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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: July 29, 2005

MEMORANDUM

TXR # 0052955

SUBJECT: Paraquat: Toxicokinetics in Dogs

FROM: Alberto Protzel, Ph.D.
Branch Senior Scientist
Toxicology Branch
Health Effects Division (7509C)

Alberto Protzel 7/29/05

THRU: Louis Scarano, Ph.D. Chief
Toxicology Branch
Health Effects Division (7509C)

Alberto Protzel for 7/29/05

TO: Hope Johnson/ Risk Manager Reviewer
James Tompkins/Risk Manager
Registration Division (7509C)

Paraquat Dichloride [PC Code: 061601]
DP Barcode: D309472

Action: Review four Toxicokinetics studies of Paraquat in Dogs

Conclusions:

1. Four toxicokinetics studies [MRIDs 46364510-11 and 4636517-18] of Paraquat formulations in dogs have been reviewed and Classified Acceptable/Non-Guideline.
2. Taken together, these studies show that inclusion of an emetic agent and a gelling agent decreases the acute oral toxicity of paraquat formulations in dogs.

3. This protection is not absolute, since dogs dosed at the highest dose may show small lung lesions consistent with paraquat toxicity.
4. Extrapolation of these results for protection of humans will require careful consideration of the actual conditions of human exposure, and the relative pharmacokinetics and pharmacodynamics of paraquat, emetic agent and gelling agent in humans versus dogs.

Detailed Considerations.

Four Toxicokinetics studies with paraquat have been submitted by Syngenta Crop Protection, Inc. The following paragraphs contain Executive Summaries of these studies.

1. Brammer, A., Heylings, J., Swain, C. (2004). Paraquat 240 G/L SL Formulation (A7813K): Toxicokinetic study in the dog. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. CTL No. XD7355. September 8, 2004. **MRID 46364510.**

EXECUTIVE SUMMARY: A non-guideline study (MRID 46364510) was conducted in which three male beagle dogs were given incremental doses (via gelatin capsule) of Paraquat 240 g/L formulation A7813K (252 g/L paraquat; 1.5 g/L PP796 emetic; Lot No. J4267/75-2). Doses were 150, 302, and 602 mg/kg (equivalent to 32, 64, and 128 mg paraquat ion/kg, respectively) given at 1, 6, and 10 weeks, respectively. Plasma kinetics (concentration-time course, rate of absorption, peak plasma concentrations, and AUC parameters) were determined. Clinical observations (emesis response and general observations), clinical chemistry, gross pathology, and histopathology of selected organs/tissues were assessed.

This study, conducted to evaluate the toxicity and kinetics of a new emetic-containing paraquat formulation (A7813K which contains a "trigger gel" allowing for greater effectiveness of the PP796 emetic agent), provided data on key plasma kinetic parameters (rate of absorption and AUC values at 1, 4 and 24 hours). Absorption rates were approximately 19, 15, and 98 ng/ml/min for the 150, 302, and 602 mg/kg doses. AUC values were not notably different between the low and mid-dose but ~2-fold greater for the high dose. The 602 mg/kg dose of A7813K resulted in a peak plasma concentration of 2.8 paraquat ion µg/ml which was similar to that achieved with a 43 mg/kg dose of Gramoxon (a contemporary formulation lacking the "trigger gel"). Study results also demonstrated that the A7813K formulation was less toxic to dogs while providing for plasma levels and a systemic dose similar to that achieved with an existing formulation (Gramoxon) which does not contain gelling agents to increase the effectiveness of the PP796 emetic component. However, small lung lesions consistent with paraquat toxicity were seen in one of the three dogs at *postmortem*.

2. Brammer, A., Heylings, J., Swain, C. (2004). Gramoxone 200 G/L SL Formulation

(A3879D): Toxicokinetic study in the dog. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. CTL No. XD7388. September 8, 2004. **MRID 46364511**. Unpublished.

EXECUTIVE SUMMARY:

A non-guideline study (MRID 46364511) was conducted to compare plasma kinetic data for Gramoxone 200 G/L SL formulation A3879D in dogs to data from an earlier study. Specifically, three male beagle dogs were given a single 43 mg/kg oral dose (via gelatin capsule) of Gramoxone 200 G/L SL formulation A3879D (195 g/L paraquat; 1.5 g/L and non-specified amount of PP796 emetic; Batch No. BSN3I1030). Plasma kinetics (concentration-time course), rate of absorption, and AUC parameters were determined for the paraquat ion and the emetic.

This was a cursory study designed to evaluate the plasma kinetics of a paraquat formulation in dogs following a single 43 mg/kg oral dose and to compare the results to data acquired from an earlier study. At the dose tested, Gramoxone was not overtly toxic to the dogs. Only one of three dogs exhibited emesis (to be expected from the emetic-containing Gramoxone) possibly indicative that emetic levels in plasma were insufficient to induce prompt emesis at the dose tested. Plasma concentration-time course data for both the paraquat ion and the PP796 emetic were variable (2-4 fold) among the three dogs, although near-complete elimination from the plasma occurred within 7 hours for the emetic, and at 12-24 hours for the paraquat ion. Peak plasma concentrations of paraquat ion ranged from 1.14 to 4.22 µg/ml. Peak plasma concentrations of the emetic ranged from 0.65 to 1.12 µg/ml. AUC values for both components were also variable due to the plasma kinetics (4.56-10.26 µg paraquat/ml-hr at 24 hours and 2.03-3.16 µg emetic/ml-hr. Most variability could be attributed to one dog. The kinetics observed in this study were similar to those reported in an earlier study.

3. Brammer, A. (2004). Paraquat 200 G/L SL Formulation (A3879BU): Toxicokinetic study in the dog. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. CTL No. XD7201. January 7, 2004. **MRID 46364517**. Unpublished.

EXECUTIVE SUMMARY:

A non-guideline study (MRID 46364517) was conducted in which three male beagle dogs were given incremental doses (via gelatin capsule) of Paraquat 200 g/L formulation A3879BU (203 g/L paraquat; 1.56 g/L PP796 emetic; Lot No. J6481/016). Doses were 46, 92, 184, 368, and 736 mg/kg (equivalent to 8, 16, 32, 64, and 128 mg paraquat ion/kg, respectively) given at 1, 5, 9, 13, and 18 weeks. Plasma kinetics (concentration-time course), rate of absorption and AUC parameters were determined. Clinical observations (emesis response and general observations), clinical chemistry, gross pathology, and histopathology of selected organs/tissues were assessed.

The paraquat A3879BU dosing regimen produced signs of toxicity only at the highest dose and primarily in one dog. Peak plasma levels (2.57, 2.00, 3.07, 1.94, and 8.21 for the low to high doses) occurred at 0.5 to 1 hour, tended to occur earlier at higher doses, and did not exhibit a quantitative dose-response. Moderate individual variability was observed among the three dogs (generally 2-3 fold differences). The time-course data showed that the paraquat ion was almost completely eliminated within 24 hours after each dose. Peak plasma concentration of the emetic agent (PP796) occurred at 0.5 to 1 hour. The dose relationship was inconsistent at the 368 mg/kg dose (notably lower plasma emetic concentration) due to compromised absorption of the test article in one dog. The plasma levels at time points up to 2 hours tended to show a dose response but the 368 mg/kg dose varied somewhat from this pattern. Although the plasma concentration-time course was variable, the emetic component was nearly completely cleared from the plasma by 24 hours after dosing. At a given time point, the paraquat ion AUC values were similar for all doses except the highest, thereby indicating that the PP796-induced emesis was limiting the systemic dose of the paraquat ion. At the highest dose, the plasma paraquat ion concentration was approaching known toxic levels as demonstrated by the effects in one dog of this dose group.

This is a cursory study designed to examine the effectiveness of a novel paraquat formulation intended to limit accumulation of the toxic paraquat ion by inducing emesis in a non-target species. Although minor problems were noted (primarily due to one of three dogs), the study provided preliminary data indicating the effectiveness of the novel formulation.

A non-guideline study (MRID 46364517) was conducted to compare plasma kinetic data for Paraquat 200 G/L formulation A3879BU in dogs with Gramoxone, a commercial standard product.

4. Heylings, J., Swain, C., Brammer, A. (2004). Paraquat: Gramoxone 200 G/L SL Formulation: Toxicokinetic study in the dog. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. CTL No. 026118. January 28, 2004. **MRID 46364518.**

EXECUTIVE SUMMARY:

A non-guideline study (MRID 46364518) was conducted to compare plasma kinetic data for Gramoxone 200 G/L SL formulation (CTL ref. no. Y00061, purity 20% assumed) in dogs administered the compound (44 mg Gramoxone/kg, equivalent to 8 mg paraquat ion/kg) via gelatin capsule or by gavage. These data were obtained from a series of studies conducted at Central Toxicology Laboratory over a period of several years. Specifically, the studies provided data for 12 dogs administered the test article via gelatin capsule and seven dogs dosed by gavage.

At the dose tested, emesis was the only treatment-related effect in the dogs. Emesis, an expected response, occurred as early as 16 minutes post dose and generally ceased

several hours post dose Paraquat ion profiles (concentration-time data and AUC estimates) were similar in dogs administered Gramoxone (44 mg/kg, equivalent to 8 mg paraquat ion) via gelatin capsule or by gavage. Peak plasma concentrations of 3-4 µg/ml were achieved at one hour post dose. The paraquat ion was almost completely cleared at 24 hours post dose in both groups. The variability in plasma concentration-time data could be attributed to individual variability among the limited number of dogs in each experimental group. AUC values over 24 hours were approximately 16 and 15 µg/ml·hr, respectively, for the gelatin capsule and gavage administrations.

The report is principally an analysis of plasma profile data from a series of earlier studies and was not designed or submitted as a guideline study.

DATA EVALUATION RECORD

PARAQUAT
STUDY TYPE: TOXICOKINETICS - DOG
[NON-GUIDELINE]
MRID 46364510

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

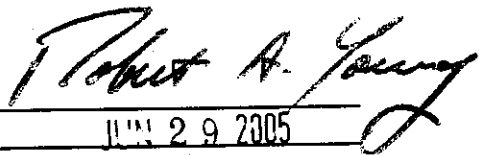
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 94-2005

Primary Reviewer:

Robert A. Young, Ph.D., D.A.B.T.

Signature:

Date:



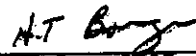
JUN 29 2005

Secondary Reviewers:

H.T. Borges, Ph.D., MT (ASCP), D.A.B.T.

Signature:

Date:

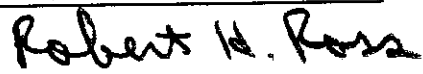


JUN 29 2005

Robert H. Ross, M.S., Group Leader

Signature:

Date:



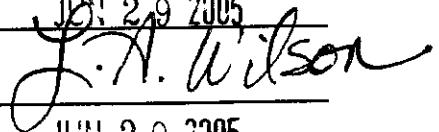
JUN 29 2005

Quality Assurance:

LeeAnn Wilson, M.A.

