



Paraquat Dichloride
Paraquat SL (A3879R)
- Acute Toxicity in Beagle Dogs with Toxicokinetic Analysis
Final Report

DATA REQUIREMENT(S):	Not Applicable
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SPONSOR:	Syngenta Crop Protection, Inc. 410 Swing Road Post Office Box 18300 Greensboro, NC 27419-8300 USA

Report Number: 1390-002

STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This nonclinical laboratory study was conducted in accordance with the United States Environmental Protection Agency (EPA), Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Good Laboratory Practice (GLP) Standards, 40 Code of Federal Regulations (CFR) Part 160. The analytical and pathology phase of the study were conducted according to the current version of the United Kingdom Principles of Good Laboratory Practice (The UK GLP Regulations) and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice (ENV/MC/CHEM(98) 17) as interpreted by Syngenta Central Toxicology Laboratory (CTL) and CEMAS. Protocol deviations are presented in Appendix 16. This report accurately reflects the raw data obtained during the performance of this study.

Ronald G. Lindahl, B.S., L.A.T.
Director, Pharmacokinetics/Toxicokinetics
Study Director

Date

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FLAGGING STATEMENT

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QUALITY ASSURANCE STATEMENT

Below are the inspections conducted by the Quality Assurance Department and the dates the inspections were reported to the Study Director and Management.

Date(s) of Inspection	Study Phase Inspected	Date(s) Reported to Study Director/Management
1/30/07	Protocol	1/30/07, 2/13/07
2/9/07	Test Material Preparation	3/13/07
2/9/07	Vehicle Dispensation	3/13/07
2/9/07	Test Material Administration	3/13/07
3/9/07	Clinical Pathology Operations	3/9/07, 4/6/07
3/15/07 to 3/16/07	Tissue Trimming	3/16/07, 4/6/07
3/15/07	Protocol Amendment 1	4/6/07
4/3/07 to 4/6/07	Data Review	4/6/07
4/3/07 to 4/6/07	Report Review	4/6/07
4/12/07 to 4/17/07	Report Review	4/17/07
4/23/07	Report Review	4/23/07
8/25/08	Report Review	8/25/08

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Study dates

Protocol Approved by Sponsor	January 8, 2007
Study Initiation Date (Protocol Signed by Study Director)	January 8, 2007
Experimental Starting Date	January 9, 2007
Experimental Start Date	February 9, 2007
Experimental Termination Date	March 9, 2007
Experimental Completion Date (Final Data Collection)	TBD
Draft Report Mail Date	May 1, 2007

Deviations from the guidelines

Not applicable

Retention of samples

All raw data, documentation, records, protocol, reserve samples and the final report generated as a result of this study will be retained at MPI Research, Inc., or an approved archive facility contracted by MPI Research, Inc., for a period of 1 year following completion of the study (final report issue date). All data generated at the laboratory of the Sponsor or CEMAS Berks, England, United Kingdom will be retained at those laboratories according to their company policies. Retention of materials after the time stated above will be subject to future contractual agreements.

A reserve sample from the batch of test article used in this study was collected and archived. Any remaining test article will be shipped to a Sponsor-designated location after completion of the study.

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SIGNATURE

This report is being submitted by the following personnel.

Ronald G. Lindahl, B.S., L.A.T.
Director, Pharmacokinetics/Toxicokinetics
Study Director

Date

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1.0 EXECUTIVE SUMMARY

1.1 Study design

The primary objective of the study, conducted for Syngenta Crop Protection, Inc., was to determine the minimum lethal dose of a 200 g paraquat ion/L SL formulation (A3879R) to the beagle dog and to describe the underlying paraquat plasma kinetics. This formulation contained a nominal concentration of 200 g/L of paraquat ion and also an emetic component (PP796) present at the nominal concentration of 0.5 g/L. Three treatment groups of four male beagle dogs were administered the test article at dose levels of 43.20, 86.40 and 129.60 mg formulation/kg body weight (equivalent to 8, 16 and 24 mg paraquat ion/kg. The formulation was administered undiluted to all groups once via oral gelatin capsule, at respective dose volumes of 0.04, 0.08 and 0.12 mL formulation/kg. Each dose level was selected based on the outcome of the previous dose.

Observations for mortality, morbidity, injury and the consumption of food and water were conducted at least twice daily for all animals. Clinical observations were conducted, body weights and food consumption were measured and recorded daily beginning in Week(-1) (relative to randomization for all animals) and on Days 1-14. Day 1 clinical observations were conducted continuously for the first 4 hours post-dose and at 5, 6, 7 and 12 hours post-dose and daily thereafter. In addition the time to emesis and the approximate volume (small, medium or large), color, consistency, presence of any capsule fragments or gel mass, of emesis or any fecal matter was recorded. Body weights and food consumption were measured and recorded daily. Complete physical examination were conducted pre-test and prior to the terminal necropsy. Blood samples for clinical pathology evaluations were collected from all animals pre-test, 24 hours post-dose and prior to necropsy. Blood samples for determination of the plasma concentrations of paraquat and emetic were collected from all animals at designated time points on Day 1. At study termination, each animal was euthanized and a necropsy examination was conducted. Photographs were taken of any lesions and microscopic examination of the lung, kidney and gastrointestinal tract was conducted. The carcasses were discarded without further evaluation.

Comment [m1]: This notation can be confusing as to what is meant. Does it mean week 1 or 1 week prior. I suggest if that is the case, should add ().

MPI please put brackets around all days/weeks prior to dosing

1.2 Results

Treatment with a single oral dose of 43.20 and 86.40 mg formulation/kg (8 and 16 mg paraquat ion/kg body weight) was tolerated with all animals surviving until scheduled study termination on Day 15. No definitive paraquat-related effects were seen in clinical chemistry or macroscopic pathology parameters. Emesis, body weight loss and reduced food consumption were noted in these groups in the period immediately after administration. However, these effects were transitory in nature and the animals were returning to a normal condition by study termination.

