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SUMMARY

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS

by

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Date of Issue:

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS

We, the undersigned, declare that this report constitutes a true record of the actions undertaken and the results obtained in the above study..

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PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS

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PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS

SUMMARY

A previous study showed that different doses of the emetic PP796 in dogs induced different times to initiation of vomiting and different degrees of emetic response. This study was performed in order to investigate the effects of different doses of PP796, in dogs, when administered simultaneously with paraquat.

Groups of 3 male dogs were dosed orally with 20mg/kg paraquat ion (as dichloride) and with 0, 0.5, 3.0 or 20mg/kg of the emetic PP796.

The effects of paraquat administration were assessed in the 4 groups by: i) peak plasma paraquat concentration, ii) area under the plasma paraquat concentration/time curve and iii) grossly observable paraquat-related lung lesions at necropsy 8 days after dosing.

There was a marked decrease in the peak plasma paraquat concentration, area under the curve and the severity of the paraquat-related lung lesions of dogs dosed with 0.5mg/kg or 3.0mg/kg PP796 + paraquat when compared with dogs dosed with paraquat alone. These reductions were dose related. The response in dogs dosed with 20mg/kg PP796 + paraquat was variable, some dogs showing a reduction in the effects of paraquat whilst others showed no decrease. One dog showed evidence of increased effects of paraquat which were considered to be due to increased paraquat absorption resulting from inhalation of regurgitated stomach contents during violent and repeated emesis.

It is considered that the optimum dose of the emetic PP796, in dogs, in terms of reducing the absorption and effects of paraquat is between 0.5mg/kg and 3.0mg/kg. High doses of PP796 provide no advantages over a dose of 3.0mg/kg and may, in some dogs, be contra-indicated.

1. INTRODUCTION

PP796 is an emetic which is believed to act centrally via the chemoreceptor trigger zone. The simultaneous administration of paraquat and an emetic dose of PP796, to dogs, resulted in lowered plasma concentrations of paraquat ion and reduced signs of toxicity when compared with dogs dosed with paraquat alone (1). PP796 is currently incorporated into some paraquat formulations in order to reduce toxicity in humans should ingestion occur. Different dose levels of PP796 have been shown to elicit different degrees of emetic response in dogs (2).

The purpose of this study was to investigate the effects of different dose levels of PP796 on the plasma profile and toxicity of paraquat in dogs, in order to select a concentration of PP796 for paraquat formulations which may provide optimum protection for humans.

The dose levels of PP796 used in this study (0.5, 3 and 20mg/kg) were selected on the basis of previous work in dogs where 20mg/kg was considered to be the maximum tolerated dose level (2). The dose level of paraquat (20mg paraquat ion/kg) was chosen since this was considered to be a non-lethal dose which would provide measurable levels of paraquat ion in plasma.

The study commenced on 17th September 1985 and the in-vivo phase was completed on 26th September 1985.

2. MATERIALS AND METHODS

2.1 Test Compounds and Dose Preparation

2.1.1 PP796: PP796 (Y00706/016/002) was supplied as the technical paste by Plant Protection Division, Fernhurst, and prepared as aqueous

solutions, correcting for purity (82.1%), by Central Dispensary as follows:

0.1% solution dosed at 0.5ml/kg	=	0.5mg/kg
0.6% solution dosed at 0.5ml/kg	=	3.0mg/kg
1% solution dosed at 2ml/kg	=	20.0mg/kg

2.1.2 Paraquat*: Paraquat dichloride (Y00061/066/001) was supplied by Mond Division as the technical liquor containing 33.07% paraquat ion. An aqueous 8% paraquat ion solution was prepared by the Central Dispensary, Central Toxicology Laboratory (CTL), and dosed at 0.25ml/kg bodyweight to give a dose level of 20mg paraquat ion/kg bodyweight.

Doses were calculated to the nearest 0.1kg and the appropriate volume of each test solution was check-weighed into syringes for administration via gavage. (A density of 1.0 was assumed for the prepared dosing solutions).

2.2 Animals and Accommodation

Thirteen male beagle dogs from separate litters, were used in this study and were 24-30 weeks old when dosed. They were obtained from the Alderley Park Dog Breeding Unit and were acclimatised to the CTL environment for at least 1 week prior to treatment. Whilst at the Breeding Unit they had received vaccinations against canine distemper, leptospirosis, canine viral hepatitis and parvovirus and treatments for possible ear mite and nematode infestations. The dogs were identified by tattooed ear numbers which were cross-referenced to experimental numbers following randomisation.

The dogs were housed in the CTL dog house and were fed 350g Laboratory Diet A (Special Diet Services Ltd, Stepfield, Witham, Essex) daily. Water was provided ad libitum except on the day of dosing. The environmental temperature ranged between 19-23°C during the course of the study.

*Throughout this report, 'paraquat' refers to 20mg paraquat ion/kg bodyweight.

2.3 Experimental Design

Twelve dogs were initially used in this study. Because of the effects seen in one group 4 dog (male 10) after dosing on day 1, another dog (male 13) was introduced to this group.

Dogs were assigned to treatment groups as follows:

Group	Treatment	Experimental Numbers
1 (control)	20mg paraquat ion/kg	1 - 3
2	20mg paraquat ion/kg + 0.5mg PP796/kg	4 - 6
3	20mg paraquat ion/kg + 3 mg PP796/kg	7 - 9
4	20mg paraquat ion/kg + 20 mg PP796/kg	10 - 13

On the day of dosing (day 1), each dog received 6g COMPLAN (Farley Health Products Ltd, Plymouth) as a 50ml aqueous suspension, by gavage (24FG, Warne Surgical Products), approximately 24 hours after the last normal feed. Immediately afterwards the appropriate volume of test solution(s) was administered via the gavage tube followed by 10ml water. Food and water were then withheld for 6 hours after dosing.

Dogs 1-12 were dosed on 17th September 1985 and dog 13 was dosed on 19th September 1985. Each dog was observed for 7 days and killed on day 8.

2.4 Clinical Investigations

The dogs were observed, continuously for the first 1-2½ hrs after dosing and then frequently during each working day, for gross clinical or behavioural abnormalities. Clinical examinations, including cardiac and pulmonary auscultation, were made pre-study, on day 1 (5-8 hrs after dosing) and terminally.