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Date:



JUN 29 2005

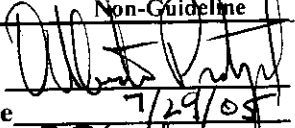
Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

PARAQUAT 240 G/L FORMULATION (A7813K)

Non-Guideline

EPA Reviewer: A. Protzel, Ph.D.
 Toxicology Branch, Health Effects Division (7509C)
 EPA Work Assignment Manager: P.V. Shah, Ph.D.
 Registration Action Branch 1, Health Effects Division (7509C)

Signature: 
 Date: 7/29/05
 Signature: P.V. Shah
 Date: 7/29/05 AP
 Template version 11/01

TXR#: 0052955

DATA EVALUATION RECORD

STUDY TYPE: Toxicokinetics - dog [Non-guideline].PC CODE: 061601DP BARCODE: 309472SUBMISSION NO.: NATEST MATERIAL (PURITY): (Paraquat 240 G/L Formulation [A7813K]; 22.3% a.i. w/w)SYNONYMS:

CITATION: Brammer, A., Heylings, J., Swain, C. (2004). Paraquat 240 G/L SL Formulation (A7813K): Toxicokinetic study in the dog. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. CTL No. XD7355. September 8, 2004. MRID 46364510. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, P. O. Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY: A non-guideline study (MRID 46364510) was conducted in which three male beagle dogs were given incremental doses (via gelatin capsule) of Paraquat 240 g/L formulation A7813K (252 g/L paraquat; 1.5 g/L PP796 emetic; Lot No. J4267/75-2). Doses were 150, 302, and 602 mg/kg (equivalent to 32, 64, and 128 mg paraquat ion/kg, respectively) given at 1, 6, and 10 weeks, respectively. Plasma kinetics (concentration-time course, rate of absorption, peak plasma concentrations, and AUC parameters) were determined. Clinical observations (emesis response and general observations), clinical chemistry, gross pathology, and histopathology of selected organs/tissues were assessed.

This study, conducted to evaluate the toxicity and kinetics of a new emetic-containing paraquat formulation (A7813K which contains a "trigger gel" allowing for greater effectiveness of the PP796 emetic agent), provided data on key plasma kinetic parameters (rate of absorption and AUC values at 1, 4 and 24 hours). Absorption rates were approximately 19, 15, and 98 ng/ml/min for the 150, 302, and 602 mg/kg doses. AUC values were not notably different between the low and mid-dose but ~2-fold greater for the high dose. The 602 mg/kg dose of A7813K resulted in a peak plasma concentration of 2.8 paraquat ion µg/ml which was similar to that achieved with a 43 mg/kg dose of Gramoxon (a contemporary formulation lacking the "trigger gel"). Study results also demonstrated that the A7813K formulation was less toxic to dogs while providing for plasma levels and a systemic dose similar to that achieved with an

PARAQUAT 240 G/L FORMULATION (A7813K)

effectiveness of the PP796 emetic component. However, small lung lesions consistent with paraquat toxicity were seen in one of the three dogs at *postmortem*.

This study (MRID 46364510) on the toxicity and plasma kinetics of Paraquat 240 g/L (A7813K formulation) in dogs is classified **Acceptable/Non-Guideline** but does not satisfy the 85-1 Guideline Requirement for a metabolism study [OPPTS 870.7485, OECD 417]. The study was neither designed nor submitted as a guideline study.

COMPLIANCE: Signed GLP, Data Confidentiality Claim, and Quality Assurance statements were provided in the study report.

I. MATERIALS AND METHODS:

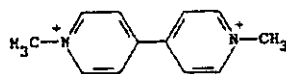
A. MATERIALS:

1. Test compound: Paraquat 240 g/L SL formulation (A7813k)

Radiolabelled test material:	Not used
Radiochemical purity	NA
Specific Activity	NA
Lot/Batch #:	NA

Non-Radiolabelled test material:

Description:	Clear green liquid
Lot/Batch #:	J4267/75-2 (CTL ref. no.: Y12693/044)
Purity:	252 g/L paraquat and 1.5 g/L PP796 emetic
Contaminants:	None noted
CAS # of TGAI:	4685-14-7
Structure:	



2. Vehicle and/or positive control: Kinetics and emesis response data for Gramoxon (an alternate formulation of paraquat) were used for comparison to the currently tested A7813K formulation.

3. Test animals:

Species:	Dog; male								
Strain:	Beagle								
Age/weight at study initiation:	47-49 weeks; 11-11.5 kg								
Source:	Conventional Animal Breeding Unit, Alderley Park, Macclesfield, UK								
Housing:	Adjacent pens, 3/pen except on treatment days when they were housed individually for 6-7 hrs for observation								
Diet:	350 g Laboratory Diet A (Special Diet Services Ltd., Stepfield, Witham, Essex, UK) daily								
Water:	Tap water <i>ad libitum</i>								
Environmental conditions:	<table border="0"> <tr> <td>Temperature:</td> <td>19±2°C</td> </tr> <tr> <td>Humidity:</td> <td>45-65% (elevation to 82% on several recorded on several occasions)</td> </tr> <tr> <td>Air changes:</td> <td>15/hr</td> </tr> <tr> <td>Photoperiod:</td> <td>12 hrs/12 hrs</td> </tr> </table>	Temperature:	19±2°C	Humidity:	45-65% (elevation to 82% on several recorded on several occasions)	Air changes:	15/hr	Photoperiod:	12 hrs/12 hrs
Temperature:	19±2°C								
Humidity:	45-65% (elevation to 82% on several recorded on several occasions)								
Air changes:	15/hr								
Photoperiod:	12 hrs/12 hrs								

Acclimation period:

4. **Dose preparations:** Amounts of A7813K required to achieve the target doses of paraquat ion (Table 1) were placed into gelatine capsules. The amount of test material was calculated as:

$$\text{mg formulation/kg} = \frac{(\text{dose volume [ml]} \times \text{specific gravity [1.13]}) \times 1000}{\text{weight (kg)}}$$

The capsules were filled immediately prior to dosing and placed in a second capsule to minimize contamination with oral/esophageal secretions. The high dose was divided between two capsules. Total dose volumes were 0.133, 0.267, and 0.533 ml/kg for the 150, 302, and 602 mg/kg doses, respectively.

Analysis of the dose preparations prior to dosing by Jealotts Hill International confirmed the paraquat ion and emetic (PP796) concentrations.

B. STUDY DESIGN AND METHODS:

1. **Group arrangements:** The experimental groups are shown in Table 1. Feed consumption was recorded (mean g feed/dog/day) for at least one week prior to treatment and throughout the treatment period. The A7813K formulation contains a "trigger gel" which increases the effectiveness of the PP796 emetic agent. The study was designed to ascertain the effectiveness of the emetic and resulting impact on plasma paraquat ion levels.

TABLE 1. Experimental protocol for toxicokinetic study in male beagle dogs given multiple oral doses of Paraquat 240 G/L SL Formulation (A7813K)			
Dose No.	A7813K dose (mg/kg) (mg paraquat ion/kg)	Week no.	Comments
1	150 (32)	1	3 dogs each received incremental doses (orally in gelatine capsules) at designated weeks; dogs fed at 4 hrs post dose and observed for 6-7 hrs
2	302 (64)	6	
3	602 (128)	10	

Data taken from p 16, MRID 46364510.

2. **Dosing and sample collection/preparation/analysis:** The dogs were dosed with the gelatine capsules (as described in §I.A.4) at approximately the same time of day and fed four hours later. Each of three dogs was dosed at 1, 6, and 10 weeks (Table 1). The dogs were weighed weekly prior to feeding, on day 1 of treatment and at weekly intervals thereafter. Clinical observations were conducted on the dogs for at least four hours following dosing and hourly thereafter. On non-dosing days, the dogs were observed at least twice daily for signs of toxicity or abnormal behavior.

Blood samples (from the jugular vein) were collected in heparinized tubes prior to feeding on the day before each dose, at 24 hours post dose and immediately prior to termination. The following clinical chemistry parameters were assessed:

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Electrolytes:		Other:	
x	Calcium	x	Albumin
x	Chloride	x	Blood creatinine
	Magnesium	x	Blood urea nitrogen
x	Phosphorus	x	Cholesterol
x	Potassium	x	Globulins
x	Sodium	x	Glucose
Enzymes		x	Total bilirubin
x	Alkaline phosphatase (ALK)	x	Total serum protein (TP)
	Cholinesterase (ChE)	x	Triglycerides
x	Creatinine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	x	A/G Ratio
x	Serum alanine aminotransferase (also SGPT)		
x	Serum aspartate aminotransferase (also SGOT)		
	Gamma glutamyl transferase (GGT)		
x	Glutamate dehydrogenase		

Blood samples were also taken for determination of the blood kinetic parameters of the test article and the emetic agent. For each dose, 2 ml blood samples were collected (in lithium heparin) on the day of dosing, 15 min, 30 minutes, and 1, 2, 4, 7, 12, and 24 hours post dose. Blood samples were centrifuged and subjected to spectrophotometric and fluorescence HPLC analysis.

At 2 weeks after the final dose, the dogs were killed by an overdose of sodium pentobarbitone), exsanguinated, and a necropsy performed. Macroscopic examinations included abnormal tissue, kidney, heart, lungs, stomach, duodenum, ileum, jejunum, liver, and esophagus. All tissues were subjected to microscopic examination.

3. Analytical techniques:

Second derivative spectrophotometric analysis: Plasma paraquat was determined by passing an aliquot (50-300 µl) of plasma through an ANSYSS SPEC PLUS PT SI cartridge. The cartridge was rinsed with HCl and the eluent collected. Dithionite reagent in NaOH was then passed through the cartridge and the eluent collected in the same cuvette as the HCl rinse. Second order derivative spectra (360-440 nm) were determined relative to a reagent blank using a Unicam UV1 spectrophotometer. The paraquat concentration was determined by reference to a standard curve for 0-10µg paraquat/ml plasma. The limit of quantification (LOQ) was 0.1 µg/ml.

Fluorescence HPLC: For plasma samples containing less than 0.1 µg paraquat/ml, fluorescence HPLC was used. Plasma samples (200 µl) were derivatized with 1% potassium ferricyanide in 9M NaOH and extracted in chloroform. The chloroform extractions were processed in silica cartridges and acetonitrile. The resulting paraquat dipyrindrone was eluted and analyzed by HPLC (Inertsil Phenyl-3 5 µ column); flow rate was 1 ml/min using a mobile phase of 30% acetonitrile and 70% water, and fluorescence detection. The amount of paraquat in each sample was determined by comparison to a standard curve (0-0.1 µg/ml). The LOQ was 10 ng/ml.

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4. **Storage stability:** Recommended storage conditions were provided but no other data were available regarding stability. Dosing formulations were prepared immediately prior to administration.
5. **Calculations/ statistical analysis:** Dose calculations and quantitative information regarding dose formulations were provided. Data were expressed as mean ± standard deviation.

II. **RESULTS:**

A. **CLINICAL OBSERVATIONS:** Scheduled veterinary examinations revealed no abnormalities. With the exception of vomiting, no clinical signs could be attributed directly to the treatment.

1. **Emesis:** The most notable (and expected) finding was emesis, the duration and severity (i.e., quantity of vomitus) of which increased with dose (Table 2). No additional effects were observed following the cessation of vomiting.