Following treatment with a single oral dose of 129.60 mg formulation/kg (24 mg paraquat ion/kg body weight), emesis was more frequent and the weight loss and reduction in food consumption were still evident at study termination. In addition, one animal was euthanized *in extremis* on Day 3 with observations of inappetence, decreased activity, salivation, loss of

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skin elasticity and skin cold to touch. The outcome in this animal was consistent with high systemic exposure to paraquat, as evidenced by the plasma paraquat 24 hour AUC (Area Under Curve). The terminal blood sample and histopathology results obtained from this dog were consistent with renal failure (demonstrated by clinical pathology and histopathology) and hepatic injury (demonstrated by clinical pathology only). This is also consistent with a high dose of paraquat and was probably the principal cause of the clinical condition of this animal.

Following oral administration of A3879R peak plasma paraquat levels were observed between 30 minutes and 1 hour post-dose. The timing of the peak plasma emetic levels was similar, between 30 minutes and 1 hour post-dose. With increasing doses of the formulation there was a dose-dependent increase in the systemic exposure to paraquat. Mean peak plasma emetic levels were observed at 1 hour post-dose for the lowest dose and at 30 minutes post-dose for the higher doses. The dose of 129.60 mg formulation/kg (equivalent to 24 mg paraquat ion/kg) resulted in the highest systemic exposure of paraquat and emetic.

The lung lesions observed in this study were typical for paraquat exposure.

1.3 Conclusion

A single oral capsule dose of 200 g paraquat ion/L SL formulation (A3879R) was tolerated at the 43.20 and 86.40 mg formulation/kg dose levels (8 and 16 mg paraquat ion/kg). Those effects observed (e.g. emesis) were transitory, and not deleterious in nature. At the highest dose level tested, 129.60 mg formulation/kg (24 mg paraquat ion/kg), one animal was euthanized *in extremis* with post-mortem findings of moderate tubular degeneration/necrosis and slight congestion and hemorrhage in the lungs. In the surviving animals at this dose, 1, reduction in body weight and food consumption were not resolved by study termination. These data indicate that 129.60 mg A3879R/kg (24 mg paraquat ion/kg) is a minimum lethal dose.

2.0 INTRODUCTION

This study was conducted in accordance with Standard Operating Procedures (SOPs) and the protocol as approved by the Sponsor. This study complied with United States Environmental Protection Agency (EPA) Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Good Laboratory Practice (GLP) Standards, and 40 Code of Federal Regulations (CFR) Part 160. The protocol and amendments are presented in Appendix 15. Procedures pertinent to this study are described in this report.

2.1 Objective

The primary objective of this study, conducted for Syngenta Crop Protection, Inc., was to determine the minimum lethal dose of a 200 g paraquat ion/L SL formulation (A3879R) to the beagle dog and to describe the underlying paraquat plasma kinetics. In addition, In addition this study also described the plasma kinetics of an emetic (PP796) component present in this formulation at a nominal concentration of 0.5 g/L. the emetic plasma kinetics and pathological findings were also determined.

2.2 Species selection

The current state of scientific knowledge does not provide acceptable alternatives, *in vitro* or otherwise, to the use of live animals to accomplish the purpose of this study. “The development of knowledge necessary for the improvement of the health and well-being of humans as well as other animals requires *in vivo* experimentation with a wide variety of animal species”.¹ “Whole animals are essential in research and testing because they best reflect the dynamic interactions between the various cells, tissues, and organs comprising the human body”.²

The beagle was selected for use in this study by the Sponsor because the dog possesses a vomit reflex and gastrointestinal tract function similar to that in man, making this an appropriate model for determining the toxicity of a formulation that contains emetic.

2.3 Justification for number of animals on study

This study was designed to use the fewest number of animals possible, consistent with the objective of the study, the scientific needs of the Sponsor, contemporary scientific standards and in consideration of applicable regulatory requirements cited in the protocol. As no major differences were expected between the sexes, males only were used to reduce animal usage.

¹ Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, Federal Register, 1985 May 20;50(97).

² Position Statement on the Use of Animals in Research, NIH Guide. 1993 Feb 26; 22(8).

3.0 MATERIALS AND METHODS

3.1 Vehicle and test article information

Pertinent test article receipt information and the Certificate of Analysis are presented in Appendix 1. The test article was dosed as supplied hence no vehicle was used on study.

The Sponsor provided documentation on the strength, purity, composition, stability and other pertinent information for the batch of test article used on study.

3.1.1 Test article preparation

The test article, 200 g paraquat ion/L SL formulation (A3879R), was used as received from the Sponsor and no adjustment was made for purity. Formulation A3879R consists of nominal concentrations of 200 g/L paraquat ion and 0.5 g/L of the emetic component PP796. The test article was administered neat (undiluted). To prepare the dosing formulation for capsule administration, the container of test article was gently inverted six times. 10 mL of the test article was measured using a syringe and transferred into an appropriately sized falcon tube and wrapped in aluminium foil. The test article was stored at ambient temperature, protected from light.

Immediately prior to dosing each animal, the falcon tube was gently inverted six times, and the required volume of the test article was placed into a size 000 gelatin capsule using a positive displacement pipette. The size 000 capsule was placed into a size 12 gelatin capsule. The number of capsules increased to accommodate the volume of test article. Capsules of the test article were prepared once and administered within 2 minutes of encapsulation for each animal. No glass was used during test article preparation due to the propensity of paraquat to adhere to glass.

3.1.2 Analysis of dosing formulations

The test article was a liquid formulation dosed as supplied. The Sponsor provided documentation (presented in Appendix 1) that the test article is a solution, homogeneous and stable for the duration of the study; therefore, no test article analyses were performed at MPI Research, Inc. On each dosing day, following inversion and prior to dispensing into the capsules, a 10 mL reserve sample of the dosing formulation was collected. Samples were collected from the middle of the container using a syringe and placed into polypropylene tubes wrapped in foil. Samples were maintained at ambient temperature at MPI Research, Inc.

3.1.3 Reserve sample and test article disposition

A reserve sample from the batch of test article used in this study was collected and archived. Any remaining test article will be shipped to a Sponsor-designated location after completion of the study.

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3.2 Experimental design

3.2.1 Animal acquisition and acclimation

Twenty-two male experimentally naïve beagle dogs, approximately 8.5 to 9.5 months of age at receipt, were received from Covance Research Products, Inc., Cumberland, Virginia/USA, on January 9, 2007. During the 31 to 45 day acclimation period, the animals were weighed approximately weekly and were observed twice daily with respect to general health and any signs of disease. All animals were given a detailed physical examination prior to selection for study. Ova and parasite evaluations on stool samples were performed, all results were negative. Results of these evaluations are not reported but are maintained in the study file. All animals had blood samples collected pretest to evaluate clinical chemistry parameters. Prior to study start, the dogs were acclimated to normal restraint techniques for bleeding and clinical observations, and to the diet and feeding regimen used on study.