2.5 Plasma Paraquat Concentrations

2ml blood samples were obtained from each dog at the following scheduled time points: pre-dose, 5, 15 and 30 mins, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 hours post-dose and placed in lithium/heparin. The plasma was stored at -20°C prior to analysis of the paraquat concentration by radioimmunoassay.

The actual sampling times were noted so that the plasma levels could be plotted accurately (particularly during the first hour).

The area under the plasma concentration, time curve (AUC), for the initial 24 hour post-dose period, was calculated by the trapezoidal rule (3).

2.6 Terminal Procedures

At termination (day 8) all surviving dogs were killed by deep pentobarbitone anaesthesia (EUTHATAL, May and Baker Ltd, Dagenham, Essex) and exsanguination. Gross necropsies were performed and the following tissues were removed and fixed:-

Adrenal gland, kidney, liver, lung, stomach, any abnormal tissue.

Tissues were fixed in 10% neutral buffered formol saline and stored.

One dog (male 10, 20mg/kg PP796 + paraquat) was killed on day 4 due to adverse clinical signs and necropsied as above. In addition fixed portions of lung were routinely processed for histology, paraffin wax embedded and 5µm sections were cut and stained with haematoxylin and eosin. Sections were examined by light microscopy.

2.7 Statistical Analysis

The following data were subjected to statistical analyses:

1) time to first vomit
2) peak plasma level

3) area under curve

4) qualitative assessment of lung lesion

Where appropriate, the data were considered using Analysis of Variance and intergroup comparisons were made on the basis of a Student's t-test following a log transformation. A higher level of variation was noted between dogs in Group 4 with regard to all 4 variables. This precluded analysis of variance into differences in mean value of peak plasma level and area under curve for this group. The Mann-Witney U-test was used to compare the assessed lung lesions (4).

3. RESULTS

Peak plasma levels, area under plasma concentration, time curve (AUC), time to first vomiting and lung lesion assessments are presented, for each animal, in Table 1. Group means and p values are presented in Table 2.

3.1 Mortalities

Male 10, Group 4 (20mg/kg PP796 + paraquat) was killed on day 4 due to adverse clinical signs.

There were no other mortalities and all other dogs, including those dosed with paraquat alone, remained in good general clinical condition during the 7 day observation period.

3.2 Clinical Observations (Tables 1 and 2, Appendix 1)

Clinical findings are summarised for each animal in Appendix 1.

All dogs dosed with PP796 showed a decreased time to first vomit after dosing when compared with those dosed with paraquat alone (group 1). Dogs dosed with 20mg/kg PP796 + paraquat showed a shorter time to vomit than those dosed with 3 or 0.5mg/kg PP796 + paraquat. There was some evidence that dogs dosed with 3mg/kg PP796 + paraquat showed a shorter mean time to vomit than dogs dosed with 0.5mg/kg PP796 + paraquat.

Clinical signs were severe in those dogs dosed with 20mg/kg PP796 + paraquat. Vomiting was accompanied by prolonged episodes of marked retching and subdued behaviour, restlessness and recumbency and these effects lasted for about 5 hours after dosing. One dog in this group (male 10) struggled violently during the dosing procedure and vomited soon after dosing (1½ minutes). Laboured, rapid respiration became evident 30 minutes after dosing. Although this improved over the next 24 hours, by day 4 the dog had a slightly productive cough, increased respiratory sounds in the lungs and upper respiratory tract, appeared slightly dehydrated and was subdued. It was killed on day 4 for humane reasons.

At 3mg/kg PP796 + paraquat, subdued behaviour, recumbency and panting accompanied vomiting but these effects were apparent only during the initial 1-2 hours post dosing.

0.5mg/kg PP796 + paraquat produced mild clinical effects. Slight hypoactivity was apparent in the first 1-2½ hours and vomiting occurred in the first hour after dosing.

Paraquat alone (20mg/kg) was tolerated well. Slight hypoactivity was seen within the first 1½ hours of dosing and the time to vomiting was extremely variable (1 hour 15 mins for male 1, 29 hours for male 2). Male 3 showed the most severe effects (vomiting occasionally over 4 days and leaving food).

3.3 Plasma Paraquat Concentrations (Tables 1 and 2, Figure 1, Appendix 2)

The peak plasma paraquat levels of dogs dosed with 0.5 or 3.0mg/kg PP796 + paraquat were markedly lower than those of dogs dosed with paraquat alone (approximately 10-fold). There was some evidence that values in dogs dosed with 3.0mg/kg PP796 + paraquat were lower than in dogs dosed with 0.5mg/kg PP796 + paraquat. The values in dogs dosed with 20mg PP796 + paraquat, with the exception of male 10, were lower than in dogs dosed with paraquat alone. However the variability in this group precluded statistical comparison with values in groups 2 and 3.

The area under the curves of dogs dosed with 0.5 or 3.0mg/kg PP796 + paraquat were also markedly lower than those of dogs dosed with paraquat alone (approximately 10-fold). There was some evidence that values in dogs dosed with 3.0mg/kg PP796 + paraquat were lower than in dogs dosed with 0.5mg/kg PP796 + paraquat. The values in dogs dosed with 20mg/kg PP796 + paraquat, with the exception of male 10, were lower than in dogs dosed with paraquat alone. Statistical analysis of these data was not performed due to the variability in this group.

3.4 Pathology (Table 3, Appendix 3)

A number of treated dogs showed gross lung lesions which were considered to be related to paraquat toxicity. These lesions took the form of dark red areas some of which were consolidated.

There was decrease in the severity of the lung lesion in dogs dosed with 0.5 and 3.0mg/kg PP796 + paraquat when compared with dogs dosed with paraquat alone. There was no statistical evidence for any difference in the severity of paraquat related lung lesions in dogs dosed with 3.0mg/kg PP796 + paraquat (group 3) when compared to those dosed with 0.5mg/kg PP796 + paraquat (group 2). However, 1 dog in group 2 showed minimal change whereas no paraquat-related lesions were present in group 3.

There was considerable variation in the severity of paraquat related lung lesions in dogs dosed with 20mg/kg PP796 + paraquat. Paraquat-related lesions were absent in male 12 whereas the lungs of male 10 (killed prematurely) showed marked changes. Overall these lesions approached the severity of those present in dogs dosed with paraquat alone and were, generally, more severe than those present in group 2 and 3.

