00 a.m. EST). Food will be restored to the animals approximately 4 hours post-dose. The test mixtures will be stored at room temperature until administration. After administration, any remaining test mixtures will be discarded appropriately.

10.2 Reason and Route of Administration

The oral route is a route of administration specified by regulatory test agencies.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## APPENDIX 9.1.1 STUDY PROTOCOL

## 10. Dose Administration - (Cont'd.)

## 10.3 Phase 1

Initially, a single dose level of 3 mg/kg will be administered to 5 males and 5 females by oral gavage. Based on the initial dose level results, two to four additional dose levels may be added in order to determine the no observable effect level for behavioral and clinical signs (including body weight effects) for each sex.

## Phase 1. Animal Identification

Dose Level (mg/kg)	Males		Females		Color Code
	Group No.	ID No.	Group No.	ID No.	
3	10	101 - 105	15	1101 - 1105	White
b	20	201 - 205	25	1201 - 1205	Blue
b	30	301 - 305	35	1301 - 1305	Green
b	40	401 - 405	45	1401 - 1405	Yellow
b	50	501 - 505	55	1501 - 1505	Red

Groups will be dosed at a volume of 5.0 ml/kg.  
b - Dose levels to be provided by amendment.

## 10.4 Phase 2

The dose level for Phase 2 will be determined following evaluation of the Phase 1 results with the Product Manager. The peak inhibition time(s) for red blood cell, serum and brain cholinesterase activity will be determined for the dose level selected.

## Phase 2. Animal Identification

Sacrifice Interval (Hours Post-Dose)	Dose Level - 0 mg/kg		Dose Level to be determined	
	Group No.	ID No.	Group No.	ID No.
2	65a	1106 - 1110	75a	1206 - 1210
4	65b	1111 - 1115	75b	1211 - 1215
6	65c	1116 - 1120	75c	1216 - 1220
24	65d	1121 - 1125	75d	1221 - 1225

Groups 65 a, b, c and d will receive only distilled water at a volume of 5.0 ml/kg. For each time interval, dosing and necropsy will be alternated by animal between the two dose levels (i.e., 1 control, 1 test).

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## APPENDIX 9.1.1 STUDY PROTOCOL

## 10. Dose Administration - (Cont'd.)

## 10.5 Phase 3

Dose levels and sacrifice time will be determined following evaluation of the Phase 1 and 2 results with the Product Manager. The dose levels and sacrifice time will be chosen to determine the no observable effect level(s) (NOEL) for inhibition of red blood cell (RBC), serum and brain cholinesterase activity. The order of dosing and necropsy of each animal will be across each dose group for each sex (i.e., 1 animal/group).

## Phase 3. Animal Identification

Dose Level (mg/kg)	Males		Females		Sacrifice Interval (Hours Post-Dose)
	Group No.	ID No.	Group No.	ID No.	
0	80	106 - 110	85	1126 - 1130	c
b	90	206 - 210	95	1226 - 1230	c
b	100	306 - 310	105	1326 - 1330	c
b	110	406 - 410	115	1426 - 1430	c
b	120	506 - 510	125	1526 - 1530	c

Groups 80 and 85 will receive only distilled water at a volume of 5.0 ml/kg.

b - Dose levels to be provided by amendment

c - Sacrifice time to be provided by amendment

## 11. In-Life Observations

## 11.1 Clinical Signs and Mortality

11.1.1 In phase 1, general physical examinations (see attached form) will be performed to record clinical signs immediately after dosing, at approximately 1, 2, 4 and 6 hours after test substance administration, and daily (a.m.) thereafter for at least 7 days. Observations may be extended when considered necessary. Throughout the study, all animals will be observed at least twice daily (a.m. and p.m.) for mortality. All rats found dead will be identified, individually placed in plastic bags, and refrigerated until necropsy. Moribund animals or animals exhibiting lesions or clinical signs that are apparently life-threatening will be sacrificed to prevent the potential loss of tissues due to autolysis. Animals that have been severely injured or have become unthrifty due to toxicity may be terminated for humane reasons. All clinical signs and mortality will be recorded.

