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Gramoxone

Supplement to Effects of Increased Emetic Levels on Toxicokinetics in the Dog

026698/Research/Supplement - 001

DATA REQUIREMENT: Not Applicable

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STUDY COMPLETION DATE: 05-Oct-2006

PERFORMING LABORATORY: Syngenta Central Toxicology Laboratory
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LABORATORY PROJECT ID: Report Number: 026698-RES-S1
Study Number: XD1328
Task Number: N/A

SPONSOR: Syngenta Limited
Alderley Park, Macclesfield
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STATEMENT OF DATA CONFIDENTIALITY CLAIM

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

I, the undersigned, declare that the objectives laid down in the protocol were achieved and that the data generated are valid. The report fully and accurately reflects the procedures used and the raw data generated in the above study.

This study was designed for research purposes and was not conducted in compliance with national or international GLP regulations but was conducted to the highest standards of research practice.

The study does not satisfy the requirements of the United States Environmental Protection Agency 40 CFR Part 160 and CFR Part 792.

C Swain	05-Oct-2006
Author	Date

FLAGGING STATEMENT

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REASON FOR SUPPLEMENT

Clarification of the comparisons made between the formulation with increased emetic examined in this study and existing formulations.

In the report, reference is made to the study of Widdop *et al* (1977) where data are reported on the toxicity of a Gramoxone formulation in the dog. This study of Widdop *et al* was considered to be representative of the Gramoxone formulations on sale at the time of the conduct of the study with a formulation with increased emetic. However, the exact composition of the formulation used by Widdop *et al* was not reported other than being a Gramoxone formulation (200g/L) sold in the United Kingdom, and the date of the publication (1977) suggests that the formulation may not have included emetic. Reference is also made to the study of Cockrill and Goburdhun (1998) where data are reported on the toxicity of a Gramoxone formulation in the dog. This study of Cockrill and Goburdhun was also considered to be representative of the Gramoxone formulations on sale at the time of the conduct of the study with a formulation with increased emetic. However, it is now realised that the formulation used in the study of Cockrill and Goburdhun did not contain emetic. While the data of Widdop *et al* and Cockrill and Goburdhun are still considered to be indicative of the general order of toxicity of a 200g/L paraquat formulation containing the standard emetic level (current standard Gramoxone formulations contain emetic at 0.5g/L), the data cannot be taken as an accurate definition of this. In comparing formulations with increased emetic with those currently on sale and containing standard emetic levels it is therefore not appropriate to draw precise quantitative comparisons relating to toxicity between the data generated in this report for the formulation with increased emetic and that of the formulations used by Widdop *et al* or Cockrill and Goburdhun.

The experimental data generated in the report for the formulation with increased emetic are unaffected by this clarification.

The data obtained for the formulation with increased emetic indicate limited paraquat absorption and no lethalities in the dog up to and including a dose of 32mg paraquat ion/kg. This is in excess of the values of 10mg/kg and 20mg/kg used in the Widdop et al and Cockrill and Goburdhun studies respectively that resulted in lethality in all of the dogs. However, lethality in two of three animals was observed following a dose of 48mg paraquat ion/kg of formulation with increased emetic.

This addendum provides clarification for the report where the Widdop *et al* and the Cockrill and Goburdhun studies are referenced and where comparisons with the formulation with increased emetic are made. As a result of the above clarification, the conclusion for the study as displayed in Sections 1.3 and 8 in the report is amended, as follows:

A dose of 16 or 32mg paraquat ion/kg of a formulation containing the equivalent of 2.4g/L emetic for a 200g/L paraquat formulation (increased emetic) was tolerated in the dog. A dose of 48mg paraquat ion/kg resulted in increased absorption of paraquat and two lethalities of three dogs dosed. This indicates a reduction in toxicity against a formulation with no emetic of approximately 3-fold.

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GRAMOXONE

EFFECTS OF INCREASED EMETIC LEVELS ON TOXICOKINETICS IN THE DOG

026698/RESEARCH/REPORT

DATA REQUIREMENT: Not Applicable

AUTHORS: C Swain
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STUDY COMPLETION DATE: 10-May-2006

PERFORMING LABORATORY: Central Toxicology Laboratory
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LABORATORY PROJECT ID: Study Number: XD1328
Report Number: 026698-RES

SPONSOR: Syngenta Limited
Alderley Park, Macclesfield
Cheshire, SK10 4TJ, UK

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

I, the undersigned, declare that the objectives laid down in the protocol were achieved and that the data generated are valid. The report fully and accurately reflects the procedures used and the raw data generated in the above study.

This study was designed for research purposes and was not conducted in compliance with any national or international GLP regulations but was conducted to the highest standards of research practice.

The study does not satisfy the requirements of the United States Environmental Protection Agency 40 CFR Part 160 and CFR Part 792.

C Swain
Author

10-May-2006
Date

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
MA Collins	Study Director (October 1987 – March 1989)
DJ Tinston	Study Director (March 1989 - March 1992)
J Heylings	Study Director (March 1992 - April 1992)
M Farnworth	Toxicokineticist
C Swain	Author

Study dates

Study initiation date: 27th October 1987
Experimental start date:
Experimental termination date:

Deviations from the guidelines

None, this was an investigative study with no applicable guidelines.

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1.0 EXECUTIVE SUMMARY

1.1 Study Design

Data have been extracted from paraquat research studies in the dog, conducted at CTL between 1987 and 1991. The data extracted from these studies provide plasma profiles following an oral dose of Gramoxone with increased levels of emetic. The formulation used in this study was a 200g paraquat ion/L with 0.5g emetic/L diluted to a 100g paraquat ion/L and fortified to 1.2g emetic/L.

Plasma samples were collected from male dogs during the 24h period after dosing and the concentration of paraquat in these plasma samples was determined. The toxicokinetic parameters AUC_{0-1}, AUC_{0-4} and AUC_{0-24} (area under the curve between time zero and 1, 4 and 24h, respectively) were calculated. . Clinical observations, including time to emesis, were made frequently during the 24h period post-dose and twice daily thereafter.

1.2 Results

Increasing the emetic concentration in the Gramoxone formulation by a factor of about 5-fold above the concentration in the current commercial product caused a measurable improvement in the oral toxicity of the formulation. The mean time to emesis was reduced from 19 minutes to 3 minutes over the dose range 16-48mg paraquat ion/kg. At 16 and 32mg paraquat ion/kg, the early emesis reduced the expected plasma paraquat levels and there were minimal clinical signs of toxicity. However, the high concentration of emetic was not protective at a dose level of 48mg paraquat ion/kg, despite the very early time to emesis of 3 minutes. Only one animal showed minimal signs of paraquat toxicity, the other 2 requiring humane intervention following either excessive or prolonged systemic exposure to paraquat, together with clinical signs of paraquat toxicity.

1.3 Conclusion

This investigation into the effect of increasing the concentration of the emetic agent in Gramoxone on the acute toxicity of paraquat in the dog has demonstrated a measurable improvement in oral toxicity. This has been brought about by causing very rapid emesis. However, even this very prompt emesis was only effective in reducing Gramoxone toxicity by approximately 3-fold.

2.0 INTRODUCTION

2.1 Purpose

Data have been extracted from paraquat research studies in the dog, conducted at CTL between 1987 and 1991. This included an assessment of toxicity for Gramoxone with increased levels of emetic and the plasma paraquat profile was determined. The formulation used in this study was a 200g paraquat ion/L with 0.5g emetic/L diluted to a 100g paraquat ion/L and fortified to 1.2g emetic/L. The aim of this study was to investigate whether increasing the concentration of the emetic agent (PP796) would reduce the systemic absorption of paraquat in the dog and thus the acute toxicity of Gramoxone.

2.2 Regulatory guidelines

These were research toxicokinetic studies to determine systemic paraquat exposure and were not designed to comply with any specific regulatory guidelines.