Other gross lesions were not considered to be treatment related.

Histopathology: There was a moderate pneumonitis in the lung of dog 10. This lesion was composed of alveolar inflammatory cells, interalveolar and peribronchiolar fibrosis and focal epithelialisation. In addition there were areas of alveolar haemorrhage and focal emphysema. One bronchus contained strands of basophilic material mixed with inflammatory cells.

4. DISCUSSION

The effects of orally administered paraquat and different dose levels of PP796, in dogs, were compared with the effects of paraquat alone by the assessment of 3 variables:

1) peak plasma concentration
2) area under the curve (AUC)
3) qualitative assessment of lung lesion

For each of these assessments there was strong evidence, supported statistically, that 0.5 and 3.0mg/kg PP796 reduced the effects of paraquat when compared with dogs dosed with paraquat alone. There was also some evidence that 3.0mg/kg PP796 induced a greater reduction in the effects of paraquat than 0.5mg/kg PP796.

It is clear that dogs dosed with 20mg/kg PP796 showed a markedly variable response to the effects of paraquat administration. In particular, the results obtained from dog 10 were abnormal for the group as a whole. The clinical signs of rapid, laboured respiration were of much greater severity and duration than those seen in any other dog in the study. Also the peak plasma concentration and area under the curve were markedly elevated above the values of other dogs in this group and, indeed, were significantly higher than values in dogs treated with paraquat alone. In addition, the lung lesions present in dog 10 were similar to the most severely affected dog in the paraquat-alone group. The plasma paraquat profile of dog 10 indicated that an abnormally high proportion of the administered dose of paraquat had been absorbed. The reason for these results in dog 10 is uncertain. There was no pathological evidence for tracheal damage or lung dosing. However the strands of basophilic material seen in the lungs on histology may indicate that some inhalation of some regurgitated stomach contents had occurred. Since the paraquat was administered separately from the COMPLAN at dosing, the abnormally high plasma paraquat values and the absence of an inhalation pneumonia may be explained by regurgitation and inhalation of the paraquat/PP796 dose and stomach retention of the COMPLAN during dosing. The difficulty

experienced in dosing this animal because of marked struggling may have enabled this differential regurgitation of the total gavage dose to have occurred.

Whilst the results obtained in dog 10 may be explained on the above basis, the results obtained in dog 11 also indicate a lesser reduction of the effects of paraquat than dogs 12 and 13. The peak plasma paraquat level was similar to the highest value in dogs dosed at 0.5mg/kg PP796 + paraquat, the AUC was considerably higher than in dogs 12 and 13 and the lung lesions were more severe than any other dog dosed with PP796 (with the exception of dog 10). This lessening in the reduction of effects of paraquat was present in spite of a very short time to first vomit. Thus although 20mg/kg PP796 produced a greater reduction in the effects of paraquat, in some dogs, when compared with 0.5mg/kg or 3.0mg/kg, dog 11 showed a reversal of this trend. There are several possible explanations for this reversed trend:

(a) excessive and violent retching and vomiting may induce inhalation of some of the administered gavage dose;

(b) excessive and violent retching and vomiting may cause minor damage to the gastric mucosa allowing enhanced absorption of paraquat;

(c) high doses of PP796 cause not only a decreased time to first vomiting and increased vomiting but also increased gastric emptying via the pylorus.

The absence of gastric haemorrhage either clinically or at necropsy in this study indicates that significant gastric damage was not present. Dogs treated with 20mg/kg PP796 showed, clinically, increased intestinal motility by virtue of the production of liquid or mucoid faeces after dosing although it is not known if this increased motility included gastric emptying into the duodenum. Evidence of inhalation of regurgitated material was present in dog 10 (the presence of basophilic material in lung histology and exceptionally high plasma paraquat levels) and it is considered that this event is the most likely explanation of the reversal of beneficial effects of PP796 when given at high doses.

The time to initiation of vomiting was progressively reduced following PP796 administration, in a dose-related manner, as reported previously (2). However the results of this study indicate that this variable alone cannot be used to assess the possible consequences of paraquat administration.

5. CONCLUSION

The emetic PP796 reduced the effects of paraquat when administered simultaneously. This agrees with previous findings (1).

In this study increased doses of PP796 progressively reduced the effects of oral paraquat administration up to a maximum dose of 3.0mg/kg PP796.

Large doses of PP796 (20mg/kg) resulted in a marked variability in the effects of paraquat, some dogs showing further reductions in the effects of paraquat whilst others showed a reversal of this trend.

Doses of the emetic PP796 in the order of 0.5mg/kg or 3.0mg/kg are clearly beneficial in the reduction of the effects of oral paraquat administration in dogs whereas large doses ie 20mg/kg are not beneficial in this respect and may be contra-indicated.

MR/AB/DC (514)
20.12.85

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TABLE 1

SUMMARY OF RESULTS

Treatment Group	Dog No.	Peak Plasma Level (µg/ml)	AUC (0-24hrs)	Time to Vomit	Lung Lesion (Dark red area)
1 Paraquat	1	8.88	26.838	1hr 15 mins	Moderate
	2	12.50	32.368	29hr	Slight
	3	7.62	48.327(1)	6hr	Marked
2 Paraquat + 0.5mg/kg PP796	4	0.72(2)	2.865(2)	24mins	NAD
	5	1.43	4.326	8mins	Minimal
	6	0.83	3.012	26mins	NAD
3 Paraquat + 3.0mg/kg PP796	7	0.12	0.465	6mins	NAD
	8	0.58	1.654	5½mins	NAD
	9	0.37	1.176	4mins	NAD
4 Paraquat + 20mg/kg PP796	10	26.70	52.964	1½mins	Marked
	11	1.42	2.478	3mins	Slight
	12	0.11	0.390	6mins	NAD
	13	0.10	0.460	3mins	Minimal

(1) excluding 8 hour results (0.04µg/ml).

(2) excluding 8 hour results (1.52 µg/ml).

