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REPORT

THE ACUTE ORAL TOXICITY AND MODE OF ACTION OF EMETIC PP796 IN CYNOMOLGUS MONKEYS, AND ITS EFFECT UPON THE ACUTE ORAL TOXICITY OF SEVERAL FORMULATIONS OF PARAQUAT

HUNTINGDON RESEARCH CENTRE

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THE ACUTE ORAL TOXICITY AND MODE OF ACTION OF EMETIC PP796 IN CYNOMOLGUS MONKEYS, AND ITS EFFECT UPON THE ACUTE ORAL TOXICITY OF SEVERAL FORMULATIONS OF PARAQUAT

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We the undersigned, hereby declare that the work was performed under our supervision according to the procedures herein described, and that this report provides a correct and faithful record of the results obtained.

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SUMMARY

SECTION I - INVESTIGATIONS ON THE METIC PP796 ALONE

Test material: ICI emetic, code number PP796

Test species: Cynomolgus monkeys (Macaca fascicularis), 12 males.

Route of administration: Oral gavage.

Observation period: 14 days after dosing.

Dosage and results:

EXPERIMENT 1 - To find the dose of PP796 which would induce vomiting within 1 hour

Group	Dose mg PP796/kg*	Number of animals	Number vomiting within 1 hour
1	1	2	1
2	2	2	2

EXPERIMENT 2 - To investigate the toxicity of PP796.

Group	Dose mg PP796/kg	Number of animals	Number vomiting within 1 hour	Clinical signs	Deaths
1	5	2	2	lethargy	0
2	10	2	1	collapse	0
3	20	2	2	collapse	0
4	30	2	2	collapse	0

EXPERIMENT 3 - To investigate the effect of subemetic doses of PP796 on gastric retention time.

Group	Dose	Number of animals	% Phenol Red remaining in stomach 1 hour after dosing
1	30 ml phenol red soln. + 10 cm^3 water	4	33.7 ± 12.8
2	30 ml phenol red soln. + 10 ml water + 0.2 mg PP796/kg	4	85.7 ± 9.4

* Refer to kg bodyweight.

SECTION II - THE EFFECT OF PP796 UPON THE TOXICITY OF A COMMERCIAL FORMULATION OF PARAQUAT (GRAMOXONE W), A 20% AQUEOUS SOLUTION OF PARAQUAT CATION CONTAINING WETTING AGENTS

Test materials: Unstenched Gramoxone (Gramoxone W - JF 1423 A)

Test species: Cynomolgus monkeys (Macaca fascicularis) 28 males.

Route of administration: Oral gavage.

Observation period: 14 days after dosing.

Dosage and results:

EXPERIMENT 4 - To find the lethal dose of Gramoxone W.

Total volume of dose: 20 ml

Group	Dose mg PQ^{2+}/kg*	Number of animals	Number vomiting within 1 hour	Deaths
1	87×	4	0	3

EXPERIMENT 5 - To test the protective effect of PP796 at the lethal dose level of Gramoxone W.

Total volume of dose: 20 ml.

Group	Dose mg PQ^{2+}/kg	Dose mg PP796/kg	Number of animals	Number vomiting within 1 hour	Deaths
1	100	0	8	0	8
2	100	2	8	6	2

EXPERIMENT 6 - To find the upper limit of paraquat dose against which PP796 can provide protection

a. Using a low total volume of dosing solution.

Total volume of dose 20 ml.

Group	Dose mg PQ^{2+}/kg	Dose mg PP796/kg	Number of animals	Number vomiting within 1 hour	Deaths
1	250	2.5	2	2	2
2	500	5	2	2	2

b. Using a high total volume of dosing solution.

Total volume of dose 100 ml.

1	250	2.5	2	1	0
2	500	5	2	2	2

* mg PQ^{2+}/kg = mg paraquat cation/kg bodyweight.

× due to the test compound containing 174 g/l instead of 200 g/l the dose was 87 mg/kg instead of 100 mg/kg

SECTION III - THE TOXICITY OF THREE FORMULATIONS OF PARAQUAT BASED ON 'STENCHED' GRAMOXONE

Test materials: ICI emetic, code number PP796.

Formulation 1 - stenched Gramoxone containing 200 g PQ^{2+}/l (JF 6043)

Formulation 2 - stenched Gramoxone containing 200 g PQ^{2+}/l + 0.5 g PP796/l (Experimental compound JF 6043 A)

Formulation 3 - Formulation II with extra PP796 added to give a concentration of 4 g PP796/l

Test species: Cynomolgus monkeys (Macaca fascicularis) 28 males.

Route of administration: Oral gavage.

Observation period: Up to 5 weeks after dosing.

Dosage and results:

EXPERIMENT 7 - To assess the toxicity of three formulations of paraquat.

Total volume of dose 100 ml.

Formulation 1

Group	Dose mg PQ^{2+}/kg	Dose mg PP796/kg	Number of animals	Number vomiting within 1 hour	Deaths
1	70	-	2	1	0
2	100	-	4	4	2
3	250	-	2	2	2

Formulation 2

1	40	0.1	8	0	5
2	70	0.175	2	2	1
3	100	0.25	2	1	2

Formulation 3

1	100	2.0	2	2	0
2	250	5.0	2	2	0
3	350	7.0	2	2	2
4	500	10.0	2	1	2

I. TRODUCTION

In a previous study of paraquat poisoni g in monkeys (HRC Report No. ICI 43/76128*) orally administered paraquat reached peak plasma l vels within 2 hours of dosing. If an emetic substance added to formulations of paraquat could prod ce vomiting within 1 hour of dosing, it is likely that an animal would be able to survive up to severa times the normal lethal dose.