2.3 Justification for test system selection

The dog is the usual non-rodent species for regulatory studies and the Alderley Park beagle was used because of the substantial background data available for this strain, in this laboratory, relating to studies of this type. In addition, the dog has a vomit reflex and the aim of this study was to investigate whether the detoxification mechanism could be improved. The oral route of exposure was chosen for the administration of paraquat, as it is the route of exposure in humans in cases of poisoning from deliberate ingestion.

2.4 Dose level selection

The dose levels of the Gramoxone formulation with increased emetic were selected as 16, 32 and 48mg paraquat ion/kg. These dose levels are 2, 4 and 6-fold higher than the non-toxic 8mg paraquat ion/kg dose previously reported for Gramoxone (Heylings *et al.*, 2004 and Brammer *et al.*, 2004).

2.5 Data storage

The study protocol, all raw data, and a copy of this report will be retained in the Archives, Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK.

3.0 TEST SUBSTANCES

Name:	Gramoxone Export
Source:	ICI, Plant Protection Division
Colour:	Green
Physical state:	Liquid
CTL test substance reference number:	Y00061/131
Purity (%w/w):	200g/L paraquat ion, 0.5g/L PP796
Density (g/L)	1.091
Storage conditions:	Ambient temperature in the dark

Name:	Emetic (PP796)
Source:	ICI, Plant Protection Division
Colour:	Off white
Physical state:	Powder
CTL test substance reference number:	Y00706/018
Purity:	90%
Storage conditions:	Refrigerated

The sample was tested as supplied without correction for purity.

4.0 EXPERIMENTAL PROCEDURES

4.1 Dose preparation

The 100g/L paraquat ion formulation with 1.2g/L emetic was prepared within CTL. The Gramoxone formulation was diluted with de-ionised water to achieve a 10% (w/v) solution containing 0.25g/L emetic, to this additional emetic was added to reach a final concentration of 1.2g/L.

Dogs were dosed with the appropriate volume of test formulation, to achieve the required oral dose in mg paraquat ion/kg, based on the most recent bodyweight. Capsules were prepared locally immediately prior to dosing, using a positive displacement pipette. The test substance was shaken vigorously prior to dispensing. For the 0.96ml/kg dose, the total volume was divided between 4 capsules and for the 0.48ml/kg dose the total volume was divided between 2 capsules due to the large dose volume required whilst for the 0.16ml/kg dose this was given in a single capsule.

4.2 Experimental design

4.2.1 Animals

Species:	Dog.
Breed:	Beagle.
Source:	Dog Animal Breeding Unit, Alderley Park, Macclesfield, Cheshire, UK.
Sex/number:	6 Males.
Specification:	16-32 weeks old on delivery, weight range at dosing was 12.6-19.2kg. Vaccinated against canine viral hepatitis, distemper, leptospirosis, canine influenza and canine parvovirus (prior to delivery to CTL). Regularly treated for possible nematode and ear-mite infestation (prior to and after delivery to CTL).
Selection:	On the basis of normal:- healthy status and bodyweight.
Supply dates:	7th September 1987 (3 males), 5th February 1990 (3 males).

4.2.2 Accommodation and husbandry

The dogs were housed individually in indoor pens, consisting of sleeping quarters (with a heated floor) and a separate exercise area, within the dog house at CTL. The dog house gave the following environmental conditions:

Temperature:	Nominally 20°C.
Relative humidity:	Not controlled.
Air:	Approximately 12 changes/hour.
Light cycle:	Artificial giving 11 hours light, 13 hours dark.

Each morning, each dog received 400g of LABORATORY DIET A (supplied by Special Diet Services Limited, Stepfield, Witham, Essex, UK), an expanded dry diet, which was left in the pen for a 24h period and any remaining food weighed. On the day of dosing no food was provided and was withheld for 24 hours. Mains water, supplied by an automatic system was available *ad libitum*, except on the day of dosing when water was withheld for 1hour before and 1 hour after dosing.

Each batch of diet was routinely analysed by the supplier for composition and for the presence of contaminants. Water was also periodically analysed for the presence of contaminants. No contaminants were found to be present in the diet or water at levels considered to be capable of interfering with the purpose of the study. Certificates of analyses are retained in the CTL Archives.

4.2.3 Acclimatisation

The animals were housed under experimental conditions for at least 1 week at CTL, prior to the start of the study.

4.2.4 Animal randomisation and identification

The dogs were randomly allocated to the treatment groups using a procedure which resulted in the even distribution of dogs to treatment groups according to bodyweight ensuring that litter mates were in different treatment groups. Animals were individually identified (whilst at the breeding unit) by tattooed ear numbers. Following randomisation, these numbers were cross-referenced to the appropriate individual experimental number (Appendix A).

On the front of each pen was a card identifying the contained animal by dose level, group number, unique ear number, experimental number, sex and study.

4.2.5 Dose levels and treatment groups

During these research studies, the formulation was dosed to groups of 3 dogs on three occasions by capsule. The experimental numbers, dose levels and dates of dosing are shown in Appendix A.

4.2.6 Dose administration

Dogs were orally dosed with gelatine capsules containing the appropriate volume of the test substance, at approximately the same time of day. Dogs were fed approximately 24 hours after dosing.

4.2.7 Duration of dose administration

The dates of dosing reported here were from 2 separate groups of dogs dosed on 13th February (Group 1), 21st February (Group 2) and 20th March 1990 (Group 1).

4.3 Clinical observations

All dogs were observed continuously for the first few hours following each dose. The timing, colour, consistency of vomit and faeces were recorded, in addition to other clinical signs.

On non-dosing days, dogs were observed at least twice daily for clinical or behavioural abnormalities (at the beginning and end of the working day). Gastro-intestinal findings were assessed daily throughout the study.

4.4 Veterinary examinations including opthalmoscopy

All dogs were given a full clinical examination by a veterinarian prior to each dose and prior to termination. The examination included cardiac and pulmonary auscultation.

4.5 Bodyweights

All dogs were weighed before feeding, on a weekly basis from arrival at CTL, prior to the day of dosing and thereafter at weekly intervals throughout the study.

4.6 Food consumption

Food residues were recorded 24 hours after feeding and any residual food was discarded. Food was withheld on the day of dosing. Food consumption was recorded throughout the study and was calculated at weekly intervals as a mean value (g food/day) for each dog.

4.7 Toxicokinetics

Blood samples were taken to determine a toxicokinetic profile of paraquat ion following each dose of formulation. Jugular vein blood samples (2ml in lithium heparin) were taken from each dog pre-dose and at 15 and 30 minutes and 1, 2, 4, 7, 12 and 24 hours after dosing. The blood was thoroughly mixed and then the plasma was separated by centrifugation.

Plasma concentrations of paraquat were determined by radioimmunoassay (SOP CT05-085, [Appendix B]). The unknown sample and a series of paraquat standards were each buffered with [^{3}H]-paraquat. Antiserum containing antibodies, raised against a derivative of monoquat, was added. After a short incubation time any free paraquat ion was adsorbed onto a bovine serum albumin-charcoal suspension. After centrifugation the antibody-[^{3}H]-paraquat ion complex in the supernatant was counted in a liquid scintillation counter and the concentration of paraquat ion in the sample was found by comparison with the standards. The LOQ was 0.006µg paraquat ion/ml plasma.

The profile of paraquat in plasma over 24h is presented and the mean (± SEM) concentrations from all the dogs within each dose group were calculated.

The toxicokinetic parameter, area under the curve (AUC) was calculated using the linear trapezoidal rule from pooled groups of animals. The toxicokinetic parameters AUC_{0-1}, AUC_{0-4} and AUC_{0-24} (area under the curve between the time zero and 1h, 4h and 24h respectively) were calculated.

4.8 Termination

Following termination the animals were not subjected to a post mortem examination and no tissues were examined histopathologically.

5.0 DATA EVALUATION

Toxicokinetic parameters were calculated using the linear trapezoidal rule. All numerical values in this report are accurate to the number of significant figures given. However, as these values

may have been rounded up or down for reporting, any calculations performed with rounded values may give results that do not correspond precisely with those reported.