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TABLE 2

STATISTICAL ANALYSIS OF GROUP MEAN DATA (STUDENT'S T-TEST)

Group and Treatment	Time to First Vomit (minutes) (Log transformed data)		Area Under Curve (Log transformed data)		Peak Plasma Level (µg/ml) (Log transformed data)	
	Transformed Mean	Mean	Transformed Mean	Mean	Transformed Mean	Mean
1 paraquat	2.56 A	725	1.541 A	35.8	0.976 A	9.67
2 0.5mg/kg PP796 + paraquat	1.23 B	19	0.524 B	3.4	-0.023 B	0.99
3 3.0mg/kg PP796 + paraquat	0.71 BC	5	-0.015 C	1.1	-0.530 C	0.36
4 20.0mg/kg PP796 + paraquat	0.48 C	3	-	14.1†	-	7.08†
Least Significant Difference‡ (LSD) p = 5%	0.70		0.379		0.463	

The outcomes of the individual intergroup comparisons using Student's t-test are annotated by the letters which accompany group means. Treatments with no letters in common are significantly different at the 5% level.

‡ This represents the between-group difference required to achieve Statistical Significance at 5%.

† Group 4 not included in Statistical Analysis due to the high level of variability

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TABLE 3

INCIDENCE OF GROSS PATHOLOGICAL FINDINGS IN LUNGS

Gross Lung Observation		Treatment Group			
		1 (Paraquat)	2 (Paraquat + 0.5mg/kg PP796)	3 (Paraquat + 3.0mg/kg PP796)	4 (Paraquat + 20mg/kg PP796)
Number of Animals Examined		3	3	3	4
No abnormality detected		0	0	1 (9)	1 (12)
Firm nodules		1 (1)	2 (4) (6)	2 (7) (8)	0
Focal pleural adhesion		0	1 (6)	0	0
Dark red area	- minimal	0	1 (5)	0	1 (13)
	- slight	1 (2)	0	0	1 (11)
	- moderate	1 (1)	0	0	0
	- marked	1 (3)	0	0	1* (10)

*Intercurrent death () animal number

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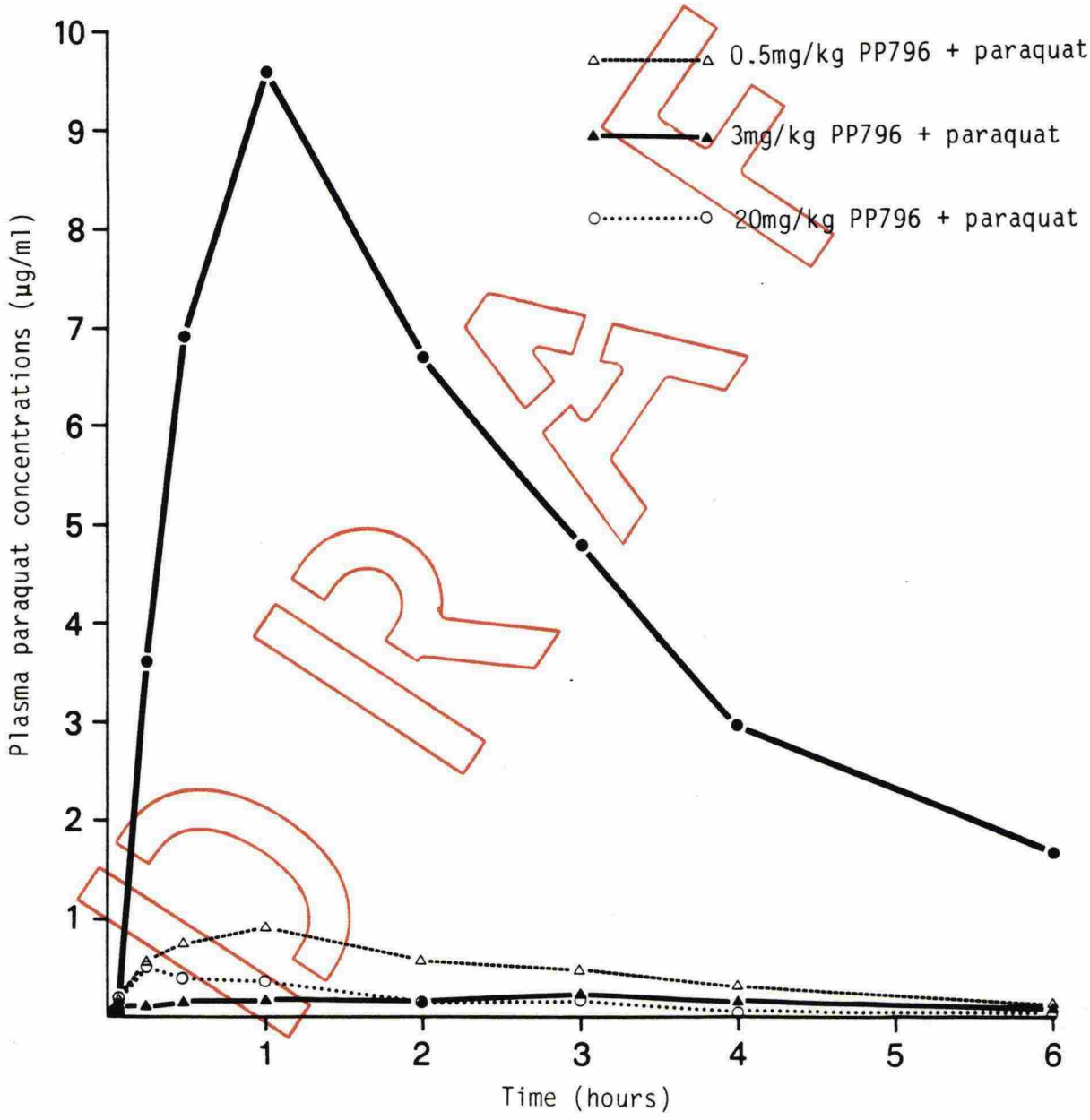
FIGURE 1

GROUP MEAN PLASMA PARAQUAT CONCENTRATION, TIME CURVES



Times are approximate up to 1 hour

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APPENDIX 1

SUMMARY OF INDIVIDUAL ANIMAL CLINICAL DATA

Key

Faecal consistency:

N = normal
S = soft
F = fluid
M = mucoid

Food residue:

/ = residues removed 5-6 hours after feeding on day -1.