3.2.2 Randomization, assignment to study and maintenance

The animals considered suitable for study were weighed. Animals assigned to study had normal clinical pathology profiles with respect to major organ systems (liver and kidney), as judged by a veterinarian. Using a simple randomization procedure, 20 male animals (weighing 8.06 to 12.32 kg at randomization) were assigned to study. As needed, animals were placed into the treatment groups identified in the following table.

Group Assignments				
Group Number	Dose Level (mg/kg)		Number of Male Animals	Animal Numbers
	Paraquat ion	Formulation		
1	8	43.20	4	101-104
2	16	86.40	4	105-108
3	24	129.60	4	109-112

Extra animals obtained for the study, but not placed on study, were transferred to the stock colony. Animals assigned to study, but not placed into treatment groups, were also transferred to the stock colony.

Each animal was assigned an animal number to be used in the Provantis™ data collection system and was implanted with a microchip bearing a unique identification number. Each dog was also identified by a permanent tattoo of a vendor animal number on an earflap. The individual animal number, implant number and study number comprised a unique identification for each animal. Each cage was identified by the animal number, study number, group number and sex. Animal identification was verified during the course of the study, as documented in the data.

Upon arrival, the dogs were housed two per run. This type of housing provided adequate room for exercise for these animals during the acclimation period. At randomization, the

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dogs were housed individually for protocol specified functions and for the duration of the study.

Fluorescent lighting was provided for approximately 12 hours per day. The dark cycle was interrupted intermittently due to scheduled blood collections. Temperature and humidity were continuously monitored and recorded. The protocol-designated ranges were 64 to 84°F (17.7 to 29°C) and 30 to 70%, respectively. The actual temperature and humidity findings are not reported but were kept within the specified range and are maintained in the study file.

Lab Diet® (Certified Canine Diet #5007, PMI Nutrition International, Inc.) was offered for approximately 2 hours each morning, prior to randomization. Beginning 1 week prior to randomization, food (approximately 300 g) was available for approximately 3 hours a day. On dosing days, the 3 hour feeding period began at approximately 4 hours post-dose. The lot number from each diet lot used for this study was recorded. Certification analysis of each diet lot was performed by the manufacturer. Tap water was available *ad libitum* via an automatic watering system. The water supply is monitored for specified contaminants at periodic intervals according to SOP. The results of food and water analyses are retained in the Archives. The Study Director is not aware of any potential contaminants likely to be present in the diet or water that would have interfered with the results of the study. Therefore, no analyses other than those stated above were conducted.

3.2.3 Test article administration

3.2.3.1 Justification for route of administration

The oral route is one of the potential routes of human exposure to this test article.

3.2.3.2 Justification of dose levels

The initial dose was selected by the Sponsor on the basis of available data from previous studies. Subsequent dose levels were selected by the Sponsor based on the results obtained from the previous dose level.

3.2.3.3 Administration

The test article was administered orally once during the study via gelatin capsule. The dose levels for the treated groups were based on the most recent body weights and are identified in the following table.

Test Article Administration					
Group	Dose level (mg paraquat ion/kg)	Dose volume (mL formulation/kg)	Achieved dose (mg formulation/kg)	Dose of emetic (mg/kg)	Animal Numbers
1	8	0.04	43.20	0.02	101-104
2	16	0.08	86.40	0.04	105-108
3	24	0.12	129.60	0.06	109-112

3.3 In-life examinations

3.3.1 Cageside observations

All animals were observed for morbidity, mortality, injury and the consumption of the available of food and water at least twice daily throughout the duration of the study. On occasion, veterinary consultations were conducted during the course of the study. All treatments and observations were recorded. The medical treatments and observations are not reported but are maintained in the study file. After consultation with the veterinarian on call and/or Study Director, animals meeting the criteria to be considered moribund due to weight loss and/or clinical findings were euthanized *in extremis* as per SOP.

Comment [m2]: No – please report any fiven fluids (lactated Ringer's solution) here and in results

Please ensure the reports for the studies in which animals received Ringers clearly indicate that they received the fluids

3.3.2 Detailed clinical observations

A detailed clinical examination of each animal was performed daily beginning on Day -7, -14, and -21 for animals at 43.20, 86.40 and 129.60 mg formulation/kg (8, 16, and 24 mg paraquat ion/kg), respectively. Clinical observations were performed through Day 15. On dosing days, clinical observations were conducted continuously for the first 4 hours post-dose, and again at 5, 6, 7, and 12 hours post-dose. On occasion, clinical observations were recorded at unscheduled intervals. Observations of retching, emesis, and defecation were recorded as observed. The appropriate volume (small, medium, or large), color, or consistency (liquid, slightly thick, or very thick) of emesis or any fecal matter was recorded. The presence of any capsule fragments or gel mass was also recorded. Any emesis or feces were immediately cleaned from the run to prevent ingestion by the dog. The observations included, but were not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, and nervous system effects including tremors, convulsions, reactivity to handling and bizarre behavior.

3.3.3 Body weights

Body weights for all animals were measured and recorded the day after receipt and approximately weekly during the acclimation period, prior to randomization, daily during the study and at necropsy. The body weights recorded during the acclimation period and at necropsy are not reported but are maintained in the study file.

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3.3.4 Food consumption

Food consumption was measured and recorded daily during the acclimation period, beginning 1 week prior to randomization. Food consumption was measured and recorded daily during the study and is reported weekly. Food consumption data measured prior to randomization are not reported but are maintained in the study file.

3.3.5 Physical examinations

A complete physical examination was conducted on all animals by a staff veterinarian pre-test and prior to terminal necropsy.

3.3.6 Clinical pathology

Clinical pathology evaluations were conducted on all animals assigned to test group pre-test, 24 hours post-dose and prior to necropsy. The animals had access to drinking water but were fasted overnight prior to sample collection. Blood samples (approximately 2 to 3 mL/sample) were collected from the jugular vein. No anticoagulant was used for the clinical chemistry samples.