TABLE 2. Time (min) to emesis and duration of emesis in male dogs following oral dosing with Paraquat A7813K				
Dose (mg formulation/kg) [mg paraquat ion/kg]	Male 1	Male 2	Male 3	Mean ^a
	Time to first emesis			
150 [32]	23	23	31	26±5
302 [64]	23	21	17	20±3
602 [128]	23	20	16	20±4
	Time to last emesis			
150 [32]	32	47	52	44±10
302 [64]	43	48	51	47±4
602 [128]	70	113	131	105±31

^a Mean ±SD (calculated by reviewer)
 Data taken from p. 20, MRID 46364510.

2. **Bodyweight** Body weight of the three dogs was not significantly affected by the treatment. Body weights at Week -1 were 11.5, 11.3, and 10.8 kg and at Week 12 were 12.2, 11.3, and 11.2 kg. Dog no.2 lost 0.3 kg during week 11 but regained the weight during the final week.
3. **Food consumption:** Feed consumption was not significantly affected by the treatment. Weekly feed consumption ranged from 307-350 g/dog/day.
4. **Clinical chemistry:** There were no treatment-related effects on clinical chemistry parameters. Dog #3 exhibited clinically insignificant elevation (~3-fold) of plasma ALP level throughout the study (Week -1 to termination).
5. **Gross pathology:** Small, discolored areas were seen in the left and right apical lung lobes of dog No. 2. There were no findings for the other two dogs.

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6. **Microscopic pathology:** Microscopic lung lesions consistent with paraquat toxicity were seen in dog No. 2 (fibrosis, interstitial pneumonitis, macrophage infiltration)

B. TOXICOKINETICS: The rate of absorption at 15 minutes and AUC values at 1, 4, and 24 hours for paraquat (A7813K) in dogs are summarized in Table 3. Peak plasma paraquat concentrations of ~1.25 µg/ml (150 and 302 mg/kg doses) and ~2.8 µg/ml (602 mg/kg dose) were achieved about 1 hour post dose. Analysis of graphic displays of plasma paraquat concentration-time data indicated increasing individual variability in peak concentrations among the three dogs in each dose group. The variability in peak plasma concentration was especially notable among the dogs in the highest dose group (approximately 1.2, 2.2, and 5.2 µg/ml). Rate of absorption and AUC values for the 150 and 302 mg/kg doses were not substantially different. Rate of absorption at the high dose was notably greater and AUC values were reflective of the greater dose. For comparison of the new formulation (A7813K), similar data (from another study) on a previously used formulation (Gramoxone A3879D) were also presented. Rate of absorption at 15 minutes for the 43 mg/kg Gramoxone dose (8 mg paraquat ion/kg) was 3.7 ng/ml/min. Paraquat AUC values at 1, 4, and 24 hours for Gramoxone were 0.89, 5.63, and 7.98 µg/ml·min, respectively.

TABLE 3. Plasma kinetics of Paraquat (A7813K) in dogs following oral administration.				
Parameter/dose (mg formulation/kg) (mg paraquat ion/kg)	15 min	1 hr	4 hrs	24 hrs
Abs. Rate (ng/ml/min)				
150 [32]	18.90±9.17	—	—	—
302 [64]	15.08±11.10	—	—	—
602 [128]	97.73±68.47	—	—	—
AUC (µg/ml·hr)				
150 [32]	—	0.77±0.15	3.02±0.23	4.65±0.36
302 [64]	—	0.78±0.31	2.56±0.56	3.69±0.66
602 [128]	—	2.04±1.02	6.15±2.49	7.96±3.19

Data taken from Table 5, p. 48, MRID 46364510.

The rate of absorption at 15 minutes and AUC values at 1, 4, and 24 hours for the emetic in dogs are summarized in Table 4. The absorption rates and AUC values for emetic in the three dose levels were similar to those observed for paraquat. Kinetic values for the emetic in Gramoxone (A3879D) were 0.012 ng/ml/min rate of absorption at 15 minutes, and AUC values of 0.41, 2.00, and 2.49 µg/ml·min for 1, 4 and 24 hours, respectively.

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TABLE 4. Plasma kinetics of for emetic component (PP796) in dogs following oral administration.				
Parameter/dose (mg formulation/kg) [mg paraquat ion/kg]	15 min	1 hr	4 hrs	24 hrs
Abs. Rate (ng/ml/min)				
150 [32]	0.262±0.134	—	—	—
302 [64]	0.205±0.090	—	—	—
602 [128]	0.549±0.306	—	—	—
AUC (µg/ml·hr)				
150 [32]	—	5.13±1.68	15.00±4.27	18.52±5.08
302 [64]	—	4.48±0.62	13.67±1.44	17.31±2.17
602 [128]	—	9.43±2.80	29.01±8.14	41.93±10.49

Data taken from Table 6, p. 49, MRID 46364510.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The investigators concluded that at the doses tested, dogs exhibited no significant signs of toxicity and that the paraquat A7813K formulation was rapidly absorbed and eliminated. At the highest dose tested (602 mg/kg equivalent to 128 mg of paraquat ion/kg), paraquat concentration in the blood did not exceed 3 µg/ml. The 24-hour AUC value indicated that the overall 24-hour systemic exposure to paraquat was 7.96 µg/ml·hr which was similar to the 24-hour AUC of 7.98 µg/ml·hr for the currently used Gramoxon formulation. The findings indicated that a substantially higher dose (602 mg/kg) of the A7813K formulation achieved plasma levels of the paraquat ion similar to those achieved with a 43 mg/kg dose of the existing Gramoxon formulation without inducing significant toxicity in dogs. The tested formulation (A7813K) also provided greater effectiveness of the emetic agent relative to the Gramoxon formulation.

The study authors also provided insight into the mechanism by which the A7813K formulation allows for greater paraquat levels in the absence of serious systemic toxicity. Specifically, the A7813K formulation contains a water soluble gelling agent (alignate), the viscosity of which increases upon contact with gastric acid. This, in turn, allows for greater effectiveness of the emetic agent (PP796) resulting in decreased toxicity in the dog.

B. REVIEWER COMMENTS: A non-guideline study (MRID 46364510) was conducted in which three male beagle dogs were given incremental doses (via gelatin capsule) of Paraquat 240 g/L formulation A7813K (252 g/L paraquat; 1.5 g/L PP796 emetic; Lot No. J4267/75-2). Doses were 150, 302, and 602 mg/kg (equivalent to 32, 64, and 128 mg paraquat ion/kg, respectively) given at 1, 6, and 10 weeks. Plasma kinetics (concentration-time course), rate of absorption and AUC parameters were determined. Clinical observations (emesis response and general observations), clinical chemistry, gross pathology, and histopathology of selected organs/tissues were assessed.

This study, conducted to evaluate the toxicity and kinetics of a new paraquat formulation, provided data on key plasma kinetic parameters (rate of absorption and AUC values at 1, 4 and 24 hours). The A7813K formulation contains a "trigger gel" which increases the effectiveness of the PP796 emetic agent. The study was specifically designed to ascertain the effectiveness of the emetic and resulting impact on plasma paraquat ion levels. Study results

also demonstrated that the A7813K formulation was less toxic to dogs while providing for plasma levels and a systemic dose similar to that achieved with an existing formulation (Gramoxon). The protection afforded by the new formulation, however, was not absolute since one of the 3 dogs showed some small lung lesions consistent with paraquat toxicity. The study appeared to be well conducted. A more concise rationale for the selection of the incremental dose regimen would be a useful component of the experimental protocol. Individual variability in clinical chemistry parameters as well as the plasma kinetics assessments were acceptable and typical for this type of study. The investigators' conclusions regarding the toxicity assessment are supported by the data. The assessment of the plasma kinetic parameters are also consistent with the graphic display of the data provided in the study report. The study authors; however, indicated that the 602 mg/kg dose of the A7831K formulation was 16-fold greater than the 43 mg/kg Gramoxon dose; the difference appears to be 14-fold. Details regarding analytical methods for determination of plasma paraquat were provided in the study report.

This study (MRID 46364510) on the toxicity and plasma kinetics of Paraquat 240 g/L (A7813K formulation) in dogs is classified **Acceptable/Non-Guideline** and does not satisfy the 85-1 Guideline Requirement for a metabolism study [OPPTS 870.7485, OECD 417]. The study was neither designed nor submitted as guideline study.

C. STUDY DEFICIENCIES: There were no apparent deficiencies in the study.

DATA EVALUATION RECORD

PARAQUAT (GRAMOXONE)
STUDY TYPE: TOXICOKINETICS - DOG
[NON-GUIDELINE]
MRID 46364511

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 94-2005

Primary Reviewer:

Robert A. Young, Ph.D., D.A.B.T.

Signature: _____

Date: _____

Robert A. Young
JUN 29 2005

Secondary Reviewers:

H.T. Borges, Ph.D., MT (ASCP), D.A.B.T.

Signature: _____

Date: _____

H.T. Borges
JUN 29 2005

Robert H. Ross, M.S., Group Leader

Signature: _____

Date: _____

Robert H. Ross
JUN 29 2005

Quality Assurance:

LeeAnn Wilson, M.A.

Signature: _____

Date: _____

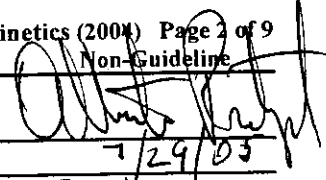
J.A. Wilson
JUN 29 2005

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

PARAQUAT 240 G/L FORMULATION (A7813K)

EPA Reviewer: A. Protzel, Ph.D.
Toxicology Branch, Health Effects Division (7509C)
EPA Work Assignment Manager: P.V. Shah, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C)

Signature: 
Date: 7/29/03
Signature: P.V. Shah
Date: 7/29/03
Template version 11/01

TXR#: 0052955

DATA EVALUATION RECORD

STUDY TYPE: Toxicokinetics - dog [Non-guideline].

PC CODE: 061601

DP BARCODE: 309472
SUBMISSION NO.: NA

TEST MATERIAL (PURITY): (Gramoxone 200 G/L SL Formulation [A3879D]; 19.5% a.i. w/w)

SYNONYMS:

CITATION: Brammer, A., Heylings, J., Swain, C. (2004). Gramoxone 200 G/L SL Formulation (A3879D): Toxicokinetic study in the dog. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. CTL No. XD7388. September 8, 2004. MRID 46364511. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, P. O. Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY:

A non-guideline study (MRID 46364511) was conducted to compare plasma kinetic data for Gramoxone 200 G/L SL formulation A3879D in dogs to data from an earlier study. Specifically, three male beagle dogs were given a single 43 mg/kg oral dose (via gelatin capsule) of Gramoxone 200 G/L SL formulation A3879D (195 g/L paraquat; 1.5 g/L and non-specified amount of PP796 emetic; Batch No. BSN3I1030). Plasma kinetics (concentration-time course), rate of absorption, and AUC parameters were determined for the paraquat ion and the emetic.