The experiments reported here were de gned to evaluate the protective effect of an emetic substance supplied by ICI (code number PP79) when added to commercial formulations of paraquat.

The programme of work consists of a se es of experiments performed between 4 May 1976 and 16 May 1977, and may be conveniently divi ed into three sections as follows:

SECTION I - INVESTIGATIONS C J THE EMETIC PP796 ALONE

EXPERIMENT 1 - To nd the dose of PP796 which would induce vomiting wit in 1 hour.

Tw animals were dosed with PP796 on two occasions.

EXPERIMENT 2 - To nvestigate the toxicity of PP796.

Tw animals were dosed repeatedly with increasing doses of P796 up to 30 mg/kg bodyweight.

EXPERIMENT 3 - To nvestigate the effect of subemetic doses of PP796 on gas ic retention time.

Tw groups of 4 animals were dosed orally with a solution of henol red. One group also received a subemetic dose of P796. The animals were killed after 1 hour and the per entages of the dose of phenol red remaining in the sto ach were measured.

SECTION II - THE EFFECT OF PP79 UPON THE TOXICITY OF A COMMERCIAL FORMULATION OF ARAQUAT (GRAMOXONE W), A 20% AQUEOUS SOLUTION OF PARA QUAT CATION CONTAINING WETTING AGENTS

EXPERIMENT 4 - (Pr iminary) To find the lethal dose of Gramoxone W. In evious experiments with paraquat (HRC Report No. ICI 0/74938**) the lowest dose of paraquat cation which has illed all monkeys to which it has been administered is 0 mg/kg, which will be termed the 'lethal dose' in thi eport.

Fou monkeys were dosed with a nominal concentration of 10(ng paraquat cation/kg as Gramoxone to confirm that the oxicity of Gramoxone was the same as that of pure par quat.

* ICI/43 The treatment of paraquat-dos d cynomolgus monkeys with forced diuresis.

** ICI/40/- The toxicity of orally anc ntravenously administered paraquat dichloride in cynomolgus monkeys.

EXPERIMENT 5 - To assess the protective effect of PP796 at the lethal dose level of Gramoxone W.

Two groups of 8 animals were dosed with 100 mg paraquat cation/kg as Gramoxone W. One group of animals also received an emetic dose of PP796.

EXPERIMENT 6 - To find the upper limit of paraquat dose against which PP796 can provide protection.

a. Using a low (20 ml) total volume of dosing solution*

b. Using a high (100 ml) total volume of dosing solution.

Pairs of animals were dosed with PP796 and Gramoxone W containing levels of paraquat cation above the lethal dose in either low or high volumes of dosing solution.

* In order to test the emetic action it was considered necessary to give the animals a standard stomach content. For Experiments 4 and 5 the dose was made up to a total volume of 20 ml with water containing 'Complan' in the ratio 1 g to 1 ml water. Complan is a soluble complete food preparation based upon skimmed milk (Glaxo-Farley Foods, Plymouth, England). No Complan was added in the case of Experiment 6a.

For Experiment 6b the 'Complan' was left out and the volume of dosing solution increased to 100 ml to see whether this would increase the efficiency of vomiting. This increased volume, without 'Complan', was then used for all subsequent experiments.

SECTION III - THE TOXICITY OF THREE FORMULATIONS OF PARAQUAT; 20% AQUEOUS SOLUTIONS OF PARAQUAT CATION CONTAINING BOTH WETTING AGENTS AND AN ADDITIVE DESIGNED TO GIVE AN UNPLEASANT SMELL AND TASTE ('STENCHED' GRAMOXONE).

Formulation 1 - containing no PP796

Formulation 2 - containing 0.05% PP796

Formulation 3 - containing 0.4% PP796

EXPERIMENT 7 - To find the approximate LD_{50} and LD_{100} of Formulations 1 - 3.

Pairs of animals were dosed with each formulation until the approximate LD_{50} and LD_{100} could be determined.

The next part of the report, the MATERIALS AND METHODS, gives details of the materials and methods used which were common to all experiments.

Accounts of Experiments 1 - 7 are then presented with specific Introductions, Methods, Results and Conclusions for individual experiments.

MATERIALS AND METHODS

General

The information given in this section is common to all experiments. The METHODS section attached to individual experiments gives details of the specific procedures used there.

Details of dose preparation for individual experiments are given in APPENDIX 1.

Animals

Seventy-six wild-caught male cynomolgus monkeys (Macaca fascicularis) aged approximately 2-5 years and weighing between 2.0 and 5.5 kg were obtained from Shamrock Farms (GB) Limited, Small Dole, Henfield, Sussex, England. The animals were held by the supplier for approximately 12 weeks prior to delivery, and arrived in small numbers as required between 23 April 1976 and 15 March 1977.

On arrival each animal was given a thorough examination by a veterinary surgeon to assess their general health and they were all found to be satisfactory. Each animal was given an intrapalpebral tuberculin test (10,000 i.u. mammalian PPD) and a chest X-ray.

Acclimatisation period

An acclimatisation period of at least 2 weeks was allowed before dosing.

Accommodation

The animals were housed individually in primate cages, constructed of aluminium and stainless steel (internal dimensions: 62 cm wide, 62 cm deep and 87 cm high). The cages were mounted on racks, in a well ventilated holding area maintained at 22 $\pm$ 3°C. Lighting was by daylight and artificial light during working hours.