11.1.2 In phase 2, general physical examinations will be performed to record clinical signs at approximately 1, 2, 4, 6 and 24 hours after test substance administration (when applicable).

11.1.3 In phase 3, to be provided by amendment.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## APPENDIX 9.1.1 STUDY PROTOCOL

## 11. In-Life Observations - (Cont'd.)

## 11.2 Body Weights

- 11.2.1 In phase 1, individual body weights will be recorded before dose administration and at 7 days after test substance administration, and at death when survival exceeds 1 day.
- 11.2.2 In phase 2, body weights will be recorded before dose administration and at termination (2, 4, 6 or 24 hours).
- 11.2.3 In phase 3, body weights will be recorded before dose administration. Any additional body weight measurements will be provided by amendment.

## 12. Clinical Laboratory Tests

## 12.1 Phase 2. Number of Animals to be Sampled at Each Interval

- 12.1.1 As indicated in the table below, blood samples will be drawn from the retro-orbital plexus under isoflurane (AErrane®) anesthesia for evaluation of red blood cell and serum cholinesterase activity.

Dose Level (mg/kg)	Interval (Hours Post-Dose)			
	2	4	6	24
0	5	5	5	5
b	5	5	5	5

b - Dose level to be provided by amendment.

- 12.1.2 The right half of the brain will be analyzed for cholinesterase activity. The other half of the brain will be kept frozen at -70° to -90°C for potential further analyses and may be discarded after completion of the study's final report.

## 12.2 Phase 3

- 12.2.1 Number of Animals: all
- 12.2.2 Method of Collection: Animals will be anesthetized with isoflurane; blood will be collected via the retro-orbital plexus.
- 12.2.3 Tests: Red blood cell cholinesterase  
Serum cholinesterase  
Brain cholinesterase (collected at scheduled sacrifice)

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1.1 STUDY PROTOCOL

13. Postmortem Examination

13.1 Euthanasia

At termination of Phase 1 (Day 7), Phase 2 (approximately 2, 4, 6, or 24 hours post-dose) and Phase 3 (hours post-dose to be provided by amendment), the non-fasted surviving animals will be anesthetized by sodium pentobarbital injected i.p. and exsanguinated via the abdominal aorta.

13.2 Necropsy

All animals that die or are euthanatized during the study will receive an abbreviated gross necropsy examination and discarded. Abnormalities will be recorded and all tissues with lesions will be collected and preserved in 10% neutral buffered formalin for possible future histological evaluation at the discretion of the Study Director and Study Pathologist.

For Phases 2 and 3, a midline sagittal cut of the brain will be made and each half will be weighed, placed on ice until transferred to a freezer set to maintain a temperature of -70°C to -90°C until analyzed for brain cholinesterase activity.

14. Statistical Analysis

Cholinesterase values will be analyzed statistically by a one-way analysis of variance and appropriate post-hoc tests (e.g., Dunnett's t-Test). Type I error (alpha) will be set at 0.05 (two-tailed). Statistical test results which reach the 0.01 level of significance will also be noted.

15. Reports

A comprehensive report will be prepared following completion of the study to include items listed below.

- Description of the test substance
- Description of the test system
- Dates of experimental initiation and termination
- Tabulation of mortality data
- Description of any toxic effects
- Tabulation of mean and individual body weights and gains by sex and dose level
- Statistical analysis
- Clinical pathology results
- Gross pathology findings
- Histopathology findings (if applicable)

16. Data Retention

The original final report and all raw data, records, protocol and specimens will be archived at the EHC for an indefinite period of time.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1.1 STUDY PROTOCOL

17. Protocol Amendments

All changes or revisions of this protocol and the reasons for the changes will be documented, signed and dated by the Study Director and maintained with the protocol.

18. Quality Assurance

The EHC Quality Assurance Unit will monitor the study in accordance with EPA FIFRA GLP standards (40 CFR, Part 160) and audit the final report.