3.3.7 Plasma analysis

Blood samples were collected from all animals via the jugular vein for determination of the plasma concentrations of paraquat and emetic.³ Samples (approximately 2.5 mL/sample) were collected from each animal at 15 and 30 minutes and at 1, 2 and 4 hours post-dose. Samples (approximately 5 mL/sample) were collected from each animal pre-dose and at 7, 12 and 24 hours post-dose. The animals were not fasted prior to blood collection, with the exception of the intervals that coincided with fasting for clinical pathology collections.

Samples were placed in plastic tubes containing lithium heparin anticoagulant and were stored on ice until centrifuged at approximately 1500 g for 10 minutes. Following centrifugation, the plasma samples were split into three approximately equal aliquots. The plasma was frozen at approximately -70°C in tightly capped pre-labeled, plastic vials. The label included the study number, relative study day, animal number and the date and time interval of collection.

The samples were stored frozen at approximately -70°C. One set of aliquots was originally maintained at MPI Research and the two remaining sets were shipped on dry ice to the Sponsor at CTL for analysis of plasma concentrations of the test article. The report and Quality Assurance Statement is presented in Appendix 10. As a result of the closure of Syngenta Central Toxicology Laboratory, the remaining aliquot was later shipped on dry ice to CEMAS, North Ascot, Berks, England, UK for analysis of plasma concentrations of emetic. The final reports and Quality Assurance Statements are presented in Appendix 10.

³ U.S. Food and Drug Administration. International Conference on Harmonisation; Guideline on the Assessment of Systemic Exposure in Toxicity Studies. 60 FR 11264-11268. 1995, March 01.

3.4 Postmortem study evaluations

3.4.1 Macroscopic

Necropsy examinations were performed under procedures approved by a veterinary pathologist on animals euthanized *in extremis* or euthanized at the scheduled terminal necropsy. Euthanasia was by an intravenous overdose of sodium pentobarbital solution followed by exsanguination via severing the femoral vessels. The animals were examined carefully for external abnormalities including palpable masses. The skin was reflected from a ventral midline incision and any abnormalities were identified and correlated with ante-mortem findings. The abdominal and thoracic cavities were examined for abnormalities and the organs removed, examined, and, where required, placed in fixative. Formalin was infused into the lung via the trachea. The mouth, including the tongue and buccal cavity, were carefully examined, and the esophagus, stomach, and the length of the small and large intestine were opened and examined for signs of mucosal irritation. The lungs were carefully examined for areas of discoloration or the presence of lesions before and after infusion with formalin. Photographs of lesions were taken at necropsy. These photographs are not included in this report but are maintained in the study file. A glossary of macroscopic terms used in this study is presented in Appendix 6.

Comment [d3]: This is not correct, appendix 6 is food consumption values, MPI please check all cross references

3.4.2 Microscopic

Representative samples of protocol-designated tissues from all animals were collected, placed in the appropriate fixative and processed to the slide stage. The processed slides were shipped to the Sponsor for examination. The microscopic examination report is included in Appendix 14. A glossary of macroscopic and microscopic terms used in this study is included in this report.

A glossary of microscopic terms used in this study is included in this report. A full list of organs and tissues collected, weighed, and examined for this study is presented in Appendix 12.

Comment [d4]: I am not finding this glossary. Please add if missing.

3.5 Statistics

Statistical analysis was not performed by MPI, however, descriptive statistics, including means and standard deviations, were calculated. For continuous endpoints, descriptive statistics consisted of means, standard deviations and group size for each treatment group and time period. For categorical endpoints, descriptive statistics consisted of incident count for each treatment group and time period.

Statistical analysis of the toxicokinetic data (mean with standard error of the mean), was performed by Syngenta Central Toxicology Laboratory and/or CEMAS and are presented in Appendix 10.

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3.6 Computer systems

The computer systems used during the conduct of this study are presented in the following table.

MPI Research Computer Systems	
Provantis™ v6.5:	Client-server, Oracle-based system primarily used for toxicology studies.
Dispense v5.0:	Automates the test material control processes.
Niagara Framework® Software	
System v2.3:	Environmental monitoring, alarming, and reporting application.
MPI Archiving System (MArcS)	In-house developed application for automated storage and retrieval information for archiveable materials (e.g., lab books, study data, wet tissues, slides, etc.).
v1.1:	
MPI Reporting System (MPIRS)	In-house developed reporting system running SAS®
v7.0:	programs primarily reporting on Provantis™ data.
Master Schedule v2.2:	Maintains the master schedule for the company.
SAS® v8.2:	The SAS® System is an integrated system of software products that enables a user to perform data entry, retrieval, data management, reporting, graphics, statistical analysis, and applications development.
Microsoft® Office 2003	Bundle of integrated productivity tools including word and data processing and communications software.
Professional:	Contains the utilities Microsoft® Access, Excel, InfoPath, Outlook, PowerPoint, Publisher, and Word.

Additional information is available in the MPI Research, Inc., company document titled “Computer Systems Information.”

4.0 RESULTS AND DISCUSSION

4.1 In-life examinations

4.1.1 Mortality

A record of animal fate and disposition is presented in Appendix 2.

Animals were dosed chronologically by group number and each subsequent dose level was selected based on the outcome of the previous dose.

All animals survived until scheduled study termination, except for animal number 109. Animal number 109, dosed at 129.60 mg formulation/kg (24 mg paraquat ion/kg body weight), was euthanized *in extremis* on Day 3. Clinical signs of inappetence, decreased activity, salivation, loss of skin elasticity and skin cold to touch were noted prior to euthanasia.

Comment [m5]: Any given fluids (lactated Ringer's solution) ?

Please ensure the reports for the studies in which animals received Ringers clearly indicate that they received the fluids

4.1.2 Clinical findings

Continuous post-dose clinical findings are summarized in Table 1. Individual continuous post-dose clinical findings are presented in Appendix 3. Clinical findings are summarized in Table 2. Individual clinical findings are presented in Appendix 4.

Emesis results are tabulated below. After dosing, emesis was seen in all animals tested except for animal number 101 at 43.20 mg formulation/kg (8 mg paraquat ion/kg). This animal had no signs of emesis during the entire study period. For the remaining animals, the mean time to first emesis decreased from 61 minutes to 28 minutes as the dose level rose from 43.20 to 86.40 mg formulation/kg (8 to 16 mg paraquat ion/kg). The mean time to first emesis at the 129.60 mg formulation/kg dose level (24 mg paraquat ion/kg) was two minutes longer than at the 86.40 mg formulation/kg dose level (16mg paraquat ion/kg), largely the result of one animal which had a much longer interval to first emesis than the other three dogs at this level. Overall, the duration of emesis increased with increasing dose.