This was a cursory study designed to evaluate the plasma kinetics of a paraquat formulation in dogs following a single 43 mg/kg oral dose and to compare the results to data acquired from an earlier study. At the dose tested, Gramoxone was not overtly toxic to the dogs. Only one of three dogs exhibited emesis (to be expected from the emetic-containing Gramoxone) possibly indicative that emetic levels in plasma were insufficient to induce prompt emesis at the dose tested. Plasma concentration-time course data for both the paraquat ion and the PP796 emetic were variable (2-4 fold) among the three dogs, although near-complete elimination from the plasma occurred within 7 hours for the emetic, and at 12-24 hours for the paraquat ion. Peak plasma concentrations of paraquat ion ranged from 1.14 to 4.22 µg/ml. Peak plasma concentrations of the emetic ranged from 0.65 to 1.12 µg/ml. AUC values for both components were also variable due to the plasma kinetics (4.56-10.26 µg paraquat/ml·hr at 24 hours and 2.03-

PARAQUAT 240 G/L FORMULATION (A7813K)

3.16 µg emetic/ml·hr. Most variability could be attributed to one dog. The kinetics observed in this study were similar to those reported in an earlier study.

This study (MRID 46364511) on the toxicity and plasma kinetics of Gramoxone 200 G/L SL formulation A3879D in dogs is classified **Acceptable/Non-Guideline** and does not satisfy the 85-1 Guideline Requirement for a metabolism study [OPPTS 870.7485, OECD 417]. It was neither designed nor submitted as a guideline study.

COMPLIANCE: Signed GLP, Data Confidentiality Claim, and Quality Assurance statements were provided in the study report.

I. MATERIALS AND METHODS:

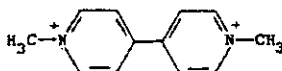
A. MATERIALS:

1. Test compound: Gramoxone 200 GL SL formulation (A3879D)

Radiolabelled test material:	Not used
Radiochemical purity	NA
Specific Activity	NA
Lot/Batch #:	NA

Non-Radiolabelled test material:

Description:	Dark green liquid
Lot/Batch #:	BSN311030 (CTL ref. no. Y12693/074)
Purity:	195 g/L paraquat
Contaminants:	None noted
CAS # of TGAI:	4685-14-7
Structure:	



2. Vehicle and/or positive control: None noted.

3. Test animals:

Species:	Dog; male								
Strain:	Beagle								
Age/weight at study initiation:	~10 months; 10.7-12.2 kg								
Source:	Conventional Animal Breeding Unit, Alderley Park, Macclesfield, UK								
Housing:	Adjacent pens, 3/pen except on treatment days when they were housed individually for 6-7 hrs for observation								
Diet:	350 g Laboratory Diet A (Special Diet Services Ltd., Stepfield, Witham, Essex, UK) daily								
Water:	Tap water <i>ad libitum</i>								
Environmental conditions:	<table border="0"> <tr> <td>Temperature:</td> <td>19±2°C</td> </tr> <tr> <td>Humidity:</td> <td>45-65% (elevation to 66-70% on several recorded on several occasions)</td> </tr> <tr> <td>Air changes:</td> <td>15/hr</td> </tr> <tr> <td>Photoperiod:</td> <td>12 hrs/12 hrs</td> </tr> </table>	Temperature:	19±2°C	Humidity:	45-65% (elevation to 66-70% on several recorded on several occasions)	Air changes:	15/hr	Photoperiod:	12 hrs/12 hrs
Temperature:	19±2°C								
Humidity:	45-65% (elevation to 66-70% on several recorded on several occasions)								
Air changes:	15/hr								
Photoperiod:	12 hrs/12 hrs								
Acclimation period:	4 Weeks								

4. **Dose preparations:** Amounts of A3879D required to achieve the target doses of paraquat ion (Table 1) were placed into gelatine capsules. The amount of test material was calculated as:

$$\text{mg formulation/kg} = \frac{(\text{dose volume [0.04 ml]} \times \text{specific gravity [1.074]}) \times 1000}{\text{weight (kg)}}$$

The capsules were filled immediately prior to dosing and placed in a second capsule to minimize contamination with oral/esophageal secretions.

Analysis of the dose preparations prior to dosing by Jealotts Hill International confirmed the paraquat ion and emetic (PP796) concentrations.

B. STUDY DESIGN AND METHODS:

1. **Group arrangements:** The experimental groups are shown in Table 1. Feed consumption was recorded (mean g feed/dog/day) for at least one week prior to treatment and throughout the 2-week treatment period.

TABLE 1. Experimental protocol for toxicokinetic study in male beagle dogs given a single oral dose of Paraquat 200 G/L SL Formulation (A3879D)	
A3879D dose (mg/kg) (mg paraquat ion/kg)	Comments
43 (8)	3 dogs each received a single dose (orally in gelatin capsules); dogs fed at 4 hrs post dose and observed for 6-7 hrs

Data taken from p 16, MRID 46364511.

2. **Dosing and sample collection/preparation/analysis:** The dogs were dosed with the gelatine capsules (as described in §I.A.4) at approximately 9:00 a.m. and fed four hours later. The dogs were weighed weekly prior to feeding, on day 1 of treatment and at weekly intervals thereafter. Clinical observations were conducted on the dogs for at least four hours following dosing and hourly thereafter. On non-dosing days, the dogs were observed at least twice daily for signs of toxicity or abnormal behavior.

Blood samples (from the jugular vein) were collected in heparinized tubes prior to feeding on the day prior to dosing. The following clinical chemistry parameters were assessed:

PARAQUAT 240 G/L FORMULATION (A7813K)

Electrolytes:		Other:	
<input checked="" type="checkbox"/>	Calcium	<input checked="" type="checkbox"/>	Albumin
<input checked="" type="checkbox"/>	Chloride	<input checked="" type="checkbox"/>	Blood creatinine
<input type="checkbox"/>	Magnesium	<input checked="" type="checkbox"/>	Blood urea nitrogen
<input checked="" type="checkbox"/>	Phosphorus	<input checked="" type="checkbox"/>	Cholesterol
<input checked="" type="checkbox"/>	Potassium	<input checked="" type="checkbox"/>	Globulins
<input checked="" type="checkbox"/>	Sodium	<input checked="" type="checkbox"/>	Glucose
Enzymes		<input checked="" type="checkbox"/>	Total bilirubin
<input checked="" type="checkbox"/>	Alkaline phosphatase (ALK)	<input checked="" type="checkbox"/>	Total serum protein (TP)
<input type="checkbox"/>	Cholinesterase (ChE)	<input checked="" type="checkbox"/>	Triglycerides
<input checked="" type="checkbox"/>	Creatinine phosphokinase	<input type="checkbox"/>	Serum protein electrophoresis
<input type="checkbox"/>	Lactic acid dehydrogenase (LDH)	<input checked="" type="checkbox"/>	A/G Ratio
<input checked="" type="checkbox"/>	Serum alanine aminotransferase (also SGPT)	<input type="checkbox"/>	
<input checked="" type="checkbox"/>	Serum aspartate aminotransferase (also SGOT)	<input type="checkbox"/>	
<input type="checkbox"/>	Gamma glutamyl transferase (GGT)	<input type="checkbox"/>	
<input checked="" type="checkbox"/>	Glutamate dehydrogenase	<input type="checkbox"/>	

Blood samples were also taken for determination of the blood kinetic parameters of the test article and the emetic agent. For each dose, 2 ml blood samples were collected (in lithium heparin) on the day of dosing, 15 min, 30 minutes, and 1, 2, 4, 7, 12, and 24 hours post dose. Blood samples were centrifuged and subjected to spectrophotometric and fluorescence HPLC analysis.

3. **Analytical techniques:**

Second derivative spectrophotometric analysis: Plasma paraquat was determined by passing an aliquot (50-300 µl) of plasma through an ANSYSS SPEC PLUS PT SI cartridge. The cartridge was rinsed with HCl and the eluent collected. Dithionite reagent in NaOH was then passed through the cartridge and the eluent collected in the same cuvette as the HCl rinse. Second order derivative spectra (360-44 nm) were determined relative to a reagent blank using a Unicam UV1 spectrophotometer. The paraquat concentration was determined by reference to a standard curve for 0-10µg paraquat/ml plasma. The limit of quantification (LOQ) was 0.1 µg/ml.

Fluorescence HPLC: For plasma samples containing less than 0.1 µg paraquat/ml, fluorescence HPLC was used. Plasma samples (200 µl) were derivatized with 1% potassium ferricyanide in 9M NaOH and extracted in chloroform. The chloroform extractions were processed in silica cartridges and acetonitrile. The resulting paraquat dipyrindrone was eluted and analyzed by HPLC (Inertsil Phenyl-3 5 µ column); flow rate was 1 ml/min using a mobile phase of 30% acetonitrile and 70% water, and fluorescence detection. The amount of paraquat in each sample was determined by comparison to a standard curve (0-0.1 µg/ml). The LOQ was 10 ng/ml.

4. **Storage stability:** Recommended storage conditions were provided but no other data were available regarding stability. Dosing formulations were prepared immediately prior to administration.

5. **Calculations/ statistical analysis:** Dose calculations and quantitative information regarding dose formulations were provided. Data were expressed as mean \pm standard deviation.

II. **RESULTS:**

- A. **CLINICAL OBSERVATIONS:** Scheduled veterinary examinations revealed no abnormalities. With the exception of vomiting, no clinical signs could be attributed directly to the treatment.

1. **Emesis:** The most notable finding was emesis (brown liquid) in one dog which occurred at 1 hour 35 minutes post dose. This was followed by a second emesis (thick frothy, cream-colored vomitus) at 3 hours 20 minutes post dose. Neither of the other dogs vomited.
2. **Bodyweight:** Body weight of the three dogs was not significantly affected by the treatment. Body weights at Day -6 were 10.7, 10.7, and 12.2 kg and at Day 15 were 10.8, 10.8, and 12.0 kg.
3. **Food consumption:** Feed consumption was not significantly affected by the treatment. Daily feed consumption ranged from 230-350 g/dog/day.
4. **Clinical chemistry:** There were no treatment-related effects on clinical chemistry parameters.

- B. **TOXICOKINETICS:** Concentration-time course data for plasma paraquat are shown in Table 2. Peak plasma levels occurred at 1-2 hours but considerable variability in the concentration-time course was observed among the three dogs. The time-course data did, however, show that paraquat was rapidly eliminated to nearly undetectable levels by 12-24 hours post dose.

TABLE 2. Plasma paraquat concentration-time ($\mu\text{g/ml}$) course in dogs given a single oral dose of 43 mg/kg (equivalent to 8 mg paraquat ion/kg).				
Time (hrs)	Animal No.			Mean \pm SD
	1	2	3	
Pre-dose	0.00	0.00	0.00	0.00 \pm 0.00
0.25	0.04	0.08	0.04	0.06 \pm 0.02
0.5	1.88	0.35	0.08	0.77 \pm 0.97
1	4.22	0.99	1.83	2.34 \pm 1.67
2	1.60	1.14	3.37	2.04 \pm 1.18
4	0.41	0.51	0.63	0.52 \pm 0.11
7	0.19	0.07	0.10	0.12 \pm 0.06
12	0.23	0.04	0.08	0.12 \pm 0.10
24	0.04	0.01	0.00	0.02 \pm 0.02

Data taken from Table 7, p. 36, MRID 46364511

The time course data for the emetic component in the Gramoxone formulation are shown in Table 3. Peak plasma concentration was achieved at one-half hours for one dog, one hour for another, and at two hours for the third. Although the plasma concentration-time course was variable during the first two hours, the emetic component was nearly completely cleared from the plasma by seven hours after dosing.