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## APPENDIX 9.1.1 STUDY PROTOCOL

## PROPOSED STUDY SCHEDULE

<u>Event</u>	<u>Date</u>
Arrival of Animals	
Phase 1	March 23, 1994
Phase 2	April 5, 1994
Phase 3	April 26, 1994
Pretest Physical	
Phase 1	By March 28, 1994
Phase 2	By April 15, 1994
Phase 3	By May 6, 1994
Assignment to Study	
Phase 1	By March 29, 1994
Phase 2	By April 18, 1994
Phase 3	By May 9, 1994
Initiate Dosing	
Phase 1	March 30, April 4, April 6, and April 8, 1994
Phase 2	April 19, 1994
Phase 3	May 10, 1994 (males) and May 12, 1994 (females)
Body Weight	
Phase 1	Initial, Day 7
Phase 2	Initial and at Term (2, 4, 6 & 24 hours post-dose)
Phase 3	Initial and by amendment
General Physical Examinations	
Phase 1	Initial, 1, 2, 4, 6 hours and daily thereafter
Phase 2	1, 2, 4, 6 and 24 hours post-dose
Phase 3	By amendment
Clinical Laboratory Tests	
Phase 2	April 19-20, 1994
Phase 3	May 10 and May 12, 1994
Necropsy	
Phase 1	April 6, April 11, April 13, and April 15, 1994
Phase 2	April 19-20, 1994
Phase 3	May 10, 1994 (males) and May 12, 1994 (females)

CIBA-GEIGY CORPORATION -66-

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1.1 STUDY PROTOCOL  
CLINICAL SIGNS FOR GENERAL PHYSICAL EXAMINATION

STUDY NUMBER: \_\_\_\_\_ DOSE GROUP: \_\_\_\_\_  
 TEST TIME AND DATE: \_\_\_\_\_ STUDY ANIMAL NUMBER: \_\_\_\_\_  
 STUDY INTERVAL: \_\_\_\_\_ TEST PERFORMED BY: \_\_\_\_\_  
 REVIEWED BY: \_\_\_\_\_ (Signature/Date)

PHYSICAL EXAM	OBSERVATION CHOICES		
POSTURE	A - Resting R - Rearing	F - Flattened S - Sitting or standing	H - Hunched V - Vertical jumping
TREMORS	1 - None	2 - Slight	3 - Markedly coarse
CONVULSIONS	1 - None	P - Present	
STEREOTYPY	N - None	P - Present	
BIZARRE BEHAVIOR	N - None	P - Present	
FECAL COLOR	N - Normal	A - Abnormal	U - None present
	1 - Normal pellets	2 - Partially formed U - None present	3 - Unformed, diarrhea
GAIT	N - Normal	M - Not mobile	B - Abnormal
EASE OF REMOVAL FROM CAGE	1 - Very Easy	2 - Easy 4 - Difficult	3 - Moderately difficult
RESPIRATORY CHARACTER	1 - Normal	2 - Slightly impaired 4 - Severely impaired	3 - Moderately impaired
HINDLIMB POSITION	N - Normal	A - Abnormal	
PUPILLARY SIZE	N - Normal	D - Mydriasis	O - Miosis
LACRIMATION	1 - Absent	2 - Slight	3 - Severe
EYE PROMINENCE	N - Normal	O - Enophthalmus	X - Exophthalmus
PALPEBRAL CLOSE	1 - Wide open	2 - Ptosis	3 - Completely shut
STAINING EARS	A - Absent	P - Present	
STAINING EYES	A - Absent	P - Present	
STAINING MOUTH	A - Absent	P - Present	
STAINING NOSE	A - Absent	P - Present	
PILOERECTION	A - Absent	P - Present	
FUR APPEARANCE	1 - Normal	2 - Slightly soiled 4 - Very soiled, crusty	2 - Moderately soiled
SALIVATION	1 - None	2 - Slight	3 - Profuse
VOCALIZATION	N - Normal/None	U - Unusual	
EASE OF HANDLING	1 - Very easy 4 - Difficult	2 - Easy	3 - Moderately difficult
OTHER			

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1.2 LIST OF PROTOCOL AMENDMENTS

Four protocol amendments were issued (3-31-94, 4-7-94, 5-2-94 and 5-5-94). There was no impact on the conduct or outcome of the study.