6.0 RESULTS

6.1 Mortalities

The following animals were killed intercurrently due to adverse clinical signs of toxicity and/or high plasma paraquat 24h AUC indicative of paraquat toxicity.

Following the 48mg paraquat ion/kg dose, dog 413 was terminated at 24h post-dose whilst dog 415 was terminated 8 days post-dose, following a period of persistent inappetance. Both terminations had observations consistent with those previously seen following an acutely toxic dose of paraquat.

6.2 Clinical observations

Observations recorded following each dose of the paraquat formulation are presented in Table 2. The principle findings were emesis and the times to first and last emesis are shown below:

Time to first emesis:

16mg paraquat ion/kg	**Dog 413**	**Dog 412**	**Dog 415**	**Mean time to 1st emesis**
	12 minutes	21 minutes	23 minutes	18.7 minutes
32mg paraquat ion/kg	**Dog 437**	**Dog 438**	**Dog 439**	**Mean time to 1st emesis**
	6 minutes	8 minutes	10 minutes	8 minutes
48mg paraquat ion/kg	**Dog 413**	**Dog 412**	**Dog 415**	**Mean time to 1st emesis**
	3 minutes	6 minutes	1 minute	3.3 minutes

Time to last emesis:

16mg paraquat ion/kg	**Dog 413**	**Dog 412**	**Dog 415**
	69 minutes	68 minutes	64 minutes
32mg paraquat ion/kg	**Dog 437**	**Dog 438**	**Dog 439**
	69 minutes	68 minutes	43 minutes
48mg paraquat ion/kg	**Dog 413**	**Dog 412**	**Dog 415**
	6 hours 58 minutes	52 minutes	11 minutes

With a higher dose of paraquat ion (and hence, increased dose of emetic), the time to first emesis was 1-6 minutes post-dosing at the highest dose compared with 12-23 minutes for the lowest dose. There were no real differences in the number of episodes of emesis across the dose levels (Table 1) with the exception of Dog 413 at the 48mg paraquat ion/kg dose where emesis was still occurring more than 6h post-dose.

6.3 Veterinary examination

There were no significant veterinary findings and no auscultatory abnormalities detected prior to dosing in any animal. At the lower dose levels all animals were clinically normal. Following the highest dose of 48mg paraquat ion/kg dog 413 was terminated for humane reasons at 24h post-dose. Dog. 415 was terminated 8 days post-dosing following a period of inappetance and a 15% weight loss following the 48mg paraquat ion/kg dose.

6.4 Bodyweights

Dog 437 lost 0.7kg following the 32mg paraquat ion/kg dose and dog 415 lost 2.8kg following the 48mg paraquat ion/kg dose (Table 2). There we no other major effects on bodyweights over the duration of the study.

6.5 Food consumption

Dog 415 became inappetant the week after the 16mg paraquat ion/kg dose but returned to normal eating patterns. However, following the 48mg paraquat ion/kg dose the animal again became inappetant and despite being given moistened diet, did not eat any of this diet.

There were no other significant effects on food consumption during the study (Table 3).

6.6 Toxicokinetics

The mean plasma paraquat concentrations are shown in Figure 1, mean plasma paraquat AUC values are shown in Figure 2 and Table 4. Individual plasma paraquat concentrations are shown in Figure 3 and Table 5, whilst AUC values are given in Table 6.

Following the 16mg paraquat ion/kg dose the mean peak plasma level occurred at 1h post-dose with levels of 4.91µg/ml detected, the level remained constant until 2h after which paraquat was steadily eliminated with levels of 0.22µg/ml detected at 7h post-dose and by 24h no paraquat was detected in the plasma.

When the dose was increased to 32mg paraquat ion/kg the mean peak plasma level occurred earlier at 30 minutes post-dose with levels of 3.81µg/ml detected, elimination of parquet from the plasma was steady until 7h with levels of 0.66µg/ml measured. After which elimination of paraquat continued at a slower rate with none detected at 24h post-dose.

Following the highest dose of 48mg paraquat ion/kg the mean peak plasma level occurred at 1h post-dose with levels of 4.95µg/ml, the elimination of paraquat from the plasma was slower at this dose than the previous doses, such that a 7h post dose there was still 1.21µg/ml in the plasma. At 12h there was a slight bounce in the profile due to increased plasma paraquat levels in dogs 413 and 415, by 24h there was a mean concentration of 0.23µg/ml. There was more variability in the individual responses at this dose level with dog 413 having a much greater absorption of paraquat across the 24h period than the other 2 animals.

The overall paraquat exposure, as measured by the AUC, was similar at the lower dose levels. Once the dose was increased to 48mg paraquat ion/kg there was more variability between the animals and the mean AUC values were greater than seen at the previous doses, this was largely influenced by dog 413 which had a 24h value of 81.03µg/ml.h.

7.0 DISCUSSION

For reference, the concentration of emetic in standard commercial Gramoxone formulation which contains 200g paraquat ion/L is 0.5g/L. In this study the commercial product was diluted to 100g paraquat ion/L and fortified to 1.2g/L emetic, equivalent to Gramoxone containing 2.4g/L emetic, or about five times that in the commercial formulation.

This investigation has demonstrated that increasing the emetic concentration in the Gramoxone formulation produces a measurable reduction in absorption of paraquat and a reduction in the oral toxicity of the formulation. However, the high concentration of emetic was only protective at dose levels of 16 and 32mg/kg paraquat ion and proved ineffective at 48mg/kg.

Previous studies have demonstrated that a single oral dose of Gramoxone equivalent to 8mg paraquat ion/kg ion is non-toxic to dogs (Heylings *et al*, 2004 and Brammer *et al.*, 2004). Since the median lethal dose for Gramoxone in this strain of dog is reported to be around 12mg paraquat ion/kg (Cockrill JB and Goburdhun R, 1988), a value that is consistent with other published studies (Widdop *et al*, 1977), we therefore evaluated a dose level just above the MLD, 16mg paraquat ion/kg with high emetic. Paraquat was removed effectively by vomiting in all dogs at 16mg/kg paraquat, following this Gramoxone formulation containing high emetic. This resulted in relatively low levels of paraquat in the blood by 7 hours after dosing. All dogs remained clinically healthy. On the basis of the minimal clinical observations at 16mg paraquat ion/kg, the dose was doubled to 32mg paraquat ion/kg in a follow up experiment. All the animals vomited very early (mean 8 minutes) following a single oral dose of 32mg paraquat ion/kg, and the remaining paraquat available for absorption was insufficient to cause toxicity. In fact, the prompt emesis resulted in a slightly lower plasma paraquat profile and AUC compared with 16mg paraquat ion/kg, despite the higher paraquat dose.

One of the animals at 32mg paraquat ion/kg had a peak paraquat level approaching 6µg/ml which from previous kinetic studies indicated potential toxicity. Although this did not prove to be the case here, a decision was taken not to double the dose to 64mg paraquat ion/kg for the next experiment, but an intermediate dose of 48mg paraquat ion/kg was selected. At this higher paraquat dose there was toxicity consistent with that observed previously with paraquat. The high emetic loading caused very rapid emesis (mean 3 minutes) but this was not enough to prevent what would be a lethal dose of paraquat from being absorbed. Dog 413 had a peak plasma paraquat above 8µg/ml with a 24h AUC of 81µg/ml.h and persistent emesis was observed in this animal up to nearly 7h post-dose. These kinetic values are consistent with a toxic systemic exposure to paraquat. An additional feature of the 48mg paraquat ion/kg dose was the elevated plasma value in another animal (Dog 415) at 12h. At non-toxic doses of paraquat, renal elimination of the chemical from the blood is very effective. When this is compromised there is often a secondary elevated blood level between 12-24h, as observed here. This animal did not fully recover from this exposure to paraquat and was humanely terminated

several days into the study due to body weight loss and persistent inappetance. The 48mg paraquat ion/kg dose level was clearly toxic to the dog in 2 out of the 3 animals and therefore represents a dose above the median lethal dose for this formulation of Gramoxone containing the high concentration of emetic. We would therefore anticipate that increasing the emetic concentration offers an improvement in oral safening of about 3-fold compared with standard Gramoxone in the dog.