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APPENDIX 1
SUMMARY OF INDIVIDUAL ANIMAL CLINICAL DATA

Treatment	Dog No.	Day No.	Body Weight (kg)	Faeces	24 Hr Food Residue	Time After Dosing	Clinical Observations/Examinations	
							Vomiting	Other Observations
20mg paraquat ion/kg	1	1	13.2	F	/	1 hr 1 hr 14 mins 1 hr 29 mins 1 hr 35 mins 2 hrs 35 mins 5 hrs	- White fluid - - - -	Fluid faeces passed. Dog restless and retching. Slightly hypoactive. Salivating, slightly hypoactive. NAD Dog examined - NAD.
		2		N/S	0	-	-	NAD
		3		N/S	0	-	-	NAD
		4		N	0	-	-	NAD
		5		N	0	-	-	NAD
		6		N/S	0	-	-	NAD
		7	12.7	N/S	0	-	-	Dog examined - increased lung sounds both sides - mid to anterior aspects.
20mg paraquat ion/kg	2	1	15.0	N	/	Approx 1 hr " 2½ hrs " 5 hrs " 8 hrs	- - - -	Slightly hypoactive, fine muscle tremors. Dog more active. Dog examined - submandibular glands palpable (as prestudy). Slightly hypoactive.
		2		N/S	0	" 29 hrs " 30 hrs	Food vomit Food vomit	- reingested Dog NAD
		3		N	75g	" 46½ hrs " 50 hrs	Dried vomit Food vomit	Dog NAD
		4		S	0	-	-	NAD
		5		N/S	0	-	-	NAD
		6		N/S	0	-	-	NAD
		7	14.5	N/S	0	-	-	Dog examined - slow breathing.

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APPENDIX 1 - continued
SUMMARY OF INDIVIDUAL ANIMAL CLINICAL DATA

Treatment	Dog No.	Day No.	Body Weight (kg)	Faeces	24 Hr Food Residue	Time After Dosing	Clinical Observations/Examinations	
							Vomiting	Other Observations
20mg paraquat ion/kg	3	1	14.9	N/F	/	39 mins Approx 2 hrs " 5 hrs " 6 hrs	- - - Pale brown and white fluid	Slightly hypoactive. Fluid faeces - ingested. Dog examined - restricted. thoracic movement during respiration. NAD
		2		N/S	125g	" 25½ hrs	Brown liquid	NAD
		3		N	275g	" 56 hrs	Yellow frothy	NAD
		4		S	295g	" 71 hrs	Brown liquid	NAD
		5		N/S	75g	-	-	NAD
		6		N/S	145g	-	-	NAD
		7	13.7	N/S	50g	-	-	Dog examined - epithelial erosion dorsal aspect of tongue. NOAD.
20mg paraquat ion/kg + 0.5mg/kg PP796	4	1	14.0	N/S	/	Predose 24 mins Approx 1½ hrs " 2½ hrs " 5 hrs	Food vomit pale, frothy - - -	Dog NAD. Dog NAD Slightly hypoactive - dog growling. Dog NAD. Dog examined - NAD.
		2		N	0	-	-	NAD
		3		N	0	-	-	NAD
		4		N	0	-	-	NAD
		5		N/M	0	-	-	NAD
		6		N/S	0	-	-	NAD
		7	13.6	N	0	-	-	Dog examined - NAD.

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS
APPENDIX 1 - continued
SUMMARY OF INDIVIDUAL ANIMAL CLINICAL DATA

Treatment	Dog No.	Day No.	Body Weight (kg)	Faeces	24 Hr Food Residue	Time After Dosing	Clinical Observations/Examinations	
							Vomiting	Other Observations
20mg paraquat ion/kg + 0.5mg/kg PP796	5	1	16.8	N/S	/	8 mins 36 mins 46 mins 1 hr 2½ hrs Approx 5 hrs	White frothy White frothy White frothy (mod volume) White frothy - -	Dog retching. Dog slightly hypoactive. Still slightly hypoactive. Dog examined - NAD.
		2		N	0	-	-	NAD
		3		N	0	-	-	NAD
		4		N	0	-	-	NAD
		5		N	0	-	-	NAD
		6		N/S	0	-	-	NAD
		7	16.6	N/S	0	-	-	Dog examined - slight increase in respiratory sounds.
20mg paraquat ion/kg + 0.5mg/kg PP796	6	1	14.3	N/S	/	26 mins Approx 1 hr " 2½ hrs " 5 hrs " 8 hrs	Large volume pale frothy fluid. - - - -	Dog NAD. Slightly hypoactive - fine muscle tremors. Dog NAD. Dog examined - pulse variable, dog subdued, spine arched. Dog NAD.
		2		N/S	0	-	-	NAD
		3		N/S	0	-	-	NAD
		4		N/S	0	-	-	NAD
		5		N	0	-	-	NAD
		6		S	0	-	-	NAD
		7	14.1	N/S	0	-	-	Dog examined - slight increase in lung sounds - mid thoracic region.

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS
APPENDIX 1 - continued
SUMMARY OF INDIVIDUAL ANIMAL CLINICAL DATA

Treatment	Dog No.	Day No.	Body Weight (kg)	Faeces	24 Hr Food Residue	Time After Dosing	Clinical Observations/Examinations	
							Vomiting	Other Observations
20mg paraquat ion/kg + 3mg/kg PP796	7	1	10.9	N/S	/	6 mins 15 mins 19 mins 32 mins Approx 1 hr " 1½ hrs " 2½ hrs " 5 hrs	White frothy White frothy White frothy White frothy - Small volume froth. - -	Dog subdued and recumbent. Dog improved slightly - fine muscle tremors. NAD. Dog examined. NAD.
		2		N/S	0	-	-	NAD
		3		N	0	-	-	NAD
		4		N/S	0	-	-	NAD
		5		N	0	-	-	NAD
		6		N/S/F	0	-	-	NAD
		7	10.7	N/S	0	-	-	Dog examined. NAD.
20mg paraquat ion/kg + 3mg/kg PP796	8	1	16.6	N/S	/	5½mins 8 mins 22 mins Approx 30 mins " 1 hr " 5 hrs	White frothy White frothy White frothy - - -	Dog very subdued. Dog improved slightly. Dog examined - abdominal breathing, thoracic movement restricted. Lung sounds pronounced as at prestudy examination.
		2		S	0	-	-	NAD
		3		N	0	-	-	NAD
		4		N/S	0	-	-	NAD
		5		N	0	-	-	NAD
		6		N	0	-	-	NAD
		7	16.3	N	0	-	-	Dog examined - lung sounds as before.