Diet

The animals were fed with a dry diet suitable for primates. A 1 : 1 ratio of "F.P.I." (Dixon and Sons Limited, Ware, Hertfordshire, England) and "Mazuri Primate diet" (BP Nutrition (UK) Limited, Stepfield, Witham, Essex, England) was prepared. Each animal was offered 150 g of this diet, 2 x 25 g "Bonio" biscuits (Spratt's Patent Limited, Barking, Essex, England) and a slice of brown bread daily.

It was therefore possible for each animal to consume 1400 g of dry diet per week.

On an average of 4 days per week each animal was offered a weighed quality of fresh fruit or vegetable produce.

Water was available at all times. In addition, each animal had access to a solution of blackcurrant juice and Cytacon* (Glaxo Laboratories Limited, Greenford, Middlesex, England), 20 ml of each in a litre of tap water.

* Contains Cyanocobalamin BP (7 µg/ml).

Test materials

Emetic (used in Sections I - III, Experiments 1 - 7)

A 4 g sample of emetic PP796, a white crystalline solid, was received on 29 April 1976 from:

ICI Central Toxicology Laboratory,
Alderley Park,
Nr. Macclesfield, Cheshire.

and was labelled as follows:

ICI 63,197 (R50796)
ADM 52075/74

This sample was used to make up all doses up to 21 March 1977. Four animals dosed on 18 April 1977 were dosed from a second batch of emetic received on 24 November 1976 which was labelled as follows:

5 grams PP796
From bottle of ICI 63,197
ADM 52124/74

Unstenched Gramoxone (Section II, Experiments 4, 5 and 6)

A 1 litre bulk sample of Gramoxone (R) was received on 10 May 1976 from:

ICI Plant Protection Division,
Jealott's Hill,
Bracknell, Berks.

The sample, a brown liquid, was labelled as follows:

GRAMOXONE W
JF 1423/A

This sample was used for making up all dosing solutions used in Section II (Experiments 4, 5 and 6).

The sample was stated to contain 200 g/l paraquat cation (PQ^{2+}) but subsequent analysis revealed the actual concentration to be 174 g/l.

Stenched Gramoxone (used in Section III, Experiment 7)

Two 1 litre bulk samples of stenched Gramoxone (R) were received on 29 September 1976 from:

ICI Plant Protection Division,
Jealott's Hill,
Bracknell, Berks.

Both were brown liquids smelling of pyridine, and were labelled as follows:

JF.6043 containing
200 g/l PARAQUATION
Ref: ERH.0309.76.JH Date 27.9.76

EXPERIMENTAL COMPOUND
JF. 6043A
PARAQUATION 200 g/l
Ref: ERH.0313 JH.76. Date 29.9.76

The experimental compound was also stated to contain emetic PP796 at a concentration of 0.25 mg/100 mg PQ^{2+}.

Storage

All test materials were stored in the dark at room temperature.

Preparation of dosing solutions

Samples of the test materials were made up into aqueous solutions containing convenient concentrations of the active ingredients. The appropriate volumes of these solutions were then measured out for each animal according to its bodyweight. Details of the preparations of these solutions and volumes used for each animal are given in APPENDIX 1.

Administration of test solutions

The animals were starved overnight for a period of not less than 16 hours. They were then weighed so that the doses of test material could be calculated. The doses were then administered by oral gavage. When the animals had received the appropriate doses of paraquat or emetic a further volume of fluid was administered through the gavage tube. This served two purposes:

1. It ensured that all the dose of active ingredients was flushed through the gavage tube into the stomach.

2. It brought the stomach contents up to a standard dosing volume.

The composition of the diluent fluid varied throughout the series of experiments; three different forms being used.

1. The dose was brought up to a total volume of 20 ml with water containing 'Complan' 1 g/cm^3.

2. The dose was brought up to a total volume of 20 ml with water.

3. The dose was brought up to a total volume of 100 ml with water.

The exact composition of the diluent fluid is given with each experiment.

Clinical signs

The animals were kept under close observation during the day of dosing. All signs of reaction to the test material were noted: particular attention was paid to vomiting 'behaviour'. The animals were retained for a 14 day observation period following dosing during which time their condition was checked twice daily. In practice, no surviving animals were killed until at least 5 weeks after dosing. For this reason two animals, which died after the initial observation period in Experiments 7 at 19 and 33 days (Animal Nos. 589 and 401, Formulation 2 Group 1), have been included in the results of the study as animals dying after dosing.

Bodyweights

The animals were weighed weekly from one week before dosing until the end of the observation period.

Food consumption

The amount of food consumed by each animal was estimated daily from one week before dosing until the end of the observation period. The consumption of dry diet only was monitored.

Results

The results of the studies are reported with each individual experiment. Details of vomiting 'behaviour' are given in APPENDIX 2. Details of food consumption data are given in APPENDIX 3 and bodyweights in APPENDIX 4.

SECTION I

INVESTIGATIONS ON THE EMETIC PP796 ALONE

EXPERIMENT 1 - To find the dose of PP796 which would induce vomiting within 1 hour.

Introduction

If PP796 is to provide effective protection against paraquat poisoning it is essential that it should be capable of producing powerful vomiting within 1 hour of dosing. This experiment was designed to find the dose which would satisfy these conditions.

Methods

Two animals were dosed with PP796 on two occasions. The dose levels used were 1 and 2 mg PP796/kg. All doses were made up to a total volume of 20 ml with water containing 'Complan' 1 g/ml. Details of dose preparation are shown in APPENDIX 1.