8.0 CONCLUSION

This investigation into the effect of increasing the concentration of the emetic agent in Gramoxone on the acute toxicity of paraquat in the dog has demonstrated a measurable improvement in oral toxicity. This has been brought about by causing very rapid emesis. However, even this very prompt emesis was only effective in reducing Gramoxone toxicity by approximately 3-fold.

9.0 REFERENCES

Brammer A, Heylings JR and Swain C (2004). Gramoxone 200g/L SL formulation (A3879D) – Toxicokinetic study in the dog. Syngenta Report Number CTL/XD7388/REGULATORY/REPORT.

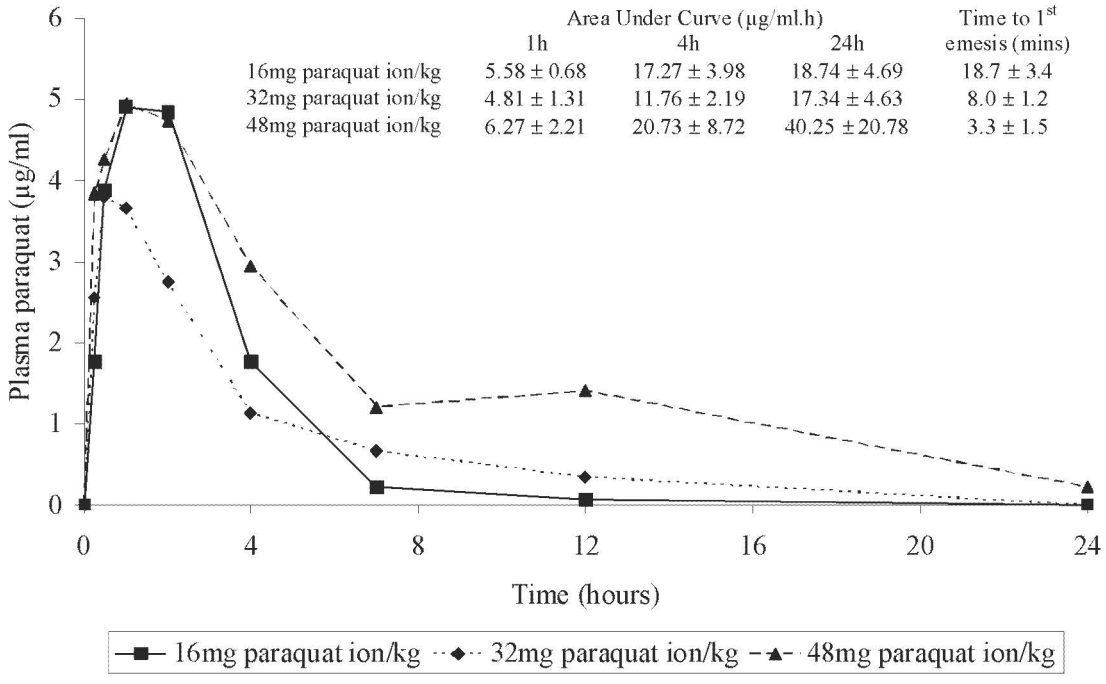
Cockrill JB and Goburdhun R (1988). Gramoxone single dose oral toxicity study in dogs. Inveresk Research International Report Number 3749. CTL/C/2103.

Heylings JR, Swain C, and Brammer A (2004). Paraquat: Gramoxone 200g/L formulation – Toxicokinetics in the dog. Syngenta Report Number CTL/026118/RESEARCH/REPORT.

Widdop B, Medd RK and Braithwaite RA (1977). Charcoal haemoperfusion in the treatment of paraquat poisoning. *Proc. Eur. Soc. Tox.*, **18,** 156-159.

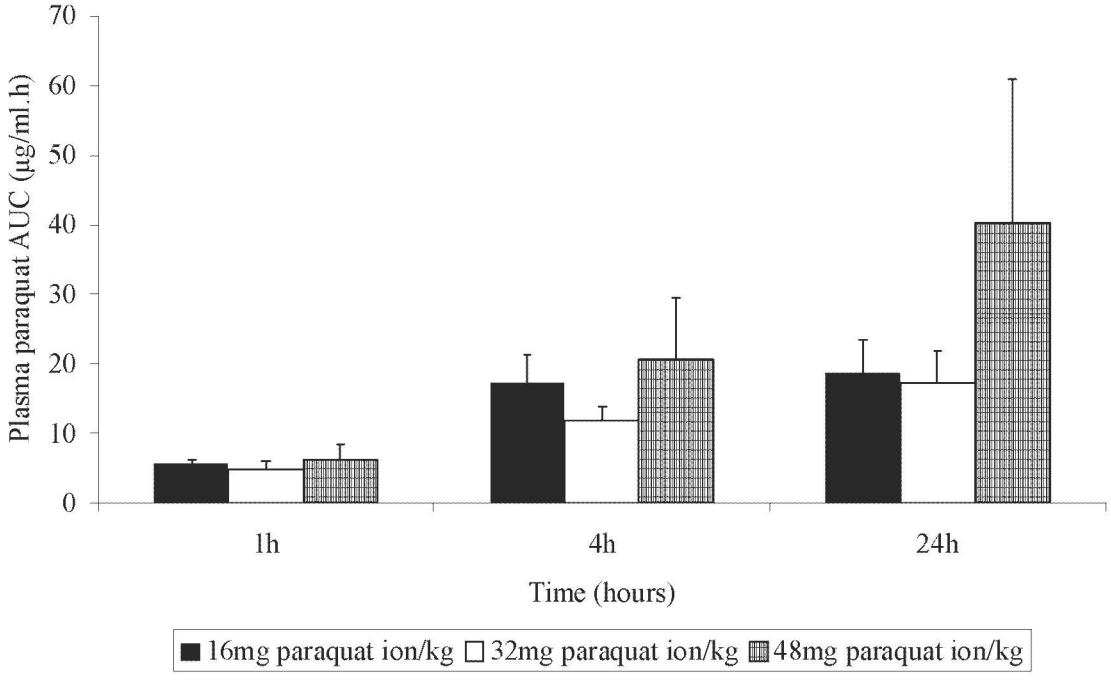
FIGURES SECTION

FIGURE 1 MEAN PLASMA PARAQUAT CONCENTRATIONS



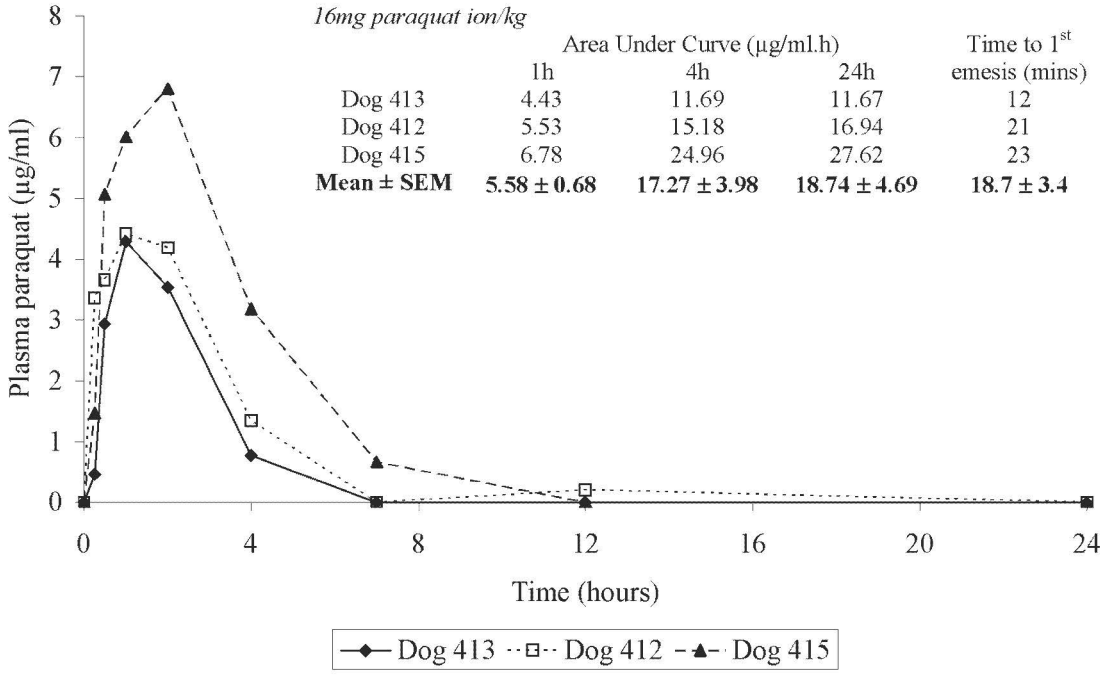
Values are means (n=3).