In general, the frequency of emesis increased with dose. At 43.20 mg formulation/kg (8 mg paraquat ion/kg), 0-2 instances of emesis were noted per animal. At 86.40 mg formulation/kg (16 mg paraquat ion/kg), the frequency increased to 2-4 instances while at the highest level tested, 129.60 mg formulation/kg (24 mg paraquat ion/kg), the frequency ranged from 1-10 instances.

Individual times to first and last emesis observed are presented in the following table.

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Clinical Observations - Emesis Findings				
Dose Level (mg formulation/kg)	Animal number	Time to First Emesis	Mean	Time to Last Emesis
43.20	101	NA		NA
	102	35 m	1 h 1 m	54 m
	103	1 h 30 m		1 h 30 m
	104	59 m		59 m
86.40	105	30 m	28 m	1 h 11 m
	106	14 m		26 m
	107	11 m		32 m
	108	56 m		57 m
129.60	109	9 m	30 m	2 h 15 m
	110	8 m		52 m
	111	26 m		45 m
	112	1 h 16 m		1 h 16 m
m – minute h – hour NA – Not Available/Not Applicable (no emesis observed)				

Aside from the observations noted above, and those seen in animal number 109 prior to euthanasia, observations were few and typical of dogs of this age.

4.1.3 Body weights

Body weight data are summarized in Table 3. Individual body weight values are presented in Appendix 5.

Animals at all dose levels tested lost weight in the days immediately after administration of A3879R. At the two lower dose levels, 43.20 and 86.40 mg formulation/kg (8 and 16 mg paraquat ion/kg), body weights returned to, or near to, Day 1 values by study termination. At the highest dose tested, 129.60 mg formulation/kg (24 mg paraquat ion/kg), body weights remained below Day 1 values throughout the study.

The animal euthanized *in extremis* lost weight steadily each day and was euthanized because of the severity of the observations after treatment.

4.1.4 Food consumption

Food consumption data are summarized in Table 4. Individual food consumption values are presented in Appendix 6.

Food consumption was decreased at all three dose levels tested in the days immediately following dose administration. At the two lower dose levels, 43.20 and 86.40 mg formulation/kg (8 and 16mg paraquat ion/kg), food consumption gradually returned to normal by the end of the study. At the highest dose level, 129.60 mg formulation/kg (24 mg

Report Number: 1390-002

Comment [m6]: Comment on 1390-001 report
The format of these tables needs to be adjusted.
When reading these tables, the reader only gets
though day 11 and then must skip a page to get rest.
(goes from page 191 to 193) Recommend that tables
either be reformatted or that at least pages be moved
so that all data for animal groups is on consecutive
pages.
Please correct as necessary on other reports – MPI
please check 002

paraquat ion/kg), food consumption improved over the two week study period but did not return completely to pre-study levels.

4.1.5 Physical examinations

Individual physical examination findings are presented in Appendix 7.

All animals appeared normal at the time of pre-test physical examination and were acceptable for use on study. The only observations noted upon physical examination prior to necropsy were findings of abrasions or sparse hair, which are common for dogs of this age.

4.1.6 Clinical chemistry and pathology

Clinical chemistry values are summarized in Table 5. Individual clinical chemistry values are presented in Appendix 9.

The clinical pathology interpretation of the results is presented below and in Appendix 8.

One animal (animal number 109) at 129.60 mg formulation/kg (24 mg paraquat ion/kg) exhibited decreased sodium, potassium and chloride, increased phosphorus, urea nitrogen, creatinine, total protein, albumin, globulins and glucose 24 hours post-dose. No other animals at this or other dose levels exhibited effects at any interval. Blood samples were obtained from animal number 109 prior to euthanasia that revealed a worsening of the above mentioned effects compatible with renal failure, as well as additional effects compatible with hepatic injury [increased aspartate aminotransferase (AST) and alanine aminotransferase (ALT)]. Triglycerides and cholesterol were also mildly to moderately elevated.

Comment [m7]: MPI please clarify if comparisons are being made pre-test.....having trouble matching the numbers with trends indicated in the text of this section with the data in the corresponding appendix or is it compared to historical control? Please clarify in a simple statement. CHECK ALL REPORTS

4.1.7 Plasma analysis

Detailed results of the analysis of plasma concentrations of paraquat and emetic are presented in Appendix 10.

Summary results of the analysis of plasma concentrations of the paraquat and emetic are summarized in the following table. The plasma paraquat 24-hour AUC values are displayed graphically in Figures 1 and 2.

Plasma Concentrations of Paraquat and Emetic

Dose received		Plasma Analysis					Mortality
mg A3879R/kg	mg paraquat ion/kg	Pq 24h AUC (µg/mL.h) ^a	Emetic 1h AUC (ng/mL.h) ^b	Time to mean peak (mins)		Mean peak Pq (µg/mL)	
				Emetic	Pq		
43.20	8	8.85 ± 1.34	6.01 ± 1.71	60	60	2.16 ± 0.52	0/4
86.40	16	11.21 ± 1.34	15.81 ± 2.66	30	30	4.71 ± 1.56	0/4
129.60	24	23.51 ± 5.87	24.72 ± 5.10	30	30	8.21 ± 2.60	1/4

^a Plasma analysis at Syngenta CTL and reported in 026791/Phase/Revision -001. Appendix 10

^b Plasma analysis at CEMAS and reported in CEMS-4058. Appendix 10

Values shown are means ± SEM (n=4).

Mean peak levels were estimated based on the levels measured at the specific timepoints. Following administration of 43.20 mg formulation/kg (8 mg paraquat ion/kg) the mean peak plasma level was observed at 1 hour post-dose with levels of 2.16 ± 0.52 µg/mL. Removal of paraquat from the plasma was gradual and by 24 hour post-dose the majority had been eliminated resulting in a mean 24 hour AUC value of 8.85 ± 1.34 µg/mL.h.