Results

The results were as follows:

Group	Dose PP796 mg/kg	Animal No.	Time to first vomit Hours	Minutes	Date dosed
1	1	281	0	16	5 May 1976
		283 between	1	00	5 May 1976
		and	1	45	5 May 1976
2	2	281	0	22	25 May 1976
		283	0	23	25 May 1976

The emetic was found to induce powerful and repeated vomiting at both 1 and 2 mg/kg. However, one animal dosed at 1 mg/kg (283) failed to vomit within the prescribed hour. Details of the vomiting 'behaviour' are given in APPENDIX 2.

Animal 281 was very sensitive to the emetic, vomiting 7 times at 1 mg/kg and 8 times at 2 mg/kg, over a period of five and a half hours at the higher dose.

Animal 283 ceased vomiting after 2 hours at the 1 mg/kg dose level, and after 1 hour 20 minutes at the 2 mg/kg dose level. At the 2 mg/kg dose level this animal vomited 3 times only.

General signs

The first sign of reaction to the emetic was usually yawning. This was followed by vomiting and bouts of non-productive retching movements accompanied by coughing. The animals lay on the cage floor in a lethargic state for 1 - 2 hours after dosing followed by a gradual and complete recovery. Frequent defaecation was also observed.

Conclusions

The emetic was found to produce powerful and repeated vomiting within 1 hour of dosing at a dose of 2 mg/kg.

The emetic appeared to have no long term adverse effects.

EXPERIMENT 2 - To investigate the toxicity of PP796.

Introduction

Having established that PP796 is an effective emetic in monkeys it was necessary to know whether higher doses might result in long term uncontrolled vomiting or other toxic effects which might obviate its use.

Method

Two animals were dosed repeatedly with increasing doses of PP796 up to 30 mg/kg.

Details of the dose preparation are given in APPENDIX 1.

The emetic doses were made up to a total volume of 100 ml with water and administered to the animals

Results

The results were as follows:

Group	Dose PP796 mg/kg	Animal No.	Time to first vomit Hours	Minutes	Signs	Date dosed
1	5	339	0	03	Lethargy	23 September 1976
		341	0	11	Lethargy	23 September 1976
2	10	339	0	05	Collapse	5 October 1976
		341	1	37	Collapse	5 October 1976
3	20	339	0	16	Collapse	7 October 1976
		341	0	14	Collapse	7 October 1976
4	30	339	0	06	Collapse	13 October 1976
		341	0	19	Collapse	13 October 1976

The emetic produced effective vomiting within a few minutes of dosing except on one occasion (Animal 341, 10 mg/kg).

In contrast to the previous test the vomiting motions continued for only a few minutes after which the animals became lethargic and, at doses of 10 mg/kg and above, collapsed by about 30 minutes after dosing. The animals remained unconscious or semiconscious for approximately 1 hour and then made a gradual and complete recovery.

Conclusions

Although the emetic appears to have powerful sedative action at high doses there was no evidence of any long term toxic effects on the animals.

There were no signs of repeated uncontrolled vomiting; in fact, probably due to the sedative effects of high doses, vomiting and retching ceased sooner at high dose levels than at low dose levels.

EXPERIMENT 3 - To investigate the effect of subemetic doses of PP796 on gastric retention time.

Introduction

From the results of experiments at the ICI Central Toxicology Laboratory (CTL)* using dogs and the results of EXPERIMENT 5 of this report it was suspected that PP796, in addition to its emetic effects, may also delay gastric emptying. In order to investigate this possibility, the rate of stomach emptying of two groups of animals, one of which received a subemetic dose of PP796, was examined.

In a preliminary test, pairs of animals were given increasing doses of PP796 to find the highest dose that would not cause vomiting. This subemetic dose was then used for the main experiment.

* Report No. CTL/R/391. The effect of administration of an emetic (PP796) on paraquat toxicity in dog and monkey.

Methods - Preliminary ex eriment

Monkeys were dosed with .1, 0.2 and 0.4 mg PP796/kg. Two animals were dosed on each occasion.

Details of dose preparatio are given in APPENDIX I. The dose was made up to a total volume of 100 ml v ith water and administered to the animals.

Results

The results were as follows

Group	Dose PP796 mg/kg	Number vomiting	Time to first vomit Hours	Minutes	Date dosed
1	0.1	1/2	0	52	18 March 1977
2	0.2	1/2	0	06	18 March 1977
3	0.4	1/2	0	15	18 March 1977

The animal vomiting at a ose level of 0.2 mg/kg did so only very slightly 6 minutes after dosing.

Conclusions

From these findings it was onsidered that a dose of 0.4 mg PP796/kg is very likely to produce vomiting, wher as a dose level of 0.2 mg PP796/kg would probably be acceptable. On this basis a dose level of 0.2 mg PP796/kg was chosen for the main experiment.

Methods - Main experime t

Details of dose preparatio are shown in APPENDIX 1.

Ten animals were dosed or ly with 30 ml of a solution of phenol red followed by 10 ml of water. Six of th e animals also received a dose of 0.2 mg PP796/kg in the test solution (2 extr animals were dosed in this group to allow for the slight possibility of emesis at this dose level). Four animals from each group were killed 1 hour after dosing. Their tomachs were ligated top and bottom, removed and weighed, and the retention f phenol red in each was analysed at ICI Central Toxicology Laboratory (CT).