FIGURE 2 MEAN PLASMA PARAQUAT AREA UNDER THE CURVE



Values are means ± SEM (n=3).

FIGURE 3 INDIVIDUAL PLASMA PARAQUAT CONCENTRATIONS



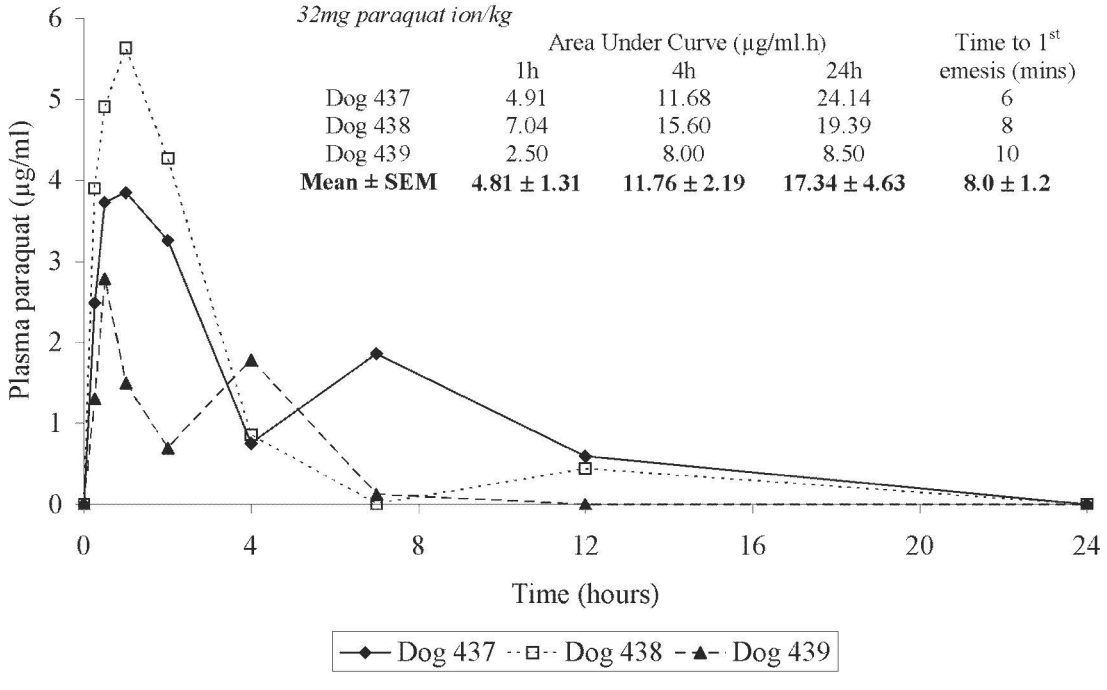
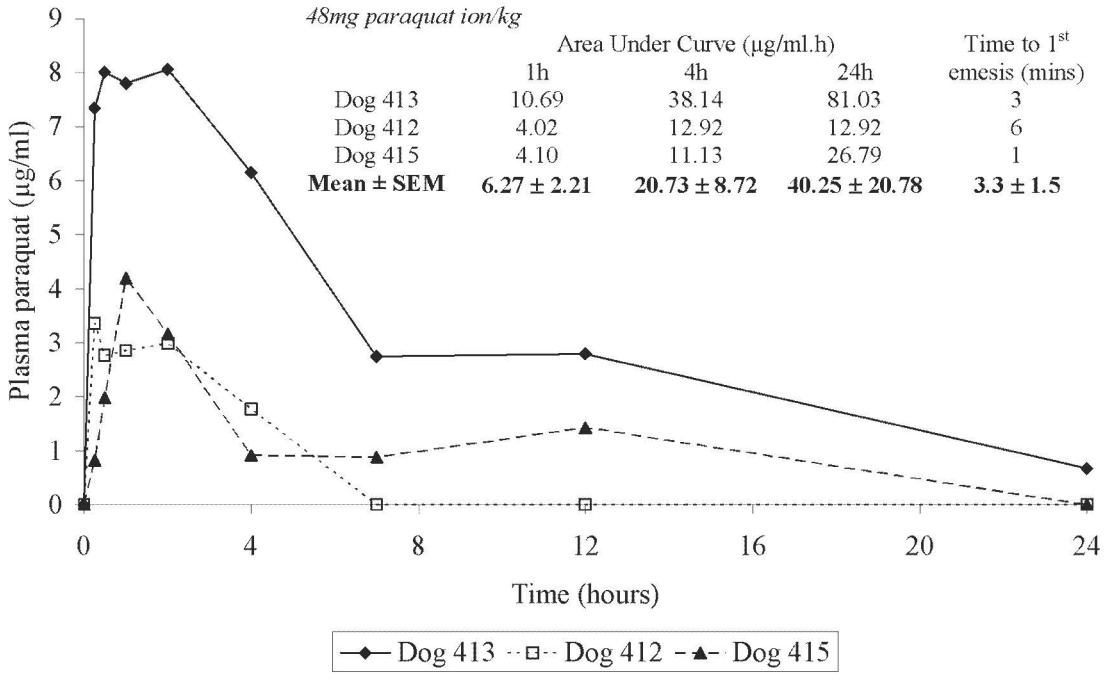


FIGURE 3 INDIVIDUAL PLASMA PARAQUAT CONCENTRATIONS


48mg paraquat ion/kg
Area Under Curve (µg/ml.h)
Time to 1st emesis (mins)
1h
4h
24h
Dog 413 10.69 38.14 81.03 3
Dog 412 4.02 12.92 12.92 6
Dog 415 4.10 11.13 26.79 1
Mean ± SEM 6.27 ± 2.21 20.73 ± 8.72 40.25 ± 20.78 3.3 ± 1.5
Plasma paraquat (µg/ml)
Time (hours)
Dog 413
Dog 412
Dog 415

TABLES SECTION

TABLE 1 INDIVIDUAL CLINICAL OBSERVATIONS

Study Number: XD1328 (E32) Date: 13th February 1990 Dose: 16mg paraquat ion/kg

Dog 413: 15.9kg

Time after dosing	Description
12 minutes	2 small pools frothy vomit
26 minutes	3 small pools frothy vomit
42 minutes	2 large pools frothy vomit
69 minutes	Large pool frothy vomit

Dog 412: 12.9kg

Time after dosing	Description
21 minutes	1 large, 1 medium and 1 small pool frothy vomit
29 minutes	Large pool frothy mucus like vomit
32 minutes	Medium pool of frothy vomit
39 minutes	Large pool thick white vomit
43 minutes	Large pool frothy vomit
46 minutes	Medium pool frothy vomit
56 minutes	1 large and 1 small pool of frothy vomit
68 minutes	Medium pool of frothy vomit