Modification:

1. Test Substance Data Description (7.3): amber crystalline solid.  
  
Experimental Design (10.3): For Phase 1, group numbers 20 and 25 will receive a dose level of 5 mg/kg. Group numbers 30 and 35 will receive a dose level of 0.3 mg/kg.  
  
Necropsy (13.2): Tissues with lesions will be collected and preserved in 10% neutral buffered formalin (NBF) or 2.5% buffered glutaraldehyde (BG).
2. Dose Administration (10.3): For Phase 1, group numbers 40, 45, 50 and 55 will not be dosed.  
  
Dose Administration (10.4): For Phase 2, group number 75 will receive a dose level of 3 mg/kg.
3. Dose Administration (10.5): For Phase 3, group numbers 90 and 95 will receive 0.01 mg/kg, group numbers 100 and 105 will receive 0.03 mg/kg, group numbers 110 and 115 will receive 0.1 mg/kg, group numbers 120 and 125 will receive 0.3 mg/kg and group numbers 130 and 135 will receive 1 mg/kg.  
  
Dose Administration (10.5): The sacrifice interval will be two hours post-dose.  
  
In-Life Observations (11.1.3): In Phase 3, general physical examinations will be performed to record clinical signs just before dosing and at approximately 1 and 2 hours post-dose.  
  
In-Life Observations (11.2.3): In Phase 3, body weights will only be recorded before dose administration. No additional body weight measurements will be taken at termination.  
  
Postmortem Examination (13.1): At termination of Phase 3 (2-hours post-dose), the non-fasted surviving animals will be anesthetized by sodium pentobarbital injected i.p. and exsanguinated via the abdominal aorta.  
  
Postmortem Examination (13.2): At termination of Phase 3, animals will not receive an abbreviated gross necropsy examination.
4. Postmortem Examination (13.1): At termination of Phase 3 (2-hours post-dose), the fasted surviving animals will be anesthetized by sodium pentobarbital injected i.p. and exsanguinated via the abdominal aorta.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.1.2 LIST OF PROTOCOL AMENDMENTS

Justification:

1. Description of material as received.

Although an effect level has been established at a dose of 3 mg/kg, there is the need to establish a NOEL for clinical signs at a lower dose and a higher non-lethal dose may provide additional clinical signs and better characterize the toxicity.

EHC SOP's require certain tissues to be initially preserved in BG.

2. The NOEL for clinical signs has been established at 0.3 mg/kg.

Protocol required dose level determination following evaluation of Phase 1 results.

3. Protocol required the determination of dose level following evaluation of Phase 1 and 2 results. An additional dose group was requested by the Product Manager to adequately assess the dose-response relationship and ensure the identification of the NOEL.

Protocol required the sacrifice interval following evaluation of Phase 1 and 2 results.

Protocol required amendment to state if and when clinical signs would be recorded.

Product Manager did not request additional body weight measurement.

Protocol required sacrifice interval to be provided by amendment.

No significant gross lesions were observed at any time interval (up to 24 hours) in phase 2.

4. Since the termination of Phase 3 is 2-hours post-dose, the animals will still be fasted at the time of anesthetization.

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.2 CHOLINESTERASE ACTIVITY - INDIVIDUAL ANIMAL

Appendix 9.2.1 Individual Animal Cholinesterase Activity - Phase 2

Appendix 9.2.2 Individual Animal Cholinesterase Activity - Phase 3

Appendix 9.2.3 Analytical Methods for Cholinesterase Activity

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## APPENDIX 9.2.1 INDIVIDUAL ANIMAL CHOLINESTERASE ACTIVITY - PHASE 2