TABLE 2. Emetic concentration-time course (ng/ml) in plasma of dogs given a single oral dose of 43 mg/kg (equivalent to 8 mg paraquat ion/kg).				
Time (hrs)	Animal No.			Mean \pm SD
	1	2	3	
Pre-dose	0.00	0.00	0.00	0.00 \pm 0.00
0.25	0.28	0.23	0.01	0.17 \pm 0.14
0.5	0.98	0.50	0.17	0.55 \pm 0.41
1	0.73	0.57	0.65	0.65 \pm 0.08
2	0.39	1.12	0.57	0.69 \pm 0.38
4	0.15	0.29	0.22	0.22 \pm 0.07
7	0.00	0.00	0.04	0.02 \pm 0.02
12	0.01	0.01	0.02	0.01 \pm 0.00
24	0.00	0.00	0.00	0.00 \pm 0.00

Data taken from Table 8, p. 37, MRID 46364511

Plasma paraquat AUC values at 1, 4, and 24 hours and rate of absorption at 15 minutes for the three dogs are shown in Table 4 and kinetics data for the emetic are shown in Table 5. Consistent with the variability observed for plasma concentrations, the plasma AUC values were also variable among the three dogs (4-fold at the 1-hr time point and ~2-fold at the 12 and 24 hour time points).

TABLE 4. Plasma kinetics for paraquat ion in dogs following administration of a single oral dose of 43 mg Gramoxone/kg (equivalent to 8 mg paraquat ion/kg).				
Animal No.	Absorption rate @ 15 min $\mu\text{g/ml/min}$	AUC ($\mu\text{g/ml}\cdot\text{hr}$)		
		1 hr	4 hrs	24 hrs
1	2.79	1.77	6.69	10.26
2	5.59	0.40	3.11	4.56
3	2.79	0.50	7.10	9.12
Mean \pm SD	3.73 \pm 1.61	0.89 \pm 0.76	5.63 \pm 2.19	7.98 \pm 3.02

Data taken from Table 5, p. 34, MRID 46364511.

The AUC values for the emetic (Table 5) in the Gramoxone formulation were also variable (up to ~3-fold), especially at the 1-hour time point. Similar to paraquat kinetics, the AUC variability was a function of the variable plasma absorption and elimination.

TABLE 4. Plasma kinetics for emetic in dogs following administration of a single oral dose of 43 mg Gramoxone/kg (equivalent to 8 mg paraquat ion/kg).				
Animal No.	Absorption rate @ 15 min ng/ml/min	AUC ($\mu\text{g/ml}\cdot\text{hr}$)		
		1 hr	4 hrs	24 hrs
1	0.019	0.62	1.72	2.03
2	0.015	0.39	2.64	3.16
3	0.001	0.23	1.63	2.29
Mean \pm SD	0.012 \pm 0.010	0.41 \pm 0.20	2.00 \pm 0.55	2.49 \pm 0.60

Data taken from Table 6, p. 35, MRID 46364511.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS

The investigators concluded that at the dose tested, dogs exhibited no significant signs of toxicity. Emesis in only one of three dogs suggested that the PP796 emetic levels in plasma were insufficient to induce prompt emesis following the single 43 mg Gramoxone/kg dose. Paraquat plasma levels following administration of the Gramoxone 200 G/L SL formulation were variable; possibly the result of one dog inasmuch as the AUC value for the other two dogs were similar to those observed in earlier studies. It was hypothesized that the variability may have been due to possible variations in capsule disintegration rate between the current and earlier study. The investigators concluded that, overall, the kinetics observed in this study were similar to those reported earlier.

- B. REVIEWER COMMENTS: A non-guideline study (MRID 46364511) was conducted to compare plasma kinetic data for Gramoxone 200 G/L SL formulation A3879D in dogs to data from an earlier study. Specifically, three male beagle dogs were given a single 43 mg/kg oral dose (via gelatin capsule) of Gramoxone 200 G/L SL formulation A3879D (195 g/L paraquat; 1.5 g/L and non-specified amount of PP796 emetic; Batch No. BSN3I1030). Plasma kinetics (concentration-time course), rate of absorption, and AUC parameters were determined for the paraquat ion and the emetic.

This was a cursory study designed to evaluate the plasma kinetics of a paraquat formulation in dogs following a single 43 mg/kg oral dose and to compare the results to data acquired from an earlier study. The study protocol and analytical techniques were well described. At the dose tested, Gramoxone was not overtly toxic to the dogs. Only one of three dogs exhibited emesis (to be expected from the emetic-containing Gramoxone). Plasma concentration-time course data for both the paraquat ion and the PP796 emetic were variable (2-4 fold) among the three dogs, although near-complete elimination from the plasma occurred within 7 hours for the emetic, and at 12-24 hours for the paraquat ion. AUC values for both components were also variable due to the plasma kinetics. Most variability could be attributed to one dog. The reviewer concurs with the conclusions of the investigators.

This study (MRID 46364511) on the toxicity and plasma kinetics of Gramoxone 200 G/L SL formulation A3879D in dogs is classified **Acceptable/Non-Guideline** and does not satisfy the 85-1 Guideline Requirement for a metabolism study [OPPTS 870.7485, OECD 417]. The study was neither designed nor submitted as a guideline study.

C. STUDY DEFICIENCIES: There were no apparent deficiencies in the study.

DATA EVALUATION RECORD

PARAQUAT (A3879BU)
STUDY TYPE: TOXICOKINETICS - DOG
[NON-GUIDELINE]
MRID 46364517

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 94-2005

Primary Reviewer:

Robert A. Young, Ph.D., D.A.B.T.

Signature:

Date:

Robert A. Young
7-5-05

Secondary Reviewers:

H.T. Borges, Ph.D., MT (ASCP), D.A.B.T.

Signature:

Date:

H.T. Borges
7-5-05

Robert H. Ross, M.S., Group Leader

Signature:

Date:

Robert H. Ross
7-5-05

Quality Assurance:

LeeAnn Wilson, M.A.

Signature:

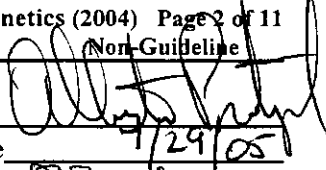
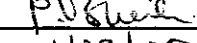
Date:

L.A. Wilson
7-5-05

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

EPA Reviewer: A. Protzel, Ph.D.
Toxicology Branch, Health Effects Division (7509C)
EPA Work Assignment Manager: P.V. Shah, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C)

Signature: 
Date: 7/29/05
Signature: 
Date: 7/29/05
Template version 11/01

TXR#: 0052955

DATA EVALUATION RECORD

STUDY TYPE: Toxicokinetics - dog [Non-guideline].

PC CODE: 061601

DP BARCODE: 309472
SUBMISSION NO.: NA

TEST MATERIAL (PURITY): (Paraquat 200 G/L SL Formulation [A3879BU]; 20.3% a.i. w/w)

SYNONYMS:

CITATION: Brammer, A. (2004). Paraquat 200 G/L SL Formulation (A3879BU): Toxicokinetic study in the dog. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. CTL No. XD7201. January 7, 2004. MRID 46364517. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, P. O. Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY:

A non-guideline study (MRID 46364517) was conducted in which three male beagle dogs were given incremental doses (via gelatin capsule) of Paraquat 200 g/L formulation A3879BU (203 g/L paraquat; 1.56 g/L PP796 emetic; Lot No. J6481/016). Doses were 46, 92, 184, 368, and 736 mg/kg (equivalent to 8, 16, 32, 64, and 128 mg paraquat ion/kg, respectively) given at 1, 5, 9, 13, and 18 weeks. Plasma kinetics (concentration-time course), rate of absorption and AUC parameters were determined. Clinical observations (emesis response and general observations), clinical chemistry, gross pathology, and histopathology of selected organs/tissues were assessed.

The paraquat A3879BU dosing regimen produced signs of toxicity only at the highest dose and primarily in one dog. Peak plasma levels (2.57, 2.00, 3.07, 1.94, and 8.21 for the low to high doses) occurred at 0.5 to 1 hour, tended to occur earlier at higher doses, and did not exhibit a quantitative dose-response. Moderate individual variability was observed among the three dogs (generally 2-3 fold differences). The time-course data showed that the paraquat ion was almost completely eliminated within 24 hours after each dose. Peak plasma concentration of the emetic agent (PP796) occurred at 0.5 to 1 hour. The dose relationship was inconsistent at the 368 mg/kg dose (notably lower plasma emetic concentration) due to compromised absorption of the test article in one dog. The plasma levels at time points up to 2 hours tended to show a dose response but the 368 mg/kg dose varied somewhat from this pattern. Although the plasma concentration-

time course was variable, the emetic component was nearly completely cleared from the plasma by 24 hours after dosing. At a given time point, the paraquat ion AUC values were similar for all doses except the highest, thereby indicating that the PP796-induced emesis was limiting the systemic dose of the paraquat ion. At the highest dose, the plasma paraquat ion concentration was approaching known toxic levels as demonstrated by the effects in one dog of this dose group.

This is a cursory study designed to examine the effectiveness of a novel paraquat formulation intended to limit accumulation of the toxic paraquat ion by inducing emesis in a non-target species. Although minor problems were noted (primarily due to one of three dogs), the study provided preliminary data indicating the effectiveness of the novel formulation.

A non-guideline study (MRID 46364517) was conducted to compare plasma kinetic data for Paraquate 200 G/L formulation A3879BU in dogs with Gramoxone, a commercial standard product. This study (MRID 46364517) on the toxicity and plasma kinetics of Paraquat 200 G/L SL formulation A3879BU in dogs is classified **Acceptable/Non-Guideline** and does not satisfy the 85-1 Guideline Requirement for a metabolism study [OPPTS 870.7485, OECD 417].

COMPLIANCE: Signed GLP, Data Confidentiality Claim, and Quality Assurance statements were provided in the study report.

I. MATERIALS AND METHODS:

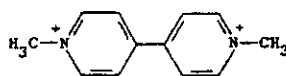
A. MATERIALS:

1. Test compound: Paraquat 200 GL SL formulation (A3879BU)

Radiolabelled test material:	not used
Radiochemical purity	NA
Specific Activity	NA
Lot/Batch #:	NA

Non-Radiolabelled test material:

Description:	dark green liquid
Lot/Batch #:	J6481/016 (CTL ref. no. Y00061/947)
Purity:	20.3% (203 g/L paraquat; 1.56 g/L PP796 emetic)
Contaminants:	none noted
CAS # of TGAI:	4685-14-7
Structure:	



2. Vehicle and/or positive control: None noted.

3. Test animals:

Species:	dog; male
Strain:	beagle
Age/weight at study initiation:	39-41 weeks; 11-13.2 kg

Paraquat 200 G/L SL Formulation (A3879BU)

Source: Conventional Animal Breeding Unit, Alderley Park, Macclesfield, UK
Housing: adjacent pens, 3/pen except on treatment days when they were housed individually for 6-7 hrs for observation
Diet: 350 g Laboratory Diet A (Special Diet Services Ltd., Stepfield, Witham, Essex, UK) daily
Water: tap water *ad libitum*
Environmental conditions: **Temperature:** 19±2°C (dropped to 16° on 2 days and increased to 24° for 2 days)
Humidity: 45-65% (elevation to 67-78% on several recorded on several occasions)
Air changes: 15/hr
Photoperiod: 12 hrs/12 hrs
Acclimation period: at least 2 weeks

4. **Dose preparations:** Amounts of A3879BU required to achieve the target doses of paraquat ion (Table 1) were placed into gelatine capsules. The amount of test material was calculated as:

$$\text{mg formulation/kg} = \frac{(\text{dose volume [ml]} \times \text{specific gravity [1.15]}) \times 1000}{\text{weight (kg)}}$$

The capsules were filled immediately prior to dosing.