Dog 415: 19.2kg

Time after dosing	Description
23 minutes	Very small pool frothy vomit
30 minutes	Large pool frothy vomit
59 minutes	2 large pools frothy vomit
64 minutes	Medium pool frothy vomit

TABLE 1 INDIVIDUAL CLINICAL OBSERVATIONS (CONTINUED)

Study Number: XD1328 (E33) Date: 21st February 1990 Dose: 32mg paraquat ion/kg

Dog 437: 19.4kg

Time after dosing	Description
6 minutes	Green vomit
11 minutes	Pool vomit
19 minutes	2 pools frothy vomit
33 minutes	White frothy vomit
49 minutes	Large pool liquid vomit
53 minutes	Large pool frothy vomit
61 minutes	Medium pool liquid vomit
69 minutes	Small pool bilious vomit

Dog 438: 17.6kg

Time after dosing	Description
8 minutes	Bright green vomit
17 minutes	Pool vomit
21 minutes	2 medium pools blue vomit
32 minutes	Pool white frothy vomit
35 minutes	Small pool white frothy vomit
41 minutes	Small pool white frothy vomit
47 minutes	Medium pool white frothy vomit
61 minutes	Medium pool white frothy vomit
68 minutes	Medium pool white viscous vomit

Dog 439: 17.1kg

Time after dosing	Description
10 minutes	Yellow-green vomit
16 minutes	Small pool yellow-green vomit
20 minutes	Pool of pale blue vomit
29 minutes	White frothy vomit
34 minutes	Small pool white frothy vomit
43 minutes	Small pool vomit

TABLE 1 INDIVIDUAL CLINICAL OBSERVATIONS (CONTINUED)

Study Number: XD1328 (E34) Date: 20th March 1990 Dose: 48mg paraquat ion/kg

Dog 413: 16.2kg

Time after dosing	Description
3 minutes	Large pool vomit
9 minutes	Vomit
19 minutes	Medium pool vomit
24 minutes	Runny green bile like vomit
25 minutes	Large pool frothy vomit
29 minutes	Medium pool frothy vomit
33 minutes	Diarrhoea
53 minutes	Panting, shaking
1 hour 10 minutes	Retching, shaking, animal lying down
2 hours 15 minutes	3 pools frothy vomit, shaking
6 hours 53 minutes	Still vomiting, shaking, panting
6 hours 58 minutes	Small pool brown frothy vomit

Dog 412: 12.6kg

Time after dosing	Description
6 minutes	2 pools vomit
12 minutes	Small pool green vomit
44 minutes	Pool frothy vomit
52 minutes	1 large and 1 medium pool frothy vomit

Dog 415: 18.8kg

Time after dosing	Description
1 minute	Medium pool vomit
3 minutes	Medium pool vomit
11 minutes	Medium pool vomit

TABLE 2 INDIVIDUAL BODYWEIGHTS (KG)

Study Number: XD1328		Experimental Number		
Date	Week Number	413	412	415
05.02.1990	116	15.9	13.1	19.3
12.02.1990	**117**	**15.9**	**12.9**	**19.2**
19.02.1990	118	16.1	12.5	18.8
26.02.1990	119	15.8	12.4	18.6
05.03.1990	120	16.1	12.5	19.0
12.03.1990	121	15.9	12.4	18.9
19.03.1990	**122**	**16.2**	**12.6**	**18.8**
26.03.1990	123		12.4	16.0
02.04.1990	124		12.7	
09.04.1990	125		12.8	
17.04.1990	126		12.9	
23.04.1990	127		12.9	
30.04.1990	128		12.5	

Study Number: XD1328		Experimental Number		
Date	Week Number	437	438	439
12.02.1990	1	19.8		17.5
19.02.1990	**2**	**19.4**	**17.6**	**17.1**
26.02.1990	3	18.7	17.3	16.7
05.03.1990	4	18.8	17.6	16.7
12.03.1990	5	18.4	17.5	16.6
19.03.1990	6	18.6	17.6	16.7
26.03.1990	7	18.3	17.7	16.5
02.04.1990	8	18.4	17.7	16.6
09.04.1990	9	18.0	17.5	16.2
17.04.1990	10	18.0	17.6	16.1
23.04.1990	11	18.0	17.6	16.2

The weighs shown in bold text are the pre-dose weights used for dose calculations.

TABLE 3 FOOD CONSUMPTION (g/dog/day)

Week Number	Experimental Number		
	413	412	415
	400	400	400
116	400	400	400
117	**400**	**400**	**400**
118	400	400	258
119	400	400	400
120	400	400	400
121	400	400	400
122	**400**	**400**	**123**
123	DEAD	400	0 (moistened)
124		400	DEAD
125		400	
126		400	
127		400	

Week Number	Experimental Number		
	437	438	439
1	400	400	400
2	**400**	**400**	**400**
3	400	400	400
4	400	400	400
5	400	400	400
6	400	400	400
7	400	400	400
8	400	400	400

Values are mean daily food consumption. The values in bold relate to the week in which the animals were dosed.

TABLE 4 PLASMA PARAQUAT AREA UNDER CURVE

Dose received	Rate of paraquat absorption at 15 minutes (ng/ml/min)	Paraquat AUC (µg/ml.h)		
		1h	4h	24h
16mg paraquat ion/kg	117.56 ± 56.62	5.58 ± 0.68	17.27 ± 3.98	18.74 ± 4.69
32mg paraquat ion/kg	170.89 ± 49.90	4.81 ± 1.31	11.76 ± 2.19	17.34 ± 4.63
48mg paraquat ion/kg	256.20 ± 126.58	6.27 ± 2.21	20.73 ± 8.72	40.25 ± 20.78

TABLE 5 INDIVIDUAL PLASMA PARAQUAT CONCENTRATIONS

Dose received	Animal number	Time (hours)/Plasma paraquat concentration (µg/ml)								
		0	0.25	0.5	1	2	4	7	12	24
16mg paraquat ion/kg	413	0.00	0.46	2.93	4.29	3.54	0.77	0.00	0.00	0.00
	412	0.00	3.36	3.66	4.42	4.20	1.34	0.00	0.21	0.00
	415	0.00	1.47	5.06	6.02	6.82	3.18	0.67	0.00	0.00
	Mean	**0.00**	**1.76**	**3.88**	**4.91**	**4.85**	**1.77**	**0.22**	**0.07**	**0.00**
	St. Dev.	**0.00**	**1.47**	**1.08**	**0.96**	**1.73**	**1.26**	**0.38**	**0.12**	**0.00**
	SEM	**0.00**	**0.85**	**0.63**	**0.55**	**1.00**	**0.73**	**0.22**	**0.07**	**0.00**
32mg paraquat ion/kg	437	0.00	2.49	3.73	3.85	3.26	0.75	1.86	0.59	0.00
	438	0.00	3.90	4.91	5.63	4.27	0.86	0.01	0.44	0.00
	439	0.00	1.31	2.79	1.50	0.70	1.79	0.12	0.00	0.00
	Mean	**0.00**	**2.56**	**3.81**	**3.66**	**2.74**	**1.13**	**0.66**	**0.34**	**0.00**
	St. Dev.	**0.00**	**1.30**	**1.06**	**2.07**	**1.84**	**0.57**	**1.04**	**0.31**	**0.00**
	SEM	**0.00**	**0.75**	**0.61**	**1.20**	**1.06**	**0.33**	**0.60**	**0.18**	**0.00**
48mg paraquat ion/kg	413	0.00	7.35	8.01	7.80	8.06	6.15	2.75	2.80	0.68
	412	0.00	3.36	2.76	2.85	2.99	1.77	0.00	0.00	0.00
	415	0.00	0.82	1.99	4.20	3.16	0.92	0.88	1.43	0.00
	Mean	**0.00**	**3.84**	**4.25**	**4.95**	**4.73**	**2.94**	**1.21**	**1.41**	**0.23**
	St. Dev.	**0.00**	**3.29**	**3.28**	**2.56**	**2.88**	**2.81**	**1.41**	**1.40**	**0.39**
	SEM	**0.00**	**1.90**	**1.89**	**1.48**	**1.66**	**1.62**	**0.81**	**0.81**	**0.23**