INTERVAL PERIOD: 2 HOURS				INTERVAL PERIOD: 6 HOURS					
ANIMAL NUMBER	S-CHE	R-CH	BR-CH	ANIMAL NUMBER	S-CHE	R-CH	BR-CH	SERUM COMMENT	
=====	=====	=====	=====	=====	=====	=====	=====	=====	
0 mg/kg				0 mg/kg					
1106	788	1260	10.96	1116	924	1740	7.68		
1107	790	1700	8.72	1117	991	1540	10.12	SH	
1108	1112	1340	9.48	1118	530	1440	9.36		
1109	966	1880	6.08	1119	776	1780	8.92		
1110	940	1700	9.20	1120	744	1380	8.84		
MEAN	919	1576	8.89	MEAN	793	1576	8.98		
S.D.	136	264	1.78	S.D.	179	178	0.89		
3 mg/kg				3 mg/kg					
1206	154	400	1.24	1216	363	660	3.60		
1207	224	400	0.80	1217	221	820	4.40	SH	
1208	177	400	1.16	1218	304	580	3.24		
1209	238	460	1.36	1219	296	640	4.08		
1210	119	580	1.04	1220	264	680	3.56		
MEAN	182	448	1.12	MEAN	290	676	3.78		
S.D.	49	78	0.21	S.D.	52	89	0.46		
INTERVAL PERIOD: 4 HOURS				INTERVAL PERIOD: 24 HOURS					
ANIMAL NUMBER	S-CHE	R-CH	BR-CH	SERUM COMMENT	ANIMAL NUMBER	S-CHE	R-CH	BR-CH	SERUM COMMENT
=====	=====	=====	=====	=====	=====	=====	=====	=====	=====
0 mg/kg					0 mg/kg				
1111	993	1440	10.24		1121	666	1300	7.92	
1112	662	1560	9.16		1122	1028	1180	8.16	SH
1113	816	1300	10.72		1123	796	1700	7.92	
1114	780	980	9.36		1124	940	1140	8.56	
1115	851	1400	9.08	SH	1125	743	1460	8.88	
MEAN	820	1336	9.71		MEAN	835	1356	8.29	
S.D.	120	220	0.73		S.D.	147	229	0.42	
3 mg/kg					3 mg/kg				
1211	213	200	1.80		1221	557	900	6.88	
1212	286	280	2.52		1222	591	840	6.20	
1213	232	360	1.96		1223	514	880	6.36	
1214	266	520	1.72		1224	570	940	4.00	
1215	291	580	2.08		1225	586	920	6.76	
MEAN	258	388	2.02		MEAN	564	896	6.04	
S.D.	34	160	0.31		S.D.	31	38	1.17	