Analysis of the dose preparations prior to dosing by Jealotts Hill International confirmed the paraquat ion and emetic agent (PP796) concentrations.

B. STUDY DESIGN AND METHODS:

1. **Group arrangements:** The experimental groups are shown in Table 1. All three dogs were given incremental doses of the test article as indicated in Table 1. Feed consumption was recorded (mean g feed/dog/day) for at least one week prior to treatment and throughout the 2-week treatment period.

Table 1. Experimental protocol for toxicokinetic study in male beagle dogs given a single oral dose of Paraquat 200 G/L SL Formulation (A3879BU)			
Dose	A3879BU dose (mg/kg) (mg paraquat ion/kg)	Week	Comments
1	46 (8)	1	All dogs dosed at approximately the same time of day and fed approximately 4 hours after dosing. Treatment commenced on May 13, 2003; dogs were terminated (overdose of sodium pentobarbitone) September 22, 2003. Blood kinetic parameters (AUC; conc.-time course) determined for all dogs. Dose volumes ranged from 0.01-0.64 ml/kg.
2	92 (16)	5	
3	184 (32)	9	
4	368 (64)	13	
5	736 (128)	18	

Data taken from p 18 and 22, MRID 46364517.

2. Dosing and sample collection/preparation/analysis:

The dogs were dosed with the gelatine capsules (as described in §I.A.4) and fed four hours later. The dogs were weighed weekly prior to feeding, on day 1 of treatment and at weekly intervals thereafter. Clinical observations (including ophthalmoscopy) were conducted on the dogs for at least four hours following dosing and hourly thereafter. On non-dosing days, the dogs were observed at least twice daily for signs of toxicity or abnormal behavior.

Blood samples (from the jugular vein) were collected in lithium heparinized tubes prior to feeding and at 24 hours post dose. Additional blood samples were taken at 3 and 6 days after the 5th dose. The following clinical chemistry parameters were assessed:

Electrolytes:	Other:
<input checked="" type="checkbox"/> Calcium	<input checked="" type="checkbox"/> Albumin
<input checked="" type="checkbox"/> Chloride	<input checked="" type="checkbox"/> Blood creatinine
<input type="checkbox"/> Magnesium	<input checked="" type="checkbox"/> Blood urea nitrogen
<input checked="" type="checkbox"/> Phosphorus	<input checked="" type="checkbox"/> Cholesterol
<input checked="" type="checkbox"/> Potassium	<input checked="" type="checkbox"/> Globulins
<input checked="" type="checkbox"/> Sodium	<input checked="" type="checkbox"/> Glucose
Enzymes	<input checked="" type="checkbox"/> Total bilirubin
<input checked="" type="checkbox"/> Alkaline phosphatase (ALK)	<input checked="" type="checkbox"/> Total serum protein (TP)
<input type="checkbox"/> Cholinesterase (ChE)	<input checked="" type="checkbox"/> Triglycerides
<input checked="" type="checkbox"/> Creatinine phosphokinase	<input type="checkbox"/> Serum protein electrophoresis
<input type="checkbox"/> Lactic acid dehydrogenase (LDH)	<input checked="" type="checkbox"/> A/G ratio
<input checked="" type="checkbox"/> Serum alanine aminotransferase (also SGPT)	
<input checked="" type="checkbox"/> Serum aspartate aminotransferase (also SGOT)	
<input type="checkbox"/> Gamma glutamyl transferase (GGT)	
<input checked="" type="checkbox"/> Glutamate dehydrogenase	

Blood samples were also taken for determination of the blood kinetic parameters (AUC_{0-1} , AUC_{0-4} , AUC_{0-24} , and conc.-time course for the paraquat ion and emetic agent). For each dose, 2 ml blood samples were collected (in lithium heparin) on the day of dosing, 15 min, 30 minutes, and 1, 2, 4, 7, 12, and 24 hours post dose. Blood samples were centrifuged and subjected to spectrophotometric and fluorescence HPLC analysis (for analysis of paraquat and emetic agent (PP796)).

At termination, the following tissues were examined *in situ*, removed and fixed for examination by light microscopy: any abnormal tissues, heart, lungs, kidney, duodenum, ileum, jejunum, stomach, and liver

3. Analytical techniques:**Second derivative spectrophotometric analysis:**

Plasma paraquat was determined by passing an aliquot (50-300 μ l) of plasma through an ANSYSS SPEC PLUS PT SI cartridge. The cartridge was rinsed with HCl and the eluent collected. Dithionite reagent in NaOH was then passed through the cartridge and the eluent collected in the same cuvette as the HCl rinse. Second order derivative spectra (360-44 nm)

were determined relative to a reagent blank using a Unicam UV1 spectrophotometer. The paraquat concentration was determined by reference to a standard curve for 0-10µg paraquat/ml plasma. The limit of quantification (LOQ) was 0.1 µg/ml.

Fluorescence HPLC:

For plasma samples containing less than 0.1 µg paraquat/ml, fluorescence HPLC was used. Plasma samples (200 µl) were derivatized with 1% potassium ferricyanide in 9M NaOH and extracted in chloroform. The chloroform extractions were processed in silica cartridges and acetonitrile. The resulting paraquat dipyridone was eluted and analyzed by HPLC (Inertsil Phenyl-3 5 µ column); flow rate was 1 ml/min using a mobile phase of 30% acetonitrile and 70% water, and fluorescence detection. The amount of paraquat in each sample was determined by comparison to a standard curve (0-0.1 µg/ml). The LOQ was 10 ng/ml.

4. Storage stability:

Recommended storage conditions were provided but no other data were available regarding stability. Dosing formulations were prepared immediately prior to administration.

5. Calculations/ statistical analysis:

Dose calculations and quantitative information regarding dose formulations were provided. Data were expressed as mean ± standard deviation.

II. RESULTS:

A. CLINICAL OBSERVATIONS

Scheduled veterinary examinations revealed no abnormalities. Vomiting was the most notable clinical sign. The dogs also exhibited slightly decreased activity, restlessness and/or excessive salivation (at the 184-768 mg/kg doses). At the highest dose, these effects were more severe and persistent (retching and/or vomiting up to 3 hours post dose).

1. Emesis

The most notable (and expected) finding was emesis, the duration and severity (i.e., quantity of vomitus) of which increased with dose (Table 2). Time to emesis decreased with dose. No additional effects were observed following the cessation of vomiting.

Table 2. Time (min) to emesis and duration of emesis in male dogs following oral dosing with Paraquat A7813K				
Dose (mg formulation/kg) [mg paraquat ion/kg]	Male 1	Male 2	Male 3	Mean*
	Time to first emesis			
46 [8]	68	36	50	51.3±16.0
92 [16]	41	28	37	35.3±6.7
184 [32]	31	23	24	26.0±4.4

368 [64]	29	44	21	31.3±11.7
736 [128]	23	16	22	20.3±3.8
	Time to last emesis			
46 [8]	88	66	67	73.7±12.4
92 [16]	95	42	46	61.0±29.5
184 [32]	72	101	60	77.7±21.1
368 [64]	65	77	59	67.0±9.2
736 [128]	127	75	133	111.7±45.1

^a Mean ±SD (calculated by reviewer)

Data taken from p. 22, MRID 46364517.

2. Bodyweight

Body weight of the three dogs was generally not significantly affected by the treatment; minor weight fluctuations were recorded throughout the study period. The most severe weight loss appeared to be dog no.3 which lost ~0.9 kg from Week 16 to Week 19.1. Body weights at Week-1 were 13.0, 11.2, and 12.3 kg and at termination were 13.1, 12.2, and 12.0 kg.

3. Food consumption

Feed consumption was not significantly affected by the treatment although dog no. 3 exhibited decreased feed consumption (221 g/day) during week 18. Daily feed consumption ranged from 221-350 g/dog/day.

4. Clinical chemistry

There were no treatment-related effects on clinical chemistry parameters.

5. Gross pathology/histopathology

One dog exhibited dark spots on two lobes of the lung which upon histopathologic examination were characterized by slight interstitial fibrosis, focal alveolar macrophage infiltration and slight focal pneumocyte hypertrophy. Minimal medullary calcifications were also noted in the kidneys of two dogs.

B. TOXICOKINETICS

Concentration-time course data for plasma paraquat are shown in Table 3. Peak plasma levels (2.57, 2.00, 3.07, 1.94, and 8.21 for the low to high doses) occurred at 0.5 to 1 hour, tended to occur earlier at higher doses, and did not exhibit a quantitative dose-response. Moderate individual variability was observed among the three dogs (generally 2-3 fold differences). The time-course data showed that paraquat was eliminated to nearly undetectable levels within 24 hours for all doses.

Table 3. Plasma paraquat concentration-time ($\mu\text{g/ml}$) course in dogs given sequential oral dose of paraquat A3879BU ^a .					
Time (hrs)	Dose (mg A3879BU/kg)				
	46	92	184	368	736
Pre-dose	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
0.25	0.28±0.16	0.13±0.15	0.60±0.27	0.92±0.94	3.31±2.99
0.5	0.74±0.16	1.24±0.58	1.97±0.81	1.94±1.51	8.21±3.65
1	2.57±0.55	2.00±1.18	3.07±0.78	1.90±0.21	5.23±2.16
2	1.64±0.27	1.04±0.29	1.44±0.29	1.36±0.57	2.53±0.87
4	0.59±0.26	0.47±0.25	0.67±0.44	0.50±0.10	0.67±0.44
7	0.12±0.05	0.19±0.17	0.15±0.04	0.12±0.04	0.17±0.12
12	0.02±0.01	0.10±0.12	0.08±0.06	0.04±0.03	0.05±0.02
24	0.01±0.00	0.01±0.00	0.03±0.02	0.01±0.00	0.03±0.00
72	-	-	-	-	0.01±0.00
144	-	-	-	-	0.00±0.00

^a Mean \pm SD of three dogs

Data taken from Appendix F, pp. 64-65, MRID 46364517.

The time course data for the emetic component in the paraquat A3879BU formulation are shown in Table 4. Peak plasma concentration for the PP796 emetic occurred at 0.5 to 1 hour with no apparent relation to dose. Plasma levels were highly variable within each time frame and dose, due primarily to one dog with substantially lower levels (up to 19-fold lower) at early time points. The plasma levels at time points up to 2 hours tended to show a dose response. The 368 mg/kg dose varied somewhat from this pattern, however. Although the plasma concentration-time course was variable, the emetic component was nearly completely cleared from the plasma by 24 hours after dosing.