After a dose of 86.40 mg formulation/kg (16 mg paraquat ion/kg) the mean peak plasma level was observed at 30 minutes with levels of 4.71 ± 1.56 µg/mL. Elimination of paraquat from the plasma was gradual and by 24 hours post-dose the majority had been eliminated resulting in a mean 24 hour AUC value of 11.21 ± 1.34 µg/mL.h. The peak plasma concentration and the 24 hour AUC value were higher than those observed from the 43.2 mg formulation/kg dose (8 mg paraquat ion/kg).

At the highest dose level of 129.60 mg formulation/kg (24 mg paraquat ion/kg) the mean peak plasma level was observed at 30 minutes post-dose with levels of 8.21 ± 2.60 µg/mL. Elimination of paraquat from the plasma was gradual and by 24 hours post-dose the majority had been eliminated resulting in a mean 24 hour AUC value of 23.51 ± 5.87 µg/mL.h. The peak plasma concentration and the 24 hour AUC value were higher than those observed from the previous doses of 43.2 and 86.40 mg formulation/kg dose (equivalent to 8 and 16 mg paraquat ion/kg).

Following administration of A3879R mean peak plasma emetic levels were observed at 1 hour post-dose for the lower dose and at 30 minutes post-dose for the higher doses.

The dose of 129.60 mg formulation/kg (24 mg paraquat ion/kg) resulted in the highest systemic exposure of emetic. At all three doses the elimination of the emetic from the plasma was gradual and essentially complete by 12 to 24 hours postdose.

4.2 Postmortem study evaluations

The pathology interpretation of the results is presented below and in Appendix 11.

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4.2.1 Macroscopic

Macroscopic observations are summarized in Table 6. The pathology interpretation of the results is presented here and detailed in Appendix 13.

There were no definitive test article-related macroscopic effects observed. Minimal to mild white discoloration of the lung and/or white focus/foci were observed in two males receiving 43.20 mg formulation/kg (8mg paraquat ion/kg). Mild tan focus/foci were observed in one male receiving 129.60 mg formulation/kg (24 mg paraquat ion/kg). A strong dose correlation was not present and similar findings were not observed within the 86.40 mg formulation/kg group (16 mg paraquat ion/kg), therefore a relationship to treatment was interpreted to be unlikely. One male receiving 129.60 mg formulation/kg (24 mg paraquat ion/kg) was terminated prior to study completion due to clinical signs; all tissues were interpreted to be within normal limits. All other macroscopic lesions were interpreted to be sporadic and incidental.

4.2.2 Microscopic

Results of the microscopic examination are presented in Appendix 14.

One dog which received 129.60 mg formulation/kg (24 mg paraquat ion/kg) was euthanised on Day 3 (number 109). The major histopathological findings in this animal were moderate renal tubular degeneration/necrosis and slight congestion and hemorrhage in the lungs. There were no lesions in the gastrointestinal tract.

In the lungs of the remaining animals that survived to Day 15, there was alveolar macrophage infiltration, pneumocyte hypertrophy and interstitial fibrosis. Overall, the lung lesions were graded as minimal or slight in severity and tended to be focal or multifocal in distribution rather than diffuse. There was no clear distinction between groups although the lesions appeared marginally more severe in the highest dose group (129.60 mg formulation/kg; equivalent to 24 mg paraquat ion/kg).

Except for dog number 109, there were only minimal changes in the kidney (tubular basophilia, tubular dilatation and interstitial inflammatory cell infiltration). There was no evidence of necrosis. A minimal degree of intratubular microlithiasis (mineralization) was a consistent feature but this is a common finding in control dogs and is considered to be unrelated to test substance administration.

There were no significant lesions in the other tissues examined.

Comment [m8]: MPI - Protocol deviation indicates gthat hfor this animal.....

"At necropsy, animal 109,.... the mouth, including the tongue and buccal cavity, were not examined, and the esophagus, stomach, and the length of the small and large intestines were not opened and examined for signs of mucosal irritation. The lungs were not examined for areas of discoloration or the presence of any lesions.

Therefore can't say within normal limits. Please correct report.

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Incidence of Key Paraquat Induced Lesions with A12837B A3879R

Microscopic Findings	Dose Level		
Dose Level (mg formulation/kg)	43	86	130
Dose Level (mg paraquat ion/kg)	8	16	24
Number examined	4	4	3 (1*)
Kidney –			
No relevant abnormalities	4	4	3 (0)
Tubular degeneration/ necrosis	0	0	0 (1)
Lung –			
No relevant abnormalities	0	0	0
Fibrosis	4	2	2 (0)
Alveolar macrophage infiltration	4	4	3 (1)
Pneumocyte hypertrophy	4	4	3 (0)

G = gavage dose

(*) – animals euthanized *in extremis*

4.3 Discussion

All animals receiving a single oral dose of 43.20 and 86.40 mg formulation/kg (8 and 16 mg paraquat ion/kg body weight) survived until scheduled study termination on Day 15. No definitive paraquat-related effects were seen in clinical chemistry or macroscopic pathology parameters. Emesis, body weight loss and reduced food consumption were noted in these groups in the period immediately after administration. However, these effects were transitory in nature and the animals were returning to a normal condition by study termination.

Emesis was more frequent and both weight loss and reduction in food consumption were still evident at study termination following treatment with a single oral dose of 129.60 mg formulation/kg (24 mg paraquat ion/kg body weight). In addition, one animal was euthanized *in extremis* on Day 3 with observations of inappetence, decreased activity, salivation, loss of skin elasticity and skin cold to touch. The outcome in this animal was consistent with high systemic exposure to paraquat, as evidenced by the plasma paraquat 24 hour AUC. The terminal blood sample and histopathology results obtained from this dog were consistent with renal failure (demonstrated by clinical pathology and histopathology) and hepatic injury (demonstrated by clinical pathology only). This is also consistent with a high dose of paraquat⁴⁵ and was probably the principal cause of the clinical condition of this animal.

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Peak plasma paraquat levels were observed between 30 minutes and 1 hour post-dose. The timing of peak plasma emetic levels was similar, between 30 minutes and 1 hour post-dose.

⁴ Lock, EA and Wilks, MF (2001). Paraquat In "Handbook of Pesticide Toxicology" 2nd Edition. (Ed. RI Krieger), Vol 2, 70 1559-1603. Academic Press

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Increasing doses of A3879R gave a dose-dependent increase in the systemic exposure to paraquat. Mean peak plasma emetic levels were observed at 1 hour post-dose for the lower dose and at 30 minutes post-dose for the higher doses. The dose of 129.60 mg formulation/kg (equivalent to 24 mg paraquat ion/kg) resulted in the highest systemic exposure of both paraquat and emetic.