TABLE 6 INDIVIDUAL PLASMA PARAQUAT ABSORPTION AND AREA UNDER CURVE

Dose received	Animal number	Rate of paraquat absorption at 15 minutes (ng/ml/min)	Paraquat AUC (µg/ml/h)		
			1h	4h	24h
16mg paraquat ion/kg	413	30.87	4.43	11.67	11.69
	412	224.00	5.53	15.18	16.94
	415	97.80	6.78	24.96	27.62
	Mean	**117.56**	**5.58**	**17.27**	**18.74**
	St. Dev.	**98.07**	**1.17**	**6.89**	**8.13**
	SEM	**56.62**	**0.68**	**3.98**	**4.69**
32mg paraquat ion/kg	437	165.67	4.91	11.68	24.14
	438	259.80	7.04	15.60	19.39
	439	87.20	2.50	8.00	8.50
	Mean	**170.89**	**4.81**	**11.76**	**17.34**
	St. Dev.	**86.42**	**2.27**	**3.80**	**8.02**
	SEM	**49.90**	**1.31**	**2.19**	**4.63**
48mg paraquat ion/kg	413	489.73	10.69	38.14	81.03
	412	224.07	4.02	12.92	12.92
	415	54.80	4.10	11.13	26.79
	Mean	**256.20**	**6.27**	**20.73**	**40.25**
	St. Dev.	**219.24**	**3.83**	**15.10**	**35.99**
	SEM	**126.58**	**2.21**	**8.72**	**20.78**

APPENDICES SECTION

APPENDIX A DETAILS OF DOGS, METHOD OF DOSING, START AND TERMINATION DATES

Experimental number	Unique ear number	Date of Birth	Delivery date	Study number	Date of dosing	Dose received	Date of termination
413	427/87	06.05.1987	07.09.1987	XD1328 (E32)	13.02.1990	16mg paraquat ion/kg by gelatine capsule	22.03.1990
412	397/87	28.04.1987	07.09.1987				03.05.1990
415	432/87	06.05.1987	07.09.1987				28.03.1990
437	721/89	30.06.1989	05.02.1990	XD1328 (E33)	21.02.1990	32mg paraquat ion/kg by gelatine capsule	16.08.1990
438	612/89	02.06.1989	05.02.1990				17.01.1991
439	759/89	09.07.1989	05.02.1990				15.04.1992
413	427/87	06.05.1987	07.09.1987	XD1328 (E34)	20.03.1990	64mg paraquat ion/kg by gelatine capsule	22.03.1990
412	397/87	28.04.1987	07.09.1987				03.05.1990
415	432/87	06.05.1987	07.09.1987				28.03.1990

APPENDIX B THE DETERMINATON OF PARAQUAT IN PLASMA BY RADIOIMMUNOASSAY

Taken from SOP CT05-085, Version 5.

1. Scope

The method described below is suitable for the determination of paraquat ion (1,1'-dimethyl-4,4'-bipyridyldiylium ion) in plasma, tissue and urine. The limits of detection are 6ng paraquat ion/ml of plasma, 30ng paraquat ion/ml of urine and 100ng paraquat ion/g tissue.

2. Method Summary

The unknown sample and a series of paraquat standards are each buffered and mixed with [^{3}H]-paraquat. Antiserum containing antibodies, raised against a derivative of monoquat, is added. After a short incubation time any free paraquat ion was adsorbed onto a bovine serum albumin-charcoal suspension. After centrifugation the antibody-[^{3}H]-paraquat ion complex in the supernatant is counted in a liquid scintillation counter and the concentration of paraquat ion in the sample was found by comparison with the standards. The assay range is 0.3-50ng paraquat ion/assay tube and the limit of detection is 60pg/assay tube.

3. Reagents

a) Analytical grade paraquat (ICI, Plant Protection Division).
b) [Methyl-^{3}H]-paraquat dichloride containing 3Ci/mmol (The Radiochemical Centre, Amersham). Kept at 4°C.
c) Horses serum inactivated, mycoplasma screened (Gibco).
d) Sodium phosphate buffer (SPB, 0.01M, pH 7.0) prepared by combining 16.5ml of a solution of 0.2M $NaH_2PO_4.2H_2O$ with 33.5ml of a solution of 0.2M Na_2HPO_4 anhydrous made up to 1L and adjusted to pH 7.0 if necessary. This may be stored at room temperature.
e) Gelatine buffer prepared by dissolving gelatine (1g/L) in SPB. Warming (30-40°C) may be necessary to dissolve the gelatine. Make sure that the solution is clear at room temperature with Ph 7.0. This buffer is stored in the refrigerator at 4°C.
f) Paraquat standards made up in gelatine buffer containing 0, 30, 80, 200, 500, 750, 1000, 2500 and 5000ng paraquat ion/ml. These are kept in a refrigerator.
g) Charcoal suspension containing Norit A charcoal (2.5g), Bovine serum albumin (300mg) and sodium azide (100mg)/100ml SPB (Norit A [Sigma Chemicals Ltd.]; NaN_3 and BSA [BDH Ltd.]). The charcoal suspension is stirred for 24h before it is used and is kept at room temperature and no more than 300ml is made up at any one time.
h) PCS, Liquid scintillant (Amersham).
i) Antiserum containing polyclonal antibodies to paraquat ion (obtainable from Acute Toxicity Section, Immunology Group). After its titre has been determined it is stored in aliquots deep-frozen.
j) 6% perchloric acid (BDH Ltd.)

k) 5M potassium hydroxide
l) Sodium hydroxide/potassium phosphate/gelatine buffer (0.1M, pH7.0) prepared by diluting 50ml of 1M KH_2PO_4 and 29.1ml of 1M NaOH, to 1L and adjusting the pH to 7.0 if necessary. Gelatine (2.5g/L) and sodium azide (2.5g/L) are then added to this solution. Heating (30-40°C) may be necessary to dissolve the gelatine. This buffer is stored at 4°C.

4. Safety Comments

Normal precautions for handling biological materials should be taken. Disposable gloves must be worn at all times. Particular care should be taken when homogenising tissue samples which must be carried out in a fume cupboard. Any spillage should be wiped up immediately and the bench washed down with savlon liquid. Handling of radioactive materials must be done in a designated area protected with Benchkote (absorbent side up) which must be replaced regularly.

Perchloric acid and potassium hydroxide are very corrosive, PCS scintillant is toluene based. Avoid inhalation of vapour.

5. Apparatus

a) Liquid scintillation counter, eg. Packard Tri Carb 2000CA.
b) Centrifuge capable of spinning tubes at 1400g, eg. MSE Mistral 3000 with 4-place windshield swing out head.
c) Tissue homogeniser, eg. Ultra-Turrax, type TP 18-10.
d) 5ml polypropylene or polystyrene disposable tubes to fit centrifuge buckets (Nunc, Gibco).
e) Vortex mixer.
f) Eppendorf multipipette 4780 with 2.5 and 5ml combitips.
g) 10ml Jencons RePette.
h) 10µl, 50µl and 1ml dispensers, eg. Gilson pipetteman P_{20}, P_{200} and P_{1000}.
i) 1ml and 10ml repeat dispensers, eg. BCL, Oxford Laboratories, Pipettor model R and model SA-L.
j) 10ml and 20ml polypropylene or polystyrene tubes with screw caps (Sterilin).
k) 1.2ml polypropylene tubes with screw on caps (Gibco).
l) Blunt needles with Luer fittings (BCL, Oxford Laboratories).
m) 20ml polyethylene scintillation vials (Packard).