Time (hrs)	Dose (mg A3879BU/kg)				
	46	92	184	368	736
Pre-dose	0.13 \pm 0.03	0.00 \pm 0.00	0.00 \pm 0.00	0.39 \pm 0.08	0.00 \pm 0.00
0.25	0.27 \pm 0.05	1.59 \pm 1.07	1.86 \pm 0.28	1.68 \pm 1.15	3.98 \pm 3.53
0.5	2.07 \pm 0.43	4.48 \pm 1.94	8.42 \pm 0.97	6.29 \pm 5.08	10.04 \pm 7.60
1	2.82 \pm 0.30	4.24 \pm 2.62	7.09 \pm 1.87	6.45 \pm 1.53	8.39 \pm 4.01
2	2.14 \pm 0.51	2.70 \pm 0.97	4.47 \pm 0.88	3.93 \pm 0.84	5.50 \pm 2.36
4	1.07 \pm 0.15	0.91 \pm 0.22	1.99 \pm 0.05	1.79 \pm 0.07	2.15 \pm 0.86
7	0.29 \pm 0.05	0.40 \pm 0.45	0.40 \pm 0.06	0.70 \pm 0.04	0.38 \pm 0.18
12	0.19 \pm 0.04	0.00 \pm 0.00	0.08 \pm 0.02	0.42 \pm 0.18	0.08 \pm 0.08
24	0.09 \pm 0.03	0.00 \pm 0.00	0.04 \pm 0.04	0.44 \pm 0.05	0.00 \pm 0.00
72	-	-	-	-	ND
144	-	-	-	-	ND

^a Mean \pm SD of three dogs

Data taken from Appendix G, pp. 66-67, MRID 46364517.

Plasma paraquat AUC values at 1, 4, and 24 hours and rate of absorption at 15 minutes for the three dogs are shown in Table 5 and kinetics data for the emetic are shown in Table 6. AUC values were reflective of early, rapid absorption and a relatively uniform overall systemic dose at all doses except the highest. The AUC value for the 368 mg/kg dose was unexpectedly low.

Dose (mg A3879BU/kg)	Absorption rate @ 15 min ng/ml/min	AUC ($\mu\text{g/ml}\cdot\text{hr}$)		
		1 hr	4 hrs	24 hrs
46	18.60 \pm 6.20	0.99 \pm 0.11	5.34 \pm 0.58	6.94 \pm 0.91
92	8.91 \pm 5.89	1.00 \pm 0.31	4.03 \pm 0.93	6.38 \pm 1.99
184	40.08 \pm 10.20	1.66 \pm 0.29	6.01 \pm 0.59	8.51 \pm 0.94
368	61.22 \pm 36.00	1.43 \pm 0.41	4.93 \pm 0.19	6.62 \pm 0.20
736	220.65 \pm 115.18	5.21 \pm 0.81	12.30 \pm 2.19	14.60 \pm 2.97

^a Values are mean \pm SD of three dogs/dose

Data taken from Table 6, p. 50, MRID 46364517.

The AUC values for the emetic (Table 6) in the paraquat formulation exhibited a dose-related

increase with the exception of the 368 mg/kg dose where the investigators noted a possible absorption deficiency for one dog. Absorption rates at 15 minutes exhibited a similar relationship.

Dose (mg A3879BU/kg)	Absorption rate @ 15 min $\mu\text{g/ml/min}$	AUC ($\mu\text{g/ml}\cdot\text{hr}$)		
		1 hr	4 hrs	24 hrs
46	0.009 \pm 0.002	1.56 \pm 0.06	7.26 \pm 0.41	12.18 \pm 0.35
92	0.106 \pm 0.041	3.14 \pm 0.73	10.22 \pm 2.08	13.19 \pm 3.28
184	0.124 \pm 0.011	5.40 \pm 0.51	17.64 \pm 1.80	23.15 \pm 1.90
368	0.086 \pm 0.044	4.44 \pm 1.47	15.35 \pm 2.62	27.10 \pm 2.01
736	0.265 \pm 0.136	6.86 \pm 2.61	21.45 \pm 6.30	26.87 \pm 7.02

^a Values are mean \pm SD of three dogs/dose
Data taken from Table 7, p. 51, MRID 46364517.

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS

The investigators concluded that at the dose regimen tested, dogs exhibited minimal to no toxic responses. Due to the emetic effect of the PP796, the plasma paraquat levels remained low. The maximum plasma concentration of $\sim 12 \mu\text{g/ml}$ occurred in one dog of the 736 mg/kg dose. Plasma paraquat levels and AUC values for the 46-368 mg/kg doses were indicative of the effectiveness of the emetic agent in limiting systemic dose of the paraquat ion. At the highest dose (736 mg/kg), mean blood levels reached $10 \mu\text{g/ml}$ (the plasma level known to induce notable toxicity and inducing minor toxicity in this study) but overt toxic effects were transient with their reduction correlating with a decreased plasma paraquat ion concentration. Pulmonary lesions (consistent with paraquat toxicity) were observed in the one dog with the $12 \mu\text{g/ml}$ plasma level. Peak plasma concentrations of the emetic agent were attained at 0.5 to 1 hour and were apparently sufficient to allow for minimizing the plasma paraquat levels. The plasma profile for the emetic exhibited an inconsistent dose relationship due primarily to the response of one dog for which emetic absorption was reportedly compromised by ingestion of feces. When compared with previously available paraquat formulations (i.e., Gramoxone), the gelling agent and emetic in the A3879BU formulation appear to allow for proper absorption of the emetic while somewhat limiting paraquat absorption, thereby reducing paraquat-induced toxicity.

B. REVIEWER COMMENTS:

A non-guideline study (MRID 46364517) was conducted in which three male beagle dogs were given incremental doses (via gelatin capsule) of Paraquat 200 g/L formulation A7813BU (203 g/L paraquat; 1.56 g/L PP796 emetic; Lot No. J6481/016). Doses were 46,

92, 184, 368, and 736 mg/kg (equivalent to 8, 16, 32, 64, and 128 mg paraquat ion/kg, respectively) given at 1, 5, 9, 13, and 18 weeks. Plasma kinetics (concentration-time course), rate of absorption and AUC parameters were determined. Clinical observations (emesis response and general observations), clinical chemistry, gross pathology, and histopathology of selected organs/tissues were assessed.

The paraquat A3879BU dosing regimen produced signs of toxicity only at the highest dose and primarily in one dog. Peak plasma levels (2.57, 2.00, 3.07, 1.94, and 8.21 for the low to high doses) occurred at 0.5 to 1 hour, tended to occur earlier at higher doses, and did not exhibit a quantitative dose-response. Moderate individual variability was observed among the three dogs (generally 2-3 fold differences). The time-course data showed that paraquat was eliminated to nearly undetectable levels within 24 hours for all doses. Peak plasma concentration of the emetic agent (PP796) occurred at 0.5 to 1 hour. The dose relationship was inconsistent at the 368 mg/kg dose due to one dog exhibiting lower plasma emetic concentrations (up to 19-fold at early time points) due to compromised absorption of the test article. The plasma levels at time points up to 2 hours tended to show a dose response. The 368 mg/kg dose varied somewhat from this pattern, however. Although the plasma concentration-time course was variable, the emetic component was nearly completely cleared from the plasma by 24 hours after dosing. At a given time point, the paraquat ion AUC values were similar for all doses except the highest, thereby indicating that the PP796-induced emesis was limiting the systemic dose of the paraquat ion. At the highest dose, the plasma paraquat ion concentration was approaching known toxic levels as demonstrated by the effects in one dog of this dose group.

This is a cursory study designed to examine the effectiveness of a novel paraquat formulation intended to limit accumulation of toxic paraquat ion by inducing emesis in a non-target species. Although minor problems were noted (primarily due to minor toxicity in one of three dogs), the study provided preliminary data indicating the effectiveness of the novel formulation.

This study (MRID 46364517) on the toxicity and plasma kinetics of Paraquat 200 G/L SL formulation A3879BU in dogs is classified **Acceptable/Non-Guideline** and does not satisfy the 85-1 Guideline Requirement for a metabolism study [OPPTS 870.7485, OECD 417].

C. STUDY DEFICIENCIES

It is curious that there were pretreatment plasma levels of emetic in dogs of the 368 mg/kg dose group. Individual plasma level data were unavailable in the study report.

DATA EVALUATION RECORD

PARAQUAT (GRAMOXONE)
STUDY TYPE: TOXICOKINETICS - DOG
[NON-GUIDELINE]
MRID 46364518

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

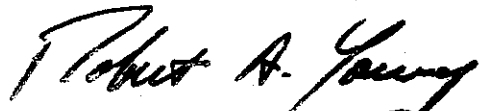
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 94-2005

Primary Reviewer:

Robert A. Young, Ph.D., D.A.B.T.

Signature:

Date:



7-5-05

Secondary Reviewers:

H.T. Borges, Ph.D., MT (ASCP), D.A.B.T.

Signature:

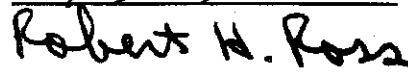
Date:


7-5-05

Robert H. Ross, M.S., Group Leader

Signature:

Date:

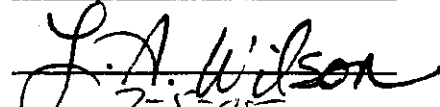

7-5-05

Quality Assurance:

LeeAnn Wilson, M.A.

Signature:

Date:


7-5-05

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

EPA Reviewer: A. Protzel, Ph.D.
Toxicology Branch, Health Effects Division (7509C)
EPA Work Assignment Manager: P.V. Shah, Ph.D.
Registration Action Branch 1, Health Effects Division (7509C)

Signature: 

Date: 7/29/05

Signature: P.V. Shah

Date: 7/29/05

Template version 11/01

TXR#: 0052955

DATA EVALUATION RECORD

STUDY TYPE: Toxicokinetics - dog [Non-guideline].

PC CODE: 061601

DP BARCODE: 309472

SUBMISSION NO.: NA

TEST MATERIAL (PURITY): (Gramoxone 200 G/L SL Formulation; 20% a.i. w/w)

SYNONYMS:

CITATION: Heylings, J., Swain, C., Brammer, A. (2004). Paraquat: Gramoxone 200 G/L SL Formulation: Toxicokinetic study in the dog. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. CTL No. 026118. January 28, 2004. MRID 46364518. Unpublished.

SPONSOR: Syngenta Crop Protection, Inc., 410 Swing Road, P. O. Box 18300, Greensboro, NC 27419.

EXECUTIVE SUMMARY:

A non-guideline study (MRID 46364518) was conducted to compare plasma kinetic data for Gramoxone 200 G/L SL formulation (CTL ref. no. Y00061, purity 20% assumed) in dogs administered the compound (44 mg Gramoxone/kg, equivalent to 8 mg paraquat ion/kg) via gelatin capsule or by gavage. These data were obtained from a series of studies conducted at Central Toxicology Laboratory over a period of several years. Specifically, the studies provided data for 12 dogs administered the test article via gelatin capsule and seven dogs dosed by gavage.

At the dose tested, emesis was the only treatment-related effect in the dogs. Emesis, an expected response, occurred as early as 16 minutes post dose and generally ceased several hours post dose. Paraquat ion profiles (concentration-time data and AUC estimates) were similar in dogs administered Garmoxone (44 mg/kg, equivalent to 8 mg paraquat ion) via gelatin capsule or by gavage. Peak plasma concentrations of 3-4 µg/ml were achieved at one hour post dose. The paraquat ion was almost completely cleared at 24 hours post dose in both groups. The variability in plasma concentration-time data could be attributed to individual variability among the limited number of dogs in each experimental group. AUC values over 24 hours were approximately 16 and 15 µg/ml·hr, respectively, for the gelatin capsule and gavage administrations.