The lung lesions observed in this study are typical findings for paraquat exposure in the dog⁴⁵.

5.0 CONCLUSION

A single oral capsule dose of 200 g paraquat ion/L SL formulation (A3879R) was tolerated at the 43.20 and 86.40 mg formulation/kg dose levels (8 and 16 mg paraquat ion/kg). Those effects observed (e.g. emesis) were transitory, and not deleterious in nature. At the highest dose level tested, 129.60 mg formulation/kg (24 mg paraquat ion/kg), one animal was euthanized *in extremis* with post-mortem findings of moderate tubular degeneration/necrosis and slight congestion and hemorrhage in the lungs. In the surviving animals at this dose, reduction in body weight and food consumption were not resolved by study termination. These data indicate that 129.60 mg A3879R/kg (24 mg paraquat ion/kg) is a minimum lethal dose.

TABLES SECTION

Report Number: 1390-002

TABLE 1 Summary of Continuous Postdose Clinical Findings

On occasion, clinical findings may have been observed more than once during the interval and were recorded accordingly. The individual continuous post-dose clinical findings table of this appendix reports the findings observed during the initial 4 hours following test article administration, not the number of times observed.

TABLE 2 Summary of Clinical Findings

On occasion, clinical findings may have been observed more than once during the interval and were recorded accordingly. The individual continuous post-dose clinical findings table of this appendix reports the findings observed during the initial 4 hours following test article administration, not the number of times observed.

Report Number: 1390-002

TABLE 3 Summary of Body Weight Values

Report Number: 1390-002

TABLE 4 Summary of Food Consumption Values

Report Number: 1390-002

TABLE 5 Summary of Clinical Chemistry Values

Report Number: 1390-002

TABLE 6 Summary of Macroscopic Observations

Report Number: 1390-002

FIGURES SECTION

Report Number: 1390-002

FIGURE 1 Plasma Paraquat 24 Hour AUC

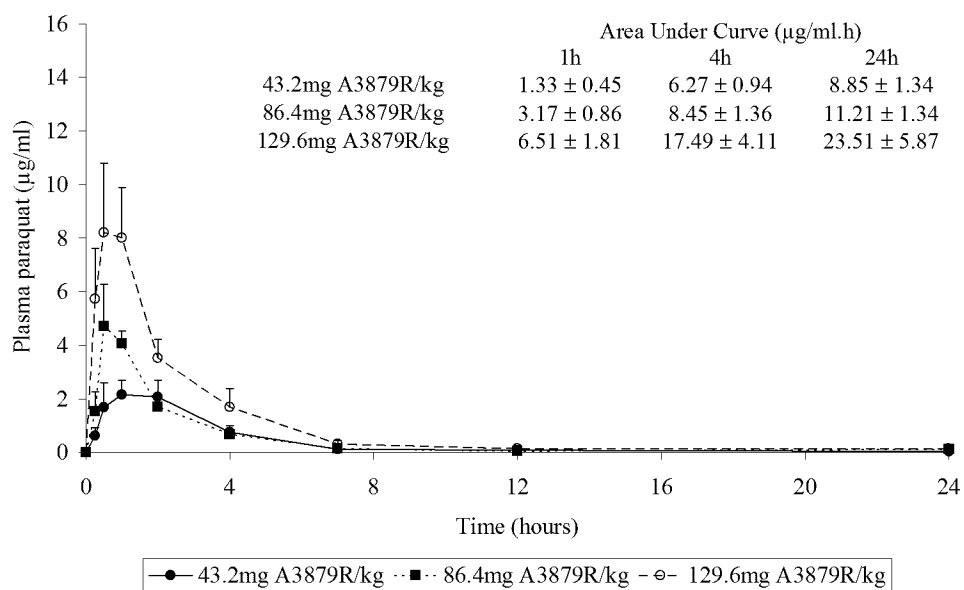
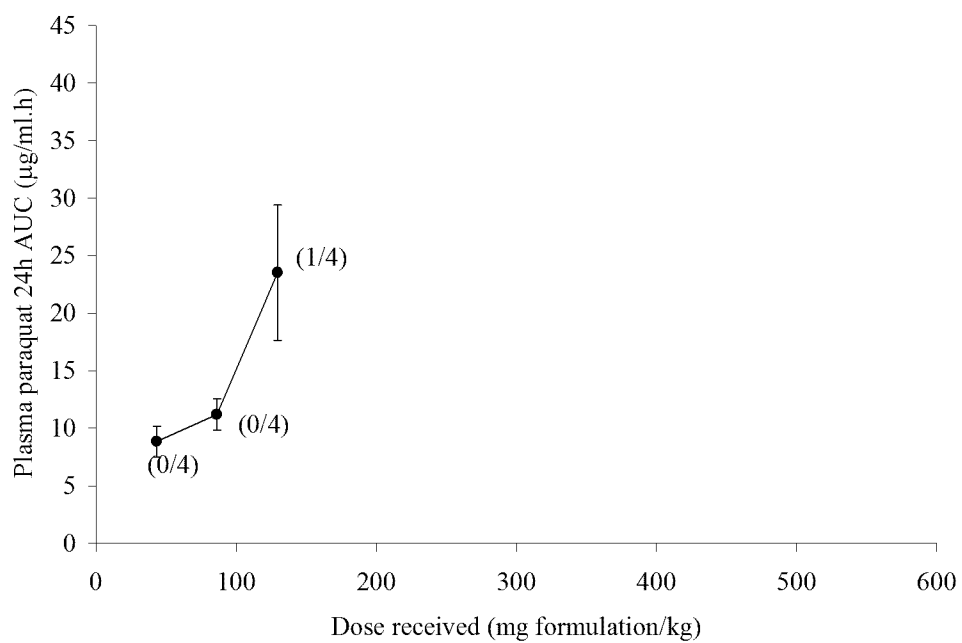


FIGURE 2 Mortality vs. Dose vs. Plasma Paraquat 24 Hour AUC



(Number of animals terminated/number of animals dosed)