SH = Slight Hemolysis

CIBA-GEIGY CORPORATION -71-

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.2.2 INDIVIDUAL ANIMAL CHOLINESTERASE ACTIVITY - PHASE 3

SEX: MALE					SEX: FEMALE				
ANIMAL NUMBER	S-CHE	R-CH	BR-CH	SERUM COMMENT	ANIMAL NUMBER	S-CHE	R-CH	BR-CH	SERUM COMMENT
=====	=====	=====	=====	=====	=====	=====	=====	=====	=====
<b>0 mg/kg</b>					<b>0 mg/kg</b>				
106	297	1820	9.16		1126	911	2300	9.32	
107	539	1700	8.40	SH	1127	728	1940	8.92	SH
108	386	2040	9.76		1128	731	1880	8.68	
109	308	1840	10.24	SH	1129	861	2280	9.48	
110	357	2020	9.08		1130	724	2300	9.48	
MEAN	377	1884	9.33		MEAN	791	2140	9.18	
S.D.	97	144	0.70		S.D.	89	211	0.36	
<b>0.01 mg/kg</b>					<b>0.01 mg/kg</b>				
206	301	1980	8.56		1226	806	2620	9.20	
207	336	2200	9.40		1227	808	1580	8.08	
208	297	1860	9.40		1228	2197 <sup>a</sup>	2100	9.08	
209	366	2040	9.32		1229	564	1980	9.24	
210	398	2740	9.36		1230	851	2220	9.04	
MEAN	340	2164	9.21		MEAN	757	2100	8.93	
S.D.	43	344	0.36		S.D.	130	377	0.48	
<b>0.03 mg/kg</b>					<b>0.03 mg/kg</b>				
306	291	1800	9.44	SH	1326	1031	2260	8.36	
307	236	2100	9.04		1327	926	2120	8.72	
308	269	1720	9.56		1328	992	1600	8.24	
309	385	1820	9.84		1329	848	1960	7.96	
310	299	1460	8.92		1330	574	2000	9.28	
MEAN	296	1780	9.36		MEAN	874	1988	8.51	
S.D.	55	229	0.38		S.D.	182	246	0.51	
<b>0.1 mg/kg</b>					<b>0.1 mg/kg</b>				
406	333	1460	5.84	SH	1426	841	2020	6.92	
407	287	1960	8.60		1427	960	1720	7.64	
408	257	1660	7.96		1428	646	2140	8.88	
409	190	1440	8.56	SH	1429	887	2080	7.84	
410	183	1720	7.92		1430	993	1940	7.60	SH
MEAN	250	1648	7.78		MEAN	865	1980	7.78	
S.D.	64	213	1.13		S.D.	136	163	0.71	
<b>0.3 mg/kg</b>					<b>0.3 mg/kg</b>				
506	217	1100	6.00		1526	751	560	7.20	
507	175	1020	5.52		1527	415	920	6.24	
508	148	1180	5.32		1528	500	820	6.64	
509	123	680	5.04		1529	552	740	7.40	
510	171	1340	4.24		1530	608	700	6.36	
MEAN	167	1064	5.22		MEAN	565	748	6.77	
S.D.	35	245	0.65		S.D.	126	135	0.51	
<b>1 mg/kg</b>					<b>1 mg/kg</b>				
606	63	800	2.52		1626	416	360	2.60	
607	72	560	2.68	SH	1627	302	860	3.60	
608	61	660	2.08		1628	347	520	2.44	MH
609	45	620	2.40	SH	1629	405	560	2.92	
610	73	600	3.24		1630	216	420	3.12	
MEAN	63	648	2.58		MEAN	337	544	2.94	
S.D.	11	92	0.43		S.D.	82	194	0.46	

a: Value excluded, being 2-3 times greater than any control value, even after repeat analysis.  
 SH = Slight Hemolysis; MH = Moderate Hemolysis

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.2.3 ANALYTICAL METHODS FOR CHOLINESTERASE ACTIVITY

Parameter(s):	<b>CHOLINESTERASE - ERYTHROCYTE (RBC CHOLINESTERASE)</b>
Instrument:	Baker Centrifichem® 500 Analyzer
Test Condition:	5 $\mu$ l of Erythrocyte hemolysate (1:20 dilution from packed cells) 50 $\mu$ l sample diluent, 250 $\mu$ l reagent, 37°C.
Unit:	International units/L packed red blood cells (U/L pRBC)
Methodology:	Blood sample was collected in EDTA-anticoagulated tubes (1 mg EDTA per 1 ml of blood). A red blood cell (RBC) hemolysate was prepared by adding 1 part of packed RBC to 19 parts of 1% triton X-100 in saline. The free sulfhydryl groups in the hemolysate were allowed to react with DTNB at room temperature for 15 min (final concentration of DTNB: $3 \times 10^{-4}$ M). Subsequently, the substrate, acetylthiocholine (final concentration: $5 \times 10^{-4}$ M in 0.1 M phosphate buffer, pH 8.0) was added and the reaction was allowed to proceed at 37°C for 30 seconds. The formation of the colored endproduct, 5-mercapto-2-nitrobenzoic acid, was measured at 405 nm. This method measures both "true" and "pseudo" cholinesterase.
References:	<ol style="list-style-type: none"><li>1. Ellman, G. L., et al., <u>Biochemical Pharmacology</u>, 7:88, 1961.</li><li>2. Runion, T. H., <u>Clinical Chemistry</u>, 30 (6):1060, 1984.</li><li>3. Wiher, R., <u>Archives of Environmental Health</u>, 6:116, April, 1963.</li><li>4. Siders, D., et al., <u>American Journal of Clinical Pathology</u>, 50(3):346, 1968.</li><li>5. Tietz, N.W., <u>Textbooks of Clinical Chemistry</u>, W.B. Saunders, Philadelphia, 1986.</li></ol>