Results

The results were reported in terms of the percentage of phenol red solution remaining in the stomach 1 hour after dosing and are shown below:

Group	Dose	Animal No.	% phenol red remaining		Date dosed
1	30 ml phenol red + 10 ml water	533	47.7	33.7 ± 12.8	21 March 1977
		535	27.1		21 March 1977
		537	40.4		21 March 1977
		539	19.4		21 March 1977
2	30 ml phenol red + 10 ml water + 0.2 mg PP796/kg	279	94.5	85.7 ± 9.4	21 March 1977
		437	84.6		21 March 1977
		543	90.7		21 March 1977
		585	72.9		21 March 1977

The results show a clearly enhanced retention of stomach contents in the group dosed with 0.2 mg PP796/kg. No animal vomited.

Conclusion

Subemetic doses of PP796 delayed stomach emptying for at least 1 hour after dosing.

SECTION II

THE EFFECT OF PP796 UPON THE TOXICITY OF A COMMERCIAL FORMULATION OF PARAQUAT (GRAMOXONE W), A 20% AQUEOUS SOLUTION OF PARAQUAT CONTAINING WETTING AGENTS

EXPERIMENT 4 - To find the lethal dose of Gramoxone W.

Introduction

In previous experiments with paraquat (HRC Reports No. ICI/40, 43) the lowest dose of paraquat cation which has killed all monkeys to which it has been administered is 100 mg/kg, and this will be termed the 'lethal dose' in this report. In the present experiment, 4 monkeys were dosed with a nominal concentration of 100 mg PQ^{2+}/kg as Gramoxone W, to confirm that the toxicity of Gramoxone W was the same as that of pure paraquat.

The sample of Gramoxone W was stated to contain 200 g PQ^{2+}/l (see page 8 However, subsequent analysis showed that the Gramoxone W sample actually contained only 174 g PQ^{2+}/l. This resulted in the animals receiving a dose of only 87 mg PQ^{2+}/kg instead of the intended 100 mg PQ^{2+}/kg.

Methods

Details of dose preparation are shown in APPENDIX 1.

Four animals were dosed with 87 mg PQ^{2+}/kg and the dose was made up to a total volume of 20 ml with water containing 'Complan' 1 g in 1 ml. Blood samples (2 ml) were taken from two animals (285 and 289). One sample was taken before dosing and the others at 1, 2, 3, 4, 6, 8 and 10 hours after dosing. The plasma from these samples was sent, at room temperature, to the Central Toxicology Laboratory of ICI for analysis of the plasma paraquat levels, by the CTL modified gas chromatographic method. The analyses were carried out by W.J.D. Laird of the Analytical Biochemistry Unit at CTL.

Results

The results were as follows:

Animal No.	Time to first vomit Hours	Minutes	Time to death	Deaths	Date dosed
285	4	04	3 days	3/4	15 May 1977
287	3	35	SURVIVED		15 May 1977
289	4	05	32 hours		15 May 1977
291	3	90	5 days		15 May 1977

The results of the plasma paraquat analysis are shown in TABLE 1. The plasma paraquat reached peak levels of 16-17 µg/ml within 4 to 6 hours of dosing and then declined to approximately 3 µg/ml within 10 hours.

The animals showed an increasing degree of lethargy and loss of co-ordination leading eventually to collapse and dyspnoea in non-survivors. Frequent defaecation was observed, which became diarrhoea by 6 hours. Vomiting 'behaviour' is detailed in APPENDIX 2.

Conclusions

The results did not fulfil the criterion of a 100% lethal dose: the test was therefore repeated at the orginally intended level, i.e. 100 mg PQ^{2+}/kg and was incorporated within EXPERIMENT 5.

EXPERIMENT 5 - To test the protective effect of PP796 at the lethal dose level of Gramoxone W.

Introduction

This experiment constitutes the main test of the protective effect of PP796 and when completed, consisted of 2 groups of 8 animals all dosed with 100 mg PQ^{2+}/kg, one group with and one group without, emetic. In practice the experiment was performed in three parts.

Initially, four animals were dosed with 100 mg PQ^{2+}/kg as Gramoxone W, (Group 1) a repeat of EXPERIMENT 4. Following this, four animals were dosed with a solution of Gramoxone W containing an emetic (Group 2) and, as the experiment at this point appeared to be successful, a further four animals were dosed in each group to bring the numbers up to a total of eight per group. Blood samples were taken as in EXPERIMENT 4 for analysis of plasma paraquat levels. This information was used to determine whether use of the emetic altered the absorption profile of paraquat from the gut.

Methods

Details of dose preparation are given in APPENDIX 1.

Two groups of eight animals were dosed with 100 mg/PQ^{2+}/kg as Gramoxone W. One group also received an emetic dose of PP796 (Group 2)*.

Blood samples (2 ml) were taken from all animals pre-dose and at 1, 2, 3, 4, 6, 8 and 10 hours after dosing. The plasma from these samples (approximately 1 ml) were sent to CTL for analysis of plasma paraquat levels. The samples were analysed for paraquat by the CTL modified gas chromatography method by W.J.D. Laird of the Analytical Biochemistry Unit at CTL.

* The doses were made up to a total volume of 20 ml with water containing 'Complan' 1 g in 1 ml.