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APPENDICES SECTION

APPENDIX 1 Vehicle and Test Article Information

Vehicle and Test Article Information

Date Received:	July 16, 2005
Supplier:	Torpac, Inc. Fairfield, New Jersey
Amount Received:	1 x 100 capsules
Label Identification:	Torpac Size # 000 Lock Ring Empty Gelatin Capsules
Lot Number:	640987
Physical Characteristics:	Empty gelatin capsules
Expiration Date:	July 16, 2008
Storage:	Room temperature
TMC Number:	3020CA

Date Received:	January 18, 2007
Supplier:	Torpac, Inc. Fairfield, New Jersey
Amount Received:	200.0000 Units
Label Identification:	#12 Torpac Gelatin Capsules
Batch Number:	1878
Physical Characteristics:	Clear light yellow capsules
Expiration Date:	September 30, 2011
Storage:	Room temperature
TMC Number:	070088

Date Received:	January 25, 2007
Supplier:	Syngenta Crop Protection, Inc. Greensboro, North Carolina
Amount Received:	500.0000 mL
Label Identification:	Paraquat
Batch Number:	J4818/175
Physical Characteristics:	Dark green liquid
Expiration Date:	August 30, 2008
Storage:	Room temperature, protected from light
TMC Number:	0700BF

Tap water was provided by MPI Research, Inc.

APPENDIX 2 Record of Animal Fate and Disposition

APPENDIX 3 Individual Continuous Postdose Clinical Findings

On occasion, clinical findings may have been observed more than once during the interval and were recorded accordingly. The individual continuous post-dose clinical findings table of this appendix reports the findings observed during the initial 4 hours following test article administration, not the number of times observed.

APPENDIX 4 Individual Clinical Findings

On occasion, clinical findings may have been observed more than once during the interval and were recorded accordingly. The individual clinical findings table of this appendix reports the findings observed, not the number of times observed.

APPENDIX 5 Individual Body Weight Values

APPENDIX 6 Individual Food Consumption Values

APPENDIX 7 Individual Physical Examination Findings

APPENDIX 8 Clinical Pathology Interpretation

MPI Research Study Number 1390-002
Paraquat 200 g/L SL (A3879R): Acute Toxicity in Beagle Dogs with Toxicokinetic
Analysis

Clinical Pathology Interpretation

Clinical Chemistry

One animal at 129.60 mg formulation/kg (animal number 109) exhibited decreased sodium, potassium and chloride, and increased phosphorus, urea nitrogen, creatinine, total protein, albumin, globulins, and glucose 24 hours postdose. No other animals at this or other dose levels exhibited effects at any interval. Blood samples were obtained from animal number 109 prior to scheduled termination that revealed a worsening of the above mentioned effects compatible with renal failure, as well as additional effects compatible with hepatic injury [increased aspartate aminotransferase (AST) and alanine aminotransferase (ALT)]. Triglycerides and cholesterol were also mildly to moderately elevated.

Jan L. VanSteenhouse, D.V.M., Ph.D., D.A.C.V.P.
Director, Clinical Pathology
Study Clinical Pathologist

Date

APPENDIX 9 Individual Clinical Chemistry Values

APPENDIX 10 Plasma Report

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APPENDIX 11 Pathology Interpretation

MPI Research Study Number 1390-002
Paraquat 200 g/L SL (A3879R): Acute Toxicity in Beagle Dogs with Toxicokinetic
Analysis

Pathology Interpretation

Macroscopic

There were no definitive test article-related effects observed. Minimal to mild white discoloration of the lung and/or white focus/foci were observed in two males receiving 43.20 mg formulation/kg and mild tan focus/foci were observed in one male receiving 129.60 mg formulation/kg; however, a strong dose correlation was not present and similar findings were not observed within the 86.40 mg formulation/kg group, therefore toxicologic significance was interpreted to be unlikely. One male receiving 129.60 mg formulation/kg died prior to study completion; all tissues were interpreted to be within normal limits. All other macroscopic lesions were interpreted to be sporadic and incidental.

Comment [d9]: Can this statement be made if lungs not examined on this dog?

Shelley L. Beazley, D.V.M., D.A.C.V.P.
Study Pathologist

Date

APPENDIX 12 Tissues and Organs Collected and Preserved

APPENDIX 13 Individual Animal Data Record: Pathology

APPENDIX 14 Microscopic Examination Report

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APPENDIX 15 Protocol and Amendment

APPENDIX 16 Deviations

DEVIATIONS

This study was conducted in accordance with the United States Environmental Protection Agency (EPA), Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), Good Laboratory Practice (GLP) Standards, 40 Code of Federal Regulations (CFR) Part 160. The analytical and pathology phase of the study were conducted according to the current version of the United Kingdom Principles of Good Laboratory Practice (The UK GLP Regulations) and the Organization for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice (ENV/MC/CHEM(98) 17) as interpreted by Syngenta Central Toxicology Laboratory (CTL), and the protocol, with the exception of the following deviations:

Protocol Deviations

Size 12 Torpac gelatin capsules were received from Fairfield, New Jersey.

Thirteen animals placed on study were younger than 9 months of age at receipt.

Prior to initiation of test article administration, on several occasions, for several animals, food was removed 1 to 8 minutes outside the allowable window.

During the week prior to dosing, several animals placed on study consumed less than 90% of the 300 g of food offered.

Two animals placed on study had body weights that fell outside $\pm 20\%$ of the mean body weight.

On the day of dosing, at 30 minutes postdose, the blood sample collected for the determination of the plasma concentration of the test article for a single animal at 86.40 mg formulation/kg (animal number 104) was clotted, resulting in serum instead of plasma.

On the day of dosing, for a single animal at 43.20 mg formulation/kg (animal number 101), no color was recorded with the observation of defecation.

On two occasions, the temperature of the animal room was outside the protocol specified range of 64 to 84°C.

At necropsy, for a single animal at 129.60 mg formulation/kg (animal number 109), the mouth, including the tongue and buccal cavity, were not examined, and the esophagus, stomach, and the length of the small and large intestines were not opened and examined for signs of mucosal irritation. The lungs were not examined for areas of discoloration or the presence of any lesions.

Comment [d10]: This statement does not seem to be consistent with discussion in the text about lung lesions of this animal. Please confirm.

In the opinion of the Study Director, these minor deviations did not affect the quality or integrity of the study.