## F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

## APPENDIX 9.2.3 ANALYTICAL METHODS FOR CHOLINESTERASE ACTIVITY

Parameter(s):	<b>CHOLINESTERASE - SERUM</b>
Instrument:	IL Monarch™ Chemistry Analyzer
Test Condition:	2 $\mu$ l serum, 18 $\mu$ l sample diluent, 220 $\mu$ l reagent, 30 °C.
Units:	International Units/Liter (U/L)
Methodology:	<p>This method measures photometrically the activities of both the "true" cholinesterase (or acetylcholinesterase, found primarily in the erythrocytes, nerve endings in the lungs, spleen and gray matter of the brain) and "pseudocholinesterase" (found in the liver, pancreas, heart, serum or plasma, and in the white matter of the brain). Acetylthiocholine, used as the substrate (<math>7 \times 10^{-4}</math> M in 0.1 M phosphate buffer, pH 8.0), is cleaved by cholinesterase into thiocholine and acetate. Thiocholine then reacts with dithiobisnitrobenzoic acid (Ellman's Reagent) to form a yellow colored thionitrobenzoic acid. Since cholinesterase activity is directly proportional to color development, the enzyme activity can be measured kinetically between 400-420 nm or at 405 nm.</p> <p style="text-align: center;">1) Acetylthiocholine <u>cholinesterase</u> &gt; thiocholine + acetate</p> <p style="text-align: center;">2) Thiocholine + dithiobisnitrobenzoic acid <math>\xrightarrow{\hspace{1cm}}</math> 5-mercapto-2-nitrobenzoic acid</p>
References:	<ol style="list-style-type: none"> <li>1. Ellman, G.L. et al., <i>Biochemical Pharmacology</i>, 7:88, 1961.</li> <li>2. Monarch Cholinesterase User Application Instrumentation Laboratory. 1987, Revision 1.</li> <li>3. Wiher, R., <u><i>Archives of Environmental Health</i></u>, 6:116, 1963.</li> <li>4. Siders, D., et al., <u><i>American Journal of Clinical Pathology</i></u>, 50(3):346, 1968.</li> </ol>

F-00189: ACUTE ORAL TOXICITY STUDY OF MONOCROTOPHOS TECHNICAL IN RATS

APPENDIX 9.2.3 ANALYTICAL METHODS FOR CHOLINESTERASE ACTIVITY

Parameter(s):	<b>CHOLINESTERASE - HALF BRAIN SAMPLES</b>
Instrument:	IL Monarch™ Chemistry Analyzer
Unit:	International units/ Gram tissue (IU/g)
Test Condition:	4 $\mu$ l tissue homogenate, 16 $\mu$ l sample diluent, 220 $\mu$ l reagent, 30° C.
Methodology:	<p>This method measures photometrically the activities of both the "true" and "pseudocholinesterase". Acetylcholine final concentration, used as the substrate, is cleaved by cholinesterase into thiocholine and acetate. Thiocholine then reacts with dithiobisnitrobenzoic acid (Ellman's Reagent) to form a yellow colored 5-mercapto-2-nitrobenzoic acid. Since cholinesterase activity is directly proportional to color development, the enzyme activity can be measured kinetically between 400-420 nm or at 405 nm.</p> <p>Fresh or previously frozen samples obtained from half the brain were diluted with ice-cold phosphate buffer (0.1 M, pH 8.0) to make a 13.35% w/v homogenate, homogenized for approximately 10 seconds (Brinkman Polytron PTA 7T generator, setting at 9) and extracted on ice for at least 30 minutes (extraction buffer consisted of 5 M NaCl, 10% Triton X-100 and purified water, 1:2:2 V/V). The extracts were then used in the assay and yielded a final extract concentration of 2.5 percent.</p>
References:	<ol style="list-style-type: none"><li>1. Ellman, G.L. et al., Biochemical Pharmacology, 7:88, 1961.</li><li>2. Boehringer Mannheim Diagnostics package insert: Cholinesterase, November 1992, No. 052222303-0692.</li><li>3. Monarch Cholinesterase User Application Instrumentation Laboratory, 1987, Revision 1.</li></ol>