Results

The results are shown below:

Group	Dose (mg/kg) PQ2+	Dose (mg/kg) PP796	Animal No.	Time to first vomit Hours	Time to first vomit Minutes	Time to death	Deaths	Date dosed
1	100	-	63	3	45	48 hours	8/8	25 May 1976
			95	4	57	7½ hours		25 May 1976
			293	4	58	24 hours		25 May 1976
			295	4	22	51½ hours		25 May 1976
			313	4	44	5¼ hours		16 June 1976
			315	4	57	23 hours		16 June 1976
			317	4	44	32 hours		16 June 1976
			319	5	00	48 hours		16 June 1976
2	100	2	297	0	55	SURVIVED	2/8	1 June 1976
			299	0	31	SURVIVED		1 June 1976
			301	0	25	SURVIVED		1 June 1976
			303	7	35	13 days		1 June 1976
			305	0	20	SURVIVED		16 June 1976
			307	4	50	48 hours		16 June 1976
			309	0	20	SURVIVED		16 June 1976
			311	0	26	SURVIVED		16 June 1976

Details of vomiting 'behaviour' are given in APPENDIX 2.

All eight monkeys dosed with paraquat alone died within 52 hours and all vomited at between 4 and 7 hours after dosing.

The first signs of paraquat poisoning occurred after 2-6 hours as a progressive loss of co-ordination leading to collapse with signs of dyspnoea followed by death.

Six of the 8 monkeys dosed with paraquat and emetic survived the 14 day observation period. Both of the animals which died failed to vomit until 4½ and 7½ hours after dosing and exhibited similar signs to the animals dosed with paraquat alone. Surviving animals all first vomited within 1 hour of dosing. Vomiting usually occurred 2-4 times during the first 1½ hours after dosing, was accompanied by powerful abdominal contractions and repeated retching motions. This was followed by a period of lethargy and ataxia with a subsequent gradual recovery.

The results of the plasma paraquat analyses are shown in TABLE II. In monkeys dosed with paraquat alone (Group 1) a plasma peak occurred at 3 hours after dosing, with a mean level of 22.36 µg/ml, and then declined to approximately 3 µg/ml by 10 hours after dosing. The plasma paraquat levels in the group dosed with emetic (Group 2) were much lower, reaching a mean peak level of 1.78 µg/ml 2 hours after dosing and declining to 0.69 µg/ml by 10 hours after dosing.

Discussion and conclusions

The results show that the emetic has a strong protective effect against paraquat poisoning at the lethal dose level of 100 mg PQ^{2+}/kg. The presence of Gramoxone W did not prevent the emetic from having a potent action on six of the animals dosed; all of which survived the 14 days observation period. It is significant that the two animals which subsequently died failed to vomit until $4\frac{1}{2}$ and $7\frac{1}{2}$ hours after dosing.

The results of the plasma paraquat analyses show that the emetic caused a dramatic reduction in the amount of paraquat absorbed, so that the mean peak level was only 1/12 of the achieved by the animals dosed with paraquat alone.

An apparent anomaly in the results is that the two animals dosed with paraquat and emetic which failed to vomit both had low plasma paraquat levels compared with animals in Group 1 which were dosed with paraquat alone, and also that they both subsequently died. In EXPERIMENT 3 (SECTION I) it has been shown that in addition to its emetic effect PP796 delayes stomach emptying, and as paraquat has been shown to be poorly absorbed from the stomach (M.S. Rose, personal communication) it is not surprising that plasma uptake should be low in these animals even though they failed to vomit. In previous work (ICI/40, ICI/43) we have produced evidence that early deaths in primates (1 - 4 days) are due to acute renal and pulmonary damage, while late deaths (1 - 3 weeks) are due to pulmonary damage and interstitial fibrosis. All of the animals in Group 1 died during the acute phase as did animal No. 307 in Group 2. Examination of the plasma profile shows that animal No. 307 reached a plasma level of 5.4 µg/ml, which was considerably higher than that achieved by any other Group 2 animal, and is close to the peak level reached by 317 in Group 1. Animal No. 307 also had a relatively high plasma paraquat level of 1.8 µg/ml at 10 hours after dosing. For these reasons animal No. 307 could be considered similar to the Group 1 animals, and probably died as a result of acute pulmonary and renal damage.

The other animal No. 303, did not achieve such a high peak plasma level, and appears to have survived the acute phase, dying at 14 days after dosing. An examination of its plasma profile shows that animal No. 303 retained a relatively high level at 10 hours after dosing. Since it has been shown (Smith et al., 1974; Rose et al., 1976) that the lung actively accumulates paraquat from the plasma over a period of days it is likely that sufficient paraquat was accumulated to cause death, probably from pulmonary fibrosis at 14 days.

EXPERIMENT 6 - To find the upper limit of paraquat dose against which PP796 can provide protection.

a. Using a low (20 ml) total volume of dosing solution.

(EXPERIMENT 6b is a repeat of EXPERIMENT 6a but using a high (100 ml) total volume of dosing solution).

Introduction

The previous experiment (EXPERIMENT 5) has shown that PP796 provides effective protection against paraquat poisoning at the lethal dose level of Gramoxone W. The present experiment (6a) was designed to find the upper limits of paraquat dose as Gramoxone W against which PP796 can provide protection.

It was decided that this experiment should mimic the situation where a human victim took large doses of a formulation containing both paraquat and emetic. For this reason the ratio of emetic concentration to paraquat concentration was kept constant at 1 mg PP796 to 100 mg PQ^{2+}. In the first part of this experiment the same total volume of dosing solution (20 ml) was used as that in the previous experiment. However, it was decided at this point to abandon the use of 'Complan'.