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS
APPENDIX 1 - continued
SUMMARY OF INDIVIDUAL ANIMAL CLINICAL DATA

Treatment	Dog No.	Day No.	Body Weight (kg)	Faeces	24 Hr Food Residue	Time After Dosing	Clinical Observations/Examinations	
							Vomiting	Other Observations
20mg paraquat ion/kg + 3mg/kg PP796	9	1	15.3	N/S	/	4 mins	White fluid	
						13 mins	White fluid	
						18 mins	White froth	
						23 mins	White froth	
						34 mins	-	Dog panting, slightly restless.
						44 mins	-	Recumbent, subdued, hyper-ventilating.
						58 mins	White fluid	
						1 hr 10 mins	-	Dog slightly improved.
						Approx 5 hrs	-	Dog examined. NAD.
		2		N	0	-	-	NAD
		3		N	0	-	-	NAD
		4		N	0	-	-	NAD
		5		N	0	-	-	NAD
		6		N	0	-	-	NAD
		7	14.9	N/S	0	-	-	Dog examined - lung sounds evident especially in mid to ventral region.
20mg paraquat ion/kg + 20mg/kg PP796	10	1	14.2	S	/			Dog very difficult to dose - struggling throughout. Staggering gait when returned to pen.
						1½-3 mins	White fluid - 3 times.	
						19 mins	-	Gait unsteady.
						26 mins	-	Dog subdued, recumbent, panting, noisy respiration.
						44 mins	-	Retching, unsteady but slightly improved.
						51 mins	-	Recumbent.
						54 mins	-	Restless, respiration rapid but not noisy.
						Approx 1½ hrs	-	Recumbent.
						" 2½ hrs	-	Unsteady, subdued, slightly hunched, fine whole body muscle tremors.
						" 3 hrs	-	Respiration slightly laboured, hunched gait but not unsteady.

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS
APPENDIX 1 - continued
SUMMARY OF INDIVIDUAL ANIMAL CLINICAL DATA

Treatment	Dog No.	Day No.	Body Weight (kg)	Faeces	24 Hr Food Residue	Time After Dosing	Clinical Observations/Examinations	
							Vomiting	Other Observations
20mg paraquat ion/kg + 20mg/kg PP796	10 (cont)	1 (cont)				Approx 5 hrs	Frothy white (small volume)	Respiration thoracic and abdominal, rate = 62/min, slightly noisy. Dog more alert but still hunched and hypoactive. Dog examined - sunken eyes, flaccid abdominal muscles, abdominal breathing, thoracic movement restricted. Respiratory sounds obvious, wheezing sounds evident/transmitted to right mid thoracic region.
						Approx 6 hrs	Frothy white (small volume)	
						" 7½ hrs	Clear liquid	Dog examined - low body temperature (37.5°C). Increased force and rate of respiration (56/min). Slight increased lung sounds particularly RHS. Occasional gagging sounds in throat.
						" 12 hrs	Clear liquid	Respiration as before, slight rasping in throat less audible than before. Dog still hypoactive, fast pulse rate (150/min).
		2		F	300g	Approx 24 hrs	-	Much improved, respiration appears normal.
						" 28-30 hrs	Brown liquid (x3)	Slightly subdued, salivating.
		3		S	330g	Approx 48 hrs	Brown liquid	Active, eyes slightly sunken, skin tone slow.
		4	12.3	N	245g (+ 295g from food given on day 4)	Approx 72 hrs	-	Dog slightly hunched otherwise no change.
						" 77 hrs	-	Dog examined - slight productive cough after being carried. Slightly dehydrated and subdued, salivating. Increased respiratory sounds lungs and upper respiratory tract. Euthanasia.

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS
APPENDIX 1 - continued
SUMMARY OF INDIVIDUAL ANIMAL CLINICAL DATA

Treatment	Dog No.	Day No.	Body Weight (kg)	Faeces	24 Hr Food Residue	Time After Dosing	Clinical Observations/Examinations	
							Vomiting	Other Observations
20mg paraquat ion/kg + 20mg/kg PP796	11	1	16.3	F/M/N	/	3 mins 20 mins 25 mins 35 mins 50 mins 2 hrs 2½ hrs 3 hrs 5 hrs	Large volume - - - - - - - -	Drooling vomit. Subdued Excess salivation, slightly hunched, rapid breathing. Very subdued, drooling, slightly hunched. Subdued, slightly restless, fine muscle tremors. Improved slightly. Yellow, mucoid liquid faeces. Dog examined - rapid, shallow abdominal breathing. Lung sounds left side.
		2		N/S	0	-	-	NAD
		3		N	0	-	-	NAD
		4		N/S	0	-	-	NAD
		5		N/S	0	-	-	NAD
		6		N/S	0	-	-	NAD
		7	16.0	N/S	0	-	-	Dog examined - NAD.
20mg paraquat ion/kg + 20mg/kg PP796	12	1	14.2	N/S	/	6 mins 8 mins 16 mins 26 mins 36 mins Approx 45 mins " 50 mins " 2½ hrs " 5 hrs	)) White) liquid) - - - - -	Dog subdued, retching, recumbent and restless. Unsteady gait, squatting. Recumbent and restless. Gait slightly unsteady, drooling, fine muscle tremors. Dog examined - salivating, pulse weak, hunched, abdominal breathing, thoracic movements restricted, shallow respiration. Lung sounds marked.