APPENDIX B THE DETERMINATON OF PARAQUAT IN PLASMA BY RADIOIMMUNOASSAY (CONTINUED)

6. Experimental procedures

6.1 Preparation of a stock solution of [^{3}H]-paraquat

Although the quantity of tritiated paraquat added to each tube is not critical it is found that the following procedure gives rise to an inhibition curve which allows a reasonable compromise between sensitivity and range for the measurement of paraquat. Dilute solutions of paraquat are absorbed rapidly onto glassware from aqueous solution. This absorption is greatly reduced by using polystyrene or polypropylene apparatus and is reduced further to negligible proportions if it is dissolved in gelatine buffer. Therefore, paraquat standards containing 5µg/ml or less and tritiated paraquat solutions are made up in gelatine buffer. Such solutions do not show any signs of deterioration when stored at 4°C for several months.

At the present time (March 1988) the sensitivity of the antiserum is such that approximately 80,000dpm/100µl [^{3}H]-methyl paraquat provides the sensitivity required. If the sensitivity alters in the future, then the amount of tracer will need to be altered, ie. if the sensitivity of the antiserum increases then the amount of tracer will be increased and vice versa.

The stock solution appears to be stable at room temperature although it is usually kept at 4°C when not in use. The solution may be frozen but the count rate should be checked when the sample is re-thawed.

6.2 Serial dilution curve to find the titre of the antiserum

The antiserum is supplied as neat plasma. It is aliquoted into plastic tubes which are then kept frozen (-20°C) until needed. Upon re-thawing a convenient volume of SPB, which has been pre-determined, is added to make a working solution of the antibody.

Method used for titre determination

Using the pipette which will be used for aliquoting the antiserum into the tubes to be frozen at -20°C, add the antiserum (50µ) to a plastic tube and the make the solution up to 1ml (SPB). This solution (Soln W) will have a final anti serum dilution of:

$$1: (1/0.05)*(1.15/0.1) = 1:230$$

Take Soln W (0.5ml) and serially dilute with SPB to give solutions X, Y, Z, etc containing antiserum of dilution 1:460, 1:920, 1:1840, etc.

Using the apparatus to be used in the assay:

a) Set the charcoal suspension stirring.
b) Add horse serum (50µl) to twelve 5ml plastic tubes. Use the eppendorf multipipette with 2.5ml combitip.
c) To tubes 1-4 add 100µl of Soln W (P_{200} reverse pipetting technique).
d) Add 100µl of solutions X,Y,Z to tubes 5 and 6, 7 and 8, 9 and 10 respectively (P_{200} reverse pipetting technique).
e) Add 100µl of SPB to tubes 11 and 12 (P_{200} reverse pipetting technique).

APPENDIX B THE DETERMINATON OF PARAQUAT IN PLASMA BY RADIOIMMUNOASSAY (CONTINUED)

f) Add 100µl of SPB to tubes 1 and 2 (P_{200} reverse pipetting technique). These tubes will determine the total counts.
g) Add 0.8ml of gelatine buffer to each of the 12 tubes (BCL, 1ml repeat dispenser). Whirlimix the tubes.
h) Add 100µl of stock [^{3}H]-paraquat solution to each of the 12 tubes. Use the eppendorf multipipette 4780 with 5.0ml combitip.
i) Whirlimix all tubes and incubate for at least 5mins (≥1h).
j) Add 100µl of charcoal suspension to tubes 3-12. Use the Jencons RePette fitted with a blunt needle.
k) Four minutes after the addition of charcoal start the centrifuge.
l) Centrifuge at 1400g for at least 15 minutes (2,500rpm in the Mistral 3000).
m) Dispense PCS scintillant (10ml) into 12 counting vials. Use a 10ml BCL repeat dispenser.
n) Pour off the supernatant from the 12 tubes into the appropriate vials. Cap the vials and shake gently until the solution is clear.
o) Count the vial for 1 minute (or longer if time permits) in the liquid scintillation counter.

Determine the percentage binding (B-N)/T * 100.

Where B = Bound radioactivity = count rates for tubes 3-10.
N = Non-specific binding = count rates for tubes 11 and 12.
T = Total radioactivity = count rates for tubes 1 and 2.

Plot percentage binding against the dilution factor. Strictly speaking the percentage binding is the ratio of the contents bound, after correction for non-specific binding, to the maximum contents bound at low dilutions of the antiserum. To avoid wasting antiserum and because we have found the maximum percentage bound to be 90-96% of the total counts we determine the titre in relation to the total counts added. Find the dilution which will give a percentage binging of 50%. Having found the dilution work out the amount of antiserum in frozen tubes ready to use (volume V_1).

$$\text{Titre } 50\% = (V_1 + V_2)/V_1 * (V_4/V_3)$$

Where V_1 = Volume of aliquot into tube before freezing (ml).
V_2 = Volume SPB added to tube after thawing to give working strength antibody solution (ml).
V_3 = Volume of working strength antiserum added to each assay tube (ml).
V_4 = Total volume of incubate in each assay tube (ml).

Eg. If we require 3ml of working strength antiserum having a titre of 1:3000, then:

$$3000 = (V_1 + 3)/V_1 * (1.15/0.1)\text{, whereby } V_1 = 11.5\mu l$$

Therefore, 11.5µl of neat antiserum is aliquoted into each tube to be frozen for storage.

6.3 Analysis of plasma

a) Prepare a series of standards in duplicate. Take two different dilutions in duplicate (10µl and 50µl) for each plasma sample (use reverse pipetting for standards and samples). Set up as detailed in Table A.
b) Add 50µl of horse serum as indicated in Table A. Use an eppendorf multipipette with 2.5ml combitip.
c) Add 100µl of stock [^{3}H]-paraquat to all tubes. Use an eppendorf multipipette with 5.0ml combitip.
d) Add 0.8ml of gelatine buffer to each tube (BCL repeat dispenser) and whirlimix all tubes.
e) Set the charcoal suspension stirring.
f) Add 100µl of antiserum to each tube. The antiserum, has been previously diluted to produce 50% binding as found by the titre. Use an eppendorf multipipette with 5.0ml combitip.
g) Whirlimix all tubes and leave to incubate for at least 5 minutes. (≥1h).
h) Add charcoal suspension to all the tubes using a Jencons RePette fitted with a blunt needle.
i) After 4 minutes centrifuge at 1400g (2,500rpm in the Mistral 3000) for at least 15 minutes. For a large number of tubes (>80) a contact time of up to 6 minutes may be used.
j) Dispense PCS scintillant (10ml) into the appropriate number of counting vials.
k) Pour off the supernatants from the standards and sample tubes into the appropriate vials, cap the vials and shake gently until the solution is clear.

Table A: Contents of assay tubes for the analysis of paraquat in plasma

Sample	Volume of sample (µl)	Volume of horse serum (µl)	Volume of [^{3}H]-paraquat (µl)	Volume of gelatine buffer (ml)	Volume of antiserum (at the initial dilution found by the titre) [µl]	Volume of charcoal suspension (µl)
Standard 0ng/ml	10	50	100	0.8	100	100
Standard 30ng/ml	10	50	100	0.8	100	100
Standard 80ng/ml	10	50	100	0.8	100	100
Standard 200ng/ml	10	50	100	0.8	100	100
Standard 500ng/ml	10	50	100	0.8	100	100
Standard 750ng/ml	10	50	100	0.8	100	100
Standard 1000ng/ml	10	50	100	0.8	100	100
Standard 2500ng/ml	10	50	100	0.8	100	100
Standard 5000ng/ml	10	50	100	0.8	100	100
Plasma sample	10	50	100	0.8	100	100
Plasma sample	50	50	100	0.8	100	100

APPENDIX B THE DETERMINATON OF PARAQUAT IN PLASMA BY RADIOIMMUNOASSAY (CONTINUED)

7. Scintillation counting and calculation of results

Count samples for 1 minute on the liquid scintillation counter. Average the duplicates and prepare a standard curve by plotting the average number of counts, against the concentration of paraquat standard (ng/ml). From the calibration curve read the concentration of paraquat in the unknowns in ng/ml.