Methods

Details of dose preparation are given in APPENDIX 1. Two pairs of animals were dosed with PP796 and Gramoxone W. One pair received 250 mg PQ^{2+}/kg + 2.5 mg PP796/kg (Group 1) and the other pair received 500 mg PQ^{2+}/kg + 5 mg PP796/kg (Group 2).

Doses were made up to a total volume of 20 ml with water.

Results

The results were as follows:

Group	Dose (mg/kg) PQ^{2+}	PP796	Animal No.	Time to first vomit Hours	Minutes	Time to death	Deaths	Date dosed
1	250	2.5	329	0	23	4 days	2/2	7 July 1976
			331	0	37	24 hours		7 July 1976
2	500	5	321	0	15	4 days	2/2	21 June 1976
			323	0	20	3 days		21 June 1976

At 250 mg PQ^{2+}/kg in spite of copious vomiting during the first hour (APPENDIX 2) both animals subsequently died. No particular signs of extreme distress were seen on the day of dosing, the animals merely showed an increasing degree of lethargy.

At 500 mg PQ^{2+}/kg both animals also died in spite of copious vomiting in the first hour (APPENDIX 2). One animal (323) went in to a state of collapse within 30 minutes of dosing and its condition, though slightly improved at $3\frac{1}{2}$ hours, continued to deteriorate until it died at 7 hours. The other animal (321) showed no particular signs on the first day but died overnight.

Conclusions

Under the dosing regime employed in this experiment, PP796 was unable to provide protection against high doses of paraquat.

b. Using a high (100 ml) total volume of dosing solution.

Introduction

The rather disappointing results of EXPERIMENT 6a lead to the suggestion that the total volume of the stomach contents could be an important factor in the toxicity of the dosing solution. Presumably, even when an animal has vomited several times, there is always a certain residual stomach content which cannot be expelled. If the concentration of the original dose solution was such that this residue contained a lethal dose of paraquat then, no matter how many times the animal vomited, it would still eventually die. For this reason it was decided to repeat the experiment with the same doses of paraquat but this time dispersed in a greater (100 ml) total volume of dosing solution. It was hoped that the residual stomach volume after vomiting would now contain a non-lethal dose of paraquat.

Methods

The details of dose preparation are given in APPENDIX 1.

Two pairs of animals were dosed as in EXPERIMENT 6a, except that the dose was made up to a total volume of 100 ml with water.

Results

The results were as follows:

Group	Dose (mg/kg) PQ^{2+}	PP796	Animal No.	Time to first vomit Hours	Minutes	Time to death	Deaths	Date dosed
1	250	2.5	325	0	10	SURVIVED	0/2	15 July 1976
			327	1	47	SURVIVED		15 July 1976
2	500	5	333	0	20	24 hours	2/2	6 August 1976
			335	0	20	24 hours		6 August 1976

Both animals dosed at 250 mg PQ^{2+}/kg survived in spite of the fact that number 327 failed to vomit until 1 hour 47 minutes after dosing (APPENDIX 2).

At 500 mg PQ^{2+}/kg both animals died in spite of copious vomiting. In both these animals vomiting was excessive continuing for 3 hours 17 minutes in the case of animal 333 and continuing for 6 hours 10 minutes in animal 335.

Conclusions

Using a larger total volume of dosing solution does seem to increase the chances of survival for animals dosed with high levels of paraquat to between 250 and 500 mg PQ^{2+}/kg. Both animals in the high dose group (500 mg PQ^{2+}/kg) died in spite of excessive vomiting which, in the case of animal 335, consisted of ten bouts of vomiting over a period of 6 hours 10 minutes.

SECTION III

THE TOXICITY OF THREE FORMULATIONS OF PARAQUAT BASED ON 'STENCHED' GRAMOXONE W

EXPERIMENT 7 - To assess the toxicity of three commerical formulations of paraquat.

Introduction

This experiment was designed to evaluate the toxicity of three formulations of paraquat based upon 'stenched' Gramoxone. The test material (see page 9) was stated to consist of a 20% aqueous solution of paraquat cation containing wetting agents and an additive designed to give an unpleasant smell and taste. The three formulations were as follows:

Formulation 1 - containing no PP796

Formulation 2 - containing a 0.05% of PP796

Formulation 3 - containing 0.4% of PP796
(Formulation 3 consisted of Formulation 2 with added PP796)

Methods

Details of the dose preparation are given in APPENDIX 1.

Pairs of animals were dosed with increasing levels of each formulation in an attempt to find the approximate LD_{50} and LD_{100} doses. The formulations consisted of the following:

Formulation 1 - containing 200 g PQ^{2+}/l

Formulation 2 - containing 200 g PQ^{2+}/l + 500 mg PP796/l

Formulation 3 - containing 200 g PQ^{2+}/l + 4 g PP796/l

All doses were made up to a total volume of 100 ml with water for administration to the animals.

Results

Details of the results are given in TABLE III, a brief synopsis of which is shown below:

Formulation 1

Group	Dose (mg/kg) PQ^{2+}	PP796	Deaths	Approximate LD_{50} (mg PQ^{2+}/kg)
1	70	-	0/2	100
2	100	-	2/4	
3	250	-	2/2	
Formulation 2				
1	40	0.1	5/8	40 - 70
2	70	0.175	1/2	
3	100	0.25	2/2	
Formulation 3				
1	100	2	0/2	250 - 350
2	250	5	0/2	
3	350	7	2/2	
4	500	10	2/2	

Vomiting 'behaviour' is shown in TABLE III and APPENDIX 2. All except one animal dosed with Formulation 1 vomited within 20 minutes. Slight vomiting occurred with Formulation 2 at 70 mg PQ^{2+}/kg and above, but the low dose group (40 mg PQ^{2+}/kg) failed to vomit until between 5 and 8 hours after dosing.