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS
APPENDIX 1 - continued
SUMMARY OF INDIVIDUAL ANIMAL CLINICAL DATA

Treatment	Dog No.	Day No.	Body Weight (kg)	Faeces	24 Hr Food Residue	Time After Dosing	Clinical Observations/Examinations	
							Vomiting	Other Observations
20mg paraquat ion/kg + 20mg/kg PP796	12 (cont)	2		N	0	-	-	NAD
		3		N	0	-	-	NAD
		4		N	0	-	-	NAD
		5		N	0	-	-	NAD
		6		N	0	-	-	NAD
		7	13.9	N	0	-	-	Dog examined - shivering, possible increased lung sounds in mid thoracic region.
20mg paraquat ion/kg + 20mg/kg PP796	13	1	12.8	N/S	/	3 mins 4 mins 7-12 mins 12-20 mins 20-30 mins 32 mins 34 mins 57 mins Approx 1½ hrs " 3 hrs " 8 hrs	White fluid White fluid Yellow frothy White froth - White vomit - White froth - - -	Sudden, projectile vomiting. Marked retching. Marked continuous retching, dog subdued and restless. Unsteady gait. Retching still very marked. Normal faeces passed. Clear liquid from anus, dog restless, retching, drooling vomit, squatting. Retching has stopped, clear liquid from anus. No change otherwise. More retching. Dog very subdued, muscle tremors, squatting. Dog improved. Excess salivation. Dog examined - NAD.
		2		N/S	0	-	-	NAD
		3		N/S	0	-	-	NAD
		4		N/F	0	-	-	NAD
		5		N/S	0	-	-	NAD
		6		N	0	-	-	NAD
		7	12.9	N	0	-	-	Dog examinaed - NAD

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS
APPENDIX 2
INDIVIDUAL ANIMAL PLASMA PARAQUAT CONCENTRATIONS (µg/ml)

Treatment	Paraquat				0.5mg/kg PP796 + Paraquat			
Scheduled Bleeding Times	Dog 1	Dog 2	Dog 3	Mean ± SD	Dog 4	Dog 5	Dog 6	Mean ± SD
0	ND	ND	ND	ND	ND	ND	ND	ND
5 min	0.03	0.04 (4)	0.08 (11)	0.05 ± 0.03	0.03 (6)	0.29 (11)	0.06 (10)	0.13 ± 0.14
15 min	4.83 (17½)	4.78 (26)	1.27 (21)	3.63 ± 2.04	0.64 (25)	0.80 (23)	0.24 (18)	0.56 ± 0.29
30 min	8.88 (40)	7.69 (35)	4.09 (31)	6.89 ± 2.49	0.72 (40)	1.06 (33)	0.43	0.74 ± 0.32
1 hr	8.88	12.50	7.44	9.61 ± 2.61	0.49	1.43	0.83	0.92 ± 0.48
2 hr	5.44	7.06	7.62	6.71 ± 1.13	0.28	0.72	0.76	0.59 ± 0.27
3 hr	3.43	4.38	6.63	4.81 ± 1.64	0.35	0.61	0.51	0.49 ± 0.13
4 hr	2.14	1.96	4.91	3.00 ± 1.65	0.31	0.36	0.30	0.32 ± 0.03
6 hr	0.96	0.91	3.29	1.72 ± 1.36	0.14	0.19	0.12	0.15 ± 0.04
8 hr	0.38	0.41	0.04	0.28 ± 0.21	1.52	0.05	<0.03	0.79 ± 1.04
12 hr	0.10	0.28	0.54	0.31 ± 0.22	0.05	0.03	0.01	0.03 ± 0.02
24 hr	0.07	0.07	0.27	0.14 ± 0.12	<0.006	0.02	0.01	0.015 ± 0.007
48 hr	0.02	0.02	0.04	0.03 ± 0.01	<0.006	<0.006	<0.006	<0.006 -
72 hr	<0.006	<0.006	0.01	0.01 -	<0.006	<0.006	<0.006	<0.006 -

Actual bleeding time in parenthesis where different from scheduled time.
ND = None detected <0.006µg/ml

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS
APPENDIX 2 - continued
INDIVIDUAL ANIMAL PLASMA PARAQUAT CONCENTRATIONS (µg/ml)

Treatment	3.0 mg/kg PP796 + Paraquat				20mg/kg PP796 + Paraquat				
Scheduled Bleeding Times	Dog 7	Dog 8	Dog 9	Mean ± SD	Dog 10	Dog 11	Dog 12	Dog 13	Mean ± SD*
0	ND	ND	ND	ND	ND	ND	ND	0.05	ND
5 min	<0.06 (4)	0.05 (9)	0.12 (9)	0.09 ± 0.05	20.55	0.36	0.08 (13)	0.04 (6)	0.16 ± 0.17
15 min	0.06 (17)	0.05 (23)	0.20 (18)	0.10 ± 0.08	26.70 (26)	1.42 (9)	0.11 (20)	0.09 (13)	0.54 ± 0.76
30 min	0.09 (40)	0.02 (33)	0.29	0.13 ± 0.14	22.40 (40)	0.90 (35)	0.11 (32)	0.10 (29)	0.37 ± 0.46
1 hr	0.12	0.03	0.37	0.17 ± 0.18	17.94	0.91	0.09	0.07	0.36 ± 0.48
2 hr	0.05	0.15	0.23	0.14 ± 0.09	8.88	0.38	0.05	0.03	0.15 ± 0.20
3 hr	0.03	0.58	0.14	0.25 ± 0.29	4.91	0.38	0.04	0.10	0.17 ± 0.18
4 hr	0.02	0.36	0.08	0.15 ± 0.18	2.63	0.09	0.03	0.04	0.05 ± 0.03
6 hr	<0.006	0.08	0.04	0.06 ± 0.03	0.88	0.03	0.01	0.02	0.02 ± 0.01
8 hr	0.04	0.02	0.03	0.03 ± 0.01	0.36	0.01	<0.006	0.01	0.01 -
12 hr	<0.006	0.01	<0.006	0.01 -	0.14	<0.006	<0.006	<0.006	<0.006 -
24 hr	<0.006	<0.006	<0.006	<0.006 -	0.12	<0.006	<0.006	<0.006	<0.006 -
48 hr	<0.006	0.01	0.01	0.01 -	0.09	0.01	0.01	<0.006	0.01 -
72 hr	<0.006	<0.006	<0.006	<0.006 -	0.008	<0.006	<0.006	<0.006	<0.006 -

Actual bleeding time in parenthesis where different from scheduled time.
ND = None detected <0.006µg/ml