This study (MRID 46364518) on the toxicity and plasma kinetics of Gramoxone 200 G/L SL formulation in dogs is classified **Acceptable/Non-Guideline** and does not satisfy the 85-1 Guideline Requirement for a metabolism study [OPPTS 870.7485, OECD 417]. The report is principally an analysis of plasma profile data from a series of earlier studies and was not designed or submitted as a guideline study.

COMPLIANCE: Signed GLP, Data Confidentiality Claim, and Quality Assurance statements were provided in the study report.

I. MATERIALS AND METHODS:

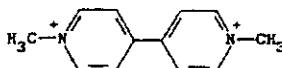
A. MATERIALS:

1. Test compound: Gramoxone 200 GL SL formulation

<u>Radiolabelled test material:</u>	[³ H]-paraquat for radioimmunoassay; no details provided
Radiochemical purity	NA
Specific Activity	NA
Lot/Batch #:	NA

Non-Radiolabelled test material:

Description:	dark green liquid
Lot/Batch #:	CTL ref. no. Y00061
Purity:	20% (assumed; emetic content not stated)
Contaminants:	none noted
CAS # of TGAI:	4685-14-7
Structure:	



2. Vehicle and/or positive control: None noted.

3. Test animals:

Species:	dog; male								
Strain:	beagle								
Age/weight at study initiation:	16-24 weeks; 13.6-17.8 kg								
Source:	Dog Animal Breeding Unit, Alderley Park, Macclesfield, UK								
Housing:	housed individually in indoor pens with a separate exercise area.								
Diet:	400 g Laboratory Diet A (Special Diet Services Ltd., Stepfield, Witham, Essex, UK) daily except for 24-hr fasting prior to treatment								
Water:	tap water <i>ad libitum</i> except for 1 hr pre-dose and 1 hr post dose								
Environmental conditions:	<table> <tr> <td>Temperature:</td> <td>20°C nominal</td> </tr> <tr> <td>Humidity:</td> <td>not controlled</td> </tr> <tr> <td>Air changes:</td> <td>15/hr</td> </tr> <tr> <td>Photoperiod:</td> <td>11 hrs light/13 hrs dark</td> </tr> </table>	Temperature:	20°C nominal	Humidity:	not controlled	Air changes:	15/hr	Photoperiod:	11 hrs light/13 hrs dark
Temperature:	20°C nominal								
Humidity:	not controlled								
Air changes:	15/hr								
Photoperiod:	11 hrs light/13 hrs dark								
Acclimation period:	at least 1 week								

4. Dose preparations: Paraquat (Gramoxone 200 g/l formulation) required to achieve a target dose of 8 mg paraquat ion/kg was administered either in a gelatine capsule or by gavage.

The amount of test material was calculated as:

$$\text{mg formulation/kg} = \frac{\text{dose volume [0.04 ml]} \times \text{specific gravity [1.1]} \times 1000}{\text{weight (kg)}}$$

B. STUDY DESIGN AND METHODS:

- 1. Group arrangements:** The reviewed study (MRID 46364518) analyzed data extracted from previous studies; the experimental groups are shown in Table 1. Plasma samples collected at 24 hours post dose during these studies were analyzed for paraquat and plasma kinetic parameters determined. The dogs in the studies were randomly allocated to the treatment groups although several experiments utilized the same dogs. Feed consumption was recorded (mean g feed/dog/day) 24 hours post dose and throughout the study period. Feed consumption was calculated at weekly intervals and expressed as g feed/day.

Table 1. Experimental sets for toxicokinetic study in male beagle dogs given Paraquat (Gramoxone 200 G/L SL Formulation)			
Study No./Date ^a	No. of dogs	Dose ^b (mg paraquat ion/kg)	Comments
XD1236 (1-28-88)	3	8	gelatin capsule (* same dogs)
XD1328 (1-28-88)	3	8	gelatin capsule
XD1328 (3-14-89)	3	8	gelatin capsule
XD1328 (1-16-91)	3	8	gelatin capsule (* same dogs)
XD1236 (6-23-87)	3	8	gavage (* same dogs)
XD1328 (1-28-88)	4	8	gavage

^a Date of dosing

^b Gramoxone dose was 44 mg/kg which provided the target 8 mg paraquat ion/kg
Data taken from Appendix A, p. 31, MRID 46364518.

All of the dogs were observed continuously for several hours post dose and at least twice daily for clinical signs or behavioral abnormalities. The timing and qualitative descriptions of vomiting and feces were recorded. All dogs were given full clinical examinations (including cardiac and pulmonary auscultation) prior to each dose and prior to termination.

2. Dosing and sample collection/preparation/analysis:

Blood samples (from the jugular vein) were collected in heparinized tubes prior to dosing, at 15 and 30 minutes post dose, and at 1, 2, 4, 7, 12, and 24 hours after dosing. Plasma was separated by centrifugation.

3. Analytical techniques:

Radioimmunoassay:

Plasma paraquat was determined by radioimmunoassay. For this procedure, the test samples and a series of standards were buffered with [³H]-paraquat. Antiserum-containing antibodies developed against a derivative of monoquat) was added. A short (non-specified) incubation period allowed free paraquat ion to be adsorbed onto bovine serum albumin-charcoal

suspension. Following centrifugation, the antibody- ^3H -paraquat ion complex in the supernatant was analyzed by LSC and the paraquat quantified by comparison to standards. Plasma profiles of paraquat ion over 24 hours were determined for each dog and mean \pm SD calculated

4. Storage stability:

Recommended storage conditions were provided but no other data were available regarding stability. Dosing formulations were prepared immediately prior to administration.

5. Calculations/ statistical analysis:

Dose calculations and quantitative information regarding dose formulations were provided. Data were expressed as mean \pm standard deviation. AUC values were calculated using the linear trapezoidal rule from pooled data. AUC_{0-1} , AUC_{0-4} , and AUC_{0-24} were calculated.

II. RESULTS:

A. CLINICAL OBSERVATIONS

There were no signs of toxicity in any of the dogs in any studies following oral administration of 44 mg Gramoxone/kg.

1. **Emesis**

The most notable finding was emesis which occurred as early as 16 minutes post dose. The dogs reportedly had no long-lasting effects (e.g., retching, additional vomiting, decreased feed consumption).

2. **Bodyweight**

There were no significant effects on body weight beyond a slight decrease that could be associated with the fasting prior to dosing.

3. **Food consumption**

Feed consumption was not significantly affected by the treatment. Daily feed consumption ranged from 230-350 g/dog/day.

4. **Clinical chemistry**

There were no treatment-related effects on clinical chemistry parameters.

B. TOXICOKINETICS

Concentration-time course data for plasma paraquat ion are shown in Table 2. The plasma profiles were similar between the gelatine capsule and gavage administration groups. The observed minimal variability could be attributed to individual variability among the dogs in each group. Peak plasma concentrations ($\sim 3-4 \mu\text{g/ml}$) of the paraquat ion occurred at 1 hour for both dosing techniques and clearance was nearly complete at 24 hours after dosing although still measurable.

Time (hrs)	Administration	
	Gelatine capsule	Gavage
Pre-dose	0.00±0.00	0.00±0.00
0.25	1.38±1.26	1.59±0.93
0.5	2.34±1.26	2.81±0.55
1	3.94±0.88	3.46±0.49
2	3.00±0.73	2.27±0.53
4	1.38±0.42	1.28±0.61
7	0.45±0.31	0.34±0.08
12	0.20±0.15	0.17±0.06
24	0.07±0.08	0.19±0.17

^a Mean±SD for 12 dogs (gelatine capsule experiments) or 7 dogs (gavage experiments); see Table 1 for experimental groups.

Data taken from Appendix B, p. 32, MRID 46364518.

Plasma AUC values for the dogs in the various experiments analyzed are shown in Table 3. Consistent with the plasma profiles, the AUC values were similar for the gelatin capsule and gavage dosing.

AUC	Administration	
	Gelatine capsule	Gavage
AUC _{0-1 hrs}	2.21±0.22	2.32±0.16
AUC _{0-4 hrs}	10.06±0.49	8.73±0.43
AUC _{0-24 hrs}	15.98±0.89	14.63±1.00

^a Mean±SD for 12 dogs (gelatine capsule experiments) or 7 dogs (gavage experiments); see Table 1 for experimental groups.

Data taken from Table 3, p. 30, MRID 46364518

III. DISCUSSION AND CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS

The investigators concluded that analysis of paraquat ion profiles (concentration-time data and AUC estimates) were similar in dogs administered Gramoxone (44 mg/kg, equivalent to 8 mg paraquat ion) via gelatin capsule or by gavage. Peak plasma concentrations of 3-4 µg/ml were achieved at one hour post dose. The paraquat ion was almost completely cleared at 24 hours post dose in both groups. At the dose tested, emesis was the only treatment-related effect in the dogs. Emesis, an expected response, occurred as early as 16 minutes post dose and generally ceased several hours post dose.

B. REVIEWER COMMENTS:

A non-guideline study (MRID 46364518) was conducted to compare plasma kinetic data for Gramoxone 200 G/L SL formulation (CTL ref. no. Y00061, purity 20% assumed) in dogs administered the compound (44 mg Gramoxone/kg, equivalent to 8 mg paraquat ion/kg) via gelatin capsule or by gavage. These data were obtained from a series of studies conducted at Central Toxicology Laboratory over a period of several years. Specifically, the studies provided data for 12 dogs administered the test article via gelatin capsule and 7 dogs dosed by gavage.

The report provided a summary of clinical effects observed for dogs receiving a single dose Gramoxone 200 GL SL formulation by gavage via gelatine capsule. Specifically, the study utilized data from previously conducted studies to compare plasma profiles for paraquat ion between the two dosing techniques. The data clearly showed peak plasma concentrations of 3-4 µg paraquat ion/ml were achieved at one hour following dosing regardless of the administration method and that similar AUC values were attained for both groups. The experiments upon which this comparative analysis was based utilized only 3-4 dogs each and some experiments utilized the same dogs, although they were performed at 1-2 year intervals. The variability in plasma concentration-time data could be attributed to individual variability among the limited number of dogs in each experimental group. Although clearly not designed or submitted as a guideline study, the report achieved its purpose of comparing plasm profile data for the two dose methods.

This study (MRID 46364518) on the toxicity and plasma kinetics of Gramoxone 200 G/L SL formulation in dogs is classified **Acceptable/Non-Guideline** and does not satisfy the 85-1 Guideline Requirement for a metabolism study [OPPTS 870.7485, OECD 417]. The report is principally an analysis of plasma profile data from a series of earlier studies and was not designed or submitted as a guideline study.

C. STUDY DEFICIENCIES

Purity is "assumed" to be 20%. The investigators noted that compound purity was the responsibility of the study sponsor (Syngenta Crop Protection) and provided no additional information. This purity, however, is similar to that used in other paraquat studies submitted by this registrant. This was not considered to be a basis for study rejection. Humidity was not controlled but assuming it was within normal ranges would not have adversely affected the study outcome.