*Excluding Dog 10

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS

APPENDIX 3

INDIVIDUAL ANIMAL PATHOLOGY DATA

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	1	Sex	Male
Dose Group	1 - 0mg/kg Control PP796 plus 20mg/kg Pq ion				
Duration	Day 8	~~Died~~/Killed		~~Intercurrent/Interim~~/Term	

MACROSCOPIC FINDINGS

Thoracic cavity: slight excess of watery fluid.
Lungs: all surfaces, all lobes, multiple dark red areas covering approximately one-third of lung surface. Left diaphragmatic 2x1mm nodules - moderate dark red areas.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	2	Sex	Male
Dose Group	1 - 0mg/kg Control PP796 plus 20mg/kg Pq ion				
Duration	Day 8	~~Died~~/Killed		~~Intercurrent/Interim~~/Term	

MACROSCOPIC FINDINGS

Lungs: left and right apical and cardiac, ventral border of left diaphragmatic - areas of dark red discolouration affecting ventral half of lobes - slight dark red areas.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	3	Sex	Male
Dose Group	1 - 0mg/kg Control PP796 plus 20mg/kg Pq ion				
Duration	Day 8	~~Died~~/Killed	~~Intercurrent/Interim~~/Term		

MACROSCOPIC FINDINGS

Tongue: no abnormalities detected.
Lungs: all surfaces of all lobes, large dark red areas of discolouration. Slightly firmer than normal covering two-thirds of lung surface - marked dark red areas.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	4	Sex	Male
Dose Group	2 - 0.5mg/kg PP796 plus 20mg/kg Pq ion				
Duration	Day 8	~~Died~~/Killed		~~Intercurrent/Interim/Term~~	

MACROSCOPIC FINDINGS

Lungs: right diaphragmatic and intermediate lobe 1mm firm nodule on each.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	5	Sex	Male
Dose Group	2 - 0.5mg/kg PP796 plus 20mg/kg Pq ion				
Duration	Day 8	~~Died~~/Killed		~~Intercurrent/Interim~~/Term	

MACROSCOPIC FINDINGS

Lungs: right cardiac 2x1mm dark red area ventral border - minimal dark red area.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	6	Sex	Male
Dose Group	2 - 0.5mg/kg PP796 plus 20mg/kg Pq ion				
Duration	Day 8	~~Died~~/Killed	~~Intercurrent/Interim~~/Term		

MACROSCOPIC FINDINGS

Lungs: all lobes - several small nodules 1-3mm diameter. Right apical costal surface focal area of pleural adhesion 5mm diameter.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	7	Sex	Male
Dose Group	3 - 3mg/kg PP796 plus 20mg/kg Pq ion				
Duration	Day 8	~~Died~~/Killed		~~Intercurrent/Interim~~/Term	

MACROSCOPIC FINDINGS

Lungs: 1mm pale firm nodules left and right apical lobe and left diaphragmatic.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	8	Sex	Male
Dose Group	3 - 3mg/kg PP796 plus 20mg/kg Pq ion				
Duration	Day 8	~~Died~~/Killed	~~Intercurrent/Interim~~/Term		

MACROSCOPIC FINDINGS

Lungs: left and right diaphragmatic 2mm dark firm nodule. Left apical 1mm dark firm nodule.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	9	Sex	Male
Dose Group	3 - 3mg/kg PP796 plus 20mg/kg Pq ion				
Duration	Day 8	~~Died~~/Killed		~~Intercurrent/Interim/~~Term	

MACROSCOPIC FINDINGS

No abnormalities detected.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	10	Sex	Male
Dose Group	4 - 20mg/kg PP796 plus 20mg/kg Pq ion				
Duration	Day 4	~~Died~~/Killed		Intercurrent/~~Interim/Term~~	

MACROSCOPIC FINDINGS

Lungs: large areas dark red consolidation, all surfaces, all lobes covering approximately half of each lobe except diaphragmatic where it covers one third - marked dark red areas
Oesophagus, larynx, trachea and mediastinum - no abnormalities detected. No evidence of misdosing or trauma.
Stomach: medium quantity of food in stomach.

MICROSCOPIC FINDINGS

Lung: moderate pneumonitis (alveolar inflammatory cells, inter-alveolar fibrosis, peribronchiolar fibrosis, focal epithelialisation) alveolar haemorrhage, focal emphysema. Basophilic material and inflammation cells in bronchus.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	11	Sex	Male
Dose Group	4 - 20mg/kg PP796 plus 20mg/kg Paq ion				
Duration	Day 8	~~Died~~/Killed		~~Intercurrent/Interim~~/Term	

MACROSCOPIC FINDINGS

Lungs: left and right cardiac - areas of dark red discolouration affecting ventral half of lobes. Left apical - dark red area 3x2cm - slight dark red area.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	12	Sex	Male
Dose Group	4 - 20mg/kg PP796 plus 20mg/kg Paq ion				
Duration	Day 8	~~Died~~/Killed		~~Intercurrent/Interim~~/Term	

MACROSCOPIC FINDINGS

No abnormalities detected.

PATHOLOGY REPORT FORM

Study Title	PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS				
Study No	XD0574	Animal Number	13	Sex	Male
Dose Group	4 - 20mg/kg PP796 plus 20mg/kg Pq ion				
Duration	Day 8	~~Died~~/Killed	~~Intercurrent/Interim~~/Term		

MACROSCOPIC FINDINGS

Heart: left ventricle, endocardial surface, small (approximately 1-2mm) linear red streaks over interventricular septum and anterior surface.
Lungs: right cardiac lobe, small circular dark red area, ventral surface. Still present after inflation - minimal dark red area.

PP796: EMETIC STUDY IN PARAQUAT TREATED DOGS

CIRCULATION

Internal

1 Report Centre Reference Copy
2 Report Centre - Spare
3 Dr I F H Purchase)
Dr S E Jaggers)
Dr R S Morrod)
4 Dr L L Smith
5 Information Bureau
6 Dr M Robinson
7 Mrs A Brammer
8 Dr J E Doe)
Dr G H Pigott)
9 Dr M C E Hodge
10 Dr M A Collins
11 Mr B H Woollen

External

12 Mr I S Chart, PSRG, PPD, Fernhurst
13 Dr P Slade, Public Affairs Department, PPD, Fernhurst
14 Dr W R T Cottrell, PPD, Fernhurst