Powerful vomiting occurred with Formulation 3 especially at 350 mg PQ^{2+}/kg where both animals dosed continued to vomit for $3\frac{1}{2}$ hours. At 500 mg PQ^{2+}/kg one animal failed to vomit and collapsed 24 minutes after dosing. The other animal collapsed after vomiting. Both subsequently died.

Discussion

The LD_{50} for pure paraquat is approximately 70 mg PQ^{2+}/kg. The lethal dose is approximately 100 mg PQ^{2+}/kg in that all animals dosed at this level died. These figures seem to be equally applicable to Gramoxone W since at 85 mg PQ^{2+}/kg one animal out of the four dosed survived, while at 100 PQ^{2+}/kg eight animals died out of eight dosed.

Formulation 1

Formulation 1 of the present study, which consists of "stenched" Gramoxone, appeared to be slightly less toxic than either pure paraquat or Gramoxone W, in that two animals out of four survived the 100 mg PQ^{2+}/kg dose and both animals dosed with 70 mg PQ^{2+}/kg survived, putting the LD_{50} dose in a region of 100 mg PQ^{2+}/kg. A possible explanation for this is that all the animals dosed with Formulation 1, except one animal at 70 mg PQ^{2+}/kg (409), vomited within 20 minutes of dosing whereas with Gramoxone W or pure paraquat, vomiting does not occur until 4 or 5 hours after dosing. This early vomiting is probably due to the 'stenching' agent and although not severe, apparently enables the animals to relieve themselves of enough paraquat to slightly enhance their chances of survival.

Formulation 2

The data obtained with Formulation 2 gives an LD_{50} in the region 40-70 mg PQ^{2+}/kg since out of eight animals dosed with 40-70 mg PQ^{2+}/kg, five died whilst one out of two animals dosed with 70 mg/kg died. However it is noteworthy that those animals dosed with 40 mg PQ^{2+}/kg either failed to vomit or vomited at a late stage (5-8 hours).

Formulation 3

The results with Formulation 3 were indentical to those obtained previously with PP796 and high levels of Gramoxone W in high dosing volumes. The LD_{50} was somewhere between 250 and 350 mg PQ^{2+}/kg or approximately 3 - 4 times less toxic than Formulation 1.

Conclusions

"Stenched" Gramoxone containing emetic doses of PP796 (Formulation 3) is less toxic than "stenched" Gramoxone without emetic (Formulation 1).

REFERENCES

L.L. Smith, W. Wright, I. Wyatt and M.S. Rose, Br. med. j., iv (1974) 569

M.S. Rose, E.A. Lock, L.L. Smith and I. Wyatt, Biochem. Pharmacol. 25 (1976) 419

TABLE I

PLASMA PARAQUAT CATION CONCENTRATIONS FOLLOWING A SINGLE ORAL DOSE OF 87 mg PQ^{2+}/kg BODYWEIGHT

Time (hours) \ Animal No.	285	289	Mean
	Concentration µg/ml		
Pre-dose	0	0	0
1	3.11	5.25	4.18
2	14.48	10.80	12.64
3	12.86	8.85	10.86
4	17.25	10.05	13.65
6	9.00	15.75	12.38
8	4.84	11.47	8.06
10	3.21	2.23	2.72

TABLE II

PLASMA PARAQUAT CATION CONCENTRATIONS FOLLOWING A SINGLE ORAL DOSE OF PARAQUAT ALONE AND PARAQUAT + EMETIC (100 mg PQ^{2+}/kg BODYWEIGHT)

Group 1 - paraquat alone (100 mg PQ^{2+}/kg)

Time (hours) \ Animal No.	63	95	293	295	319	317	315	313	Mean	Standard deviation
	Plasma concentration µg/ml									
Pre-dose	<0.02	<0.02	<0.02	<0.02	<0.02	<0.5	<0.02	<0.02	0.08	0.17
1	11.6	3.8	12.1	7.0	3.0	6.0	5.2	3.0	6.46	3.61
2	12.6	11.7	25.0	4.4	10.5	6.8	10.5	4.3	10.73	6.60
3	21.8	16.4	15.0	6.75	7.2	5.2	16.5	90.0	22.36	27.95
4	13.2	38.3	13.0	7.9	9.8	4.5	8.2	Animal	13.56	11.32
6	8.1	33.3	7.2	2.7	4.5	3.8	3.7	died	9.04	10.88
8	3.1	-	5.3	1.3	2.4	1.5	3.1		2.78	1.45
10	4.3	-	4.1	1.2	2.2	0.9	6.0		3.12	2.01

Group 2 - paraquat + emetic (100 mg PQ^{2+} + 2 mg PP796/kg)

Time (hours) \ Animal No.	303	299	297	301	305	307	309	311	Mean	Standard deviation
	Plasma concentration µg/ml									
Pre-dose	<0.02	<0.02	<0.02	<0.02	0.03	<0.02	0.03	<0.02	0.02	0.00
1	1.8	0.8	1.4	2.0	1.68	2.4	0.60	1.29	1.50	0.60
2	1.9	0.2	1.1	1.1	1.39	5.4	-	1.39	1.78	1.68
3	2.9	0.1	1.3	1.1	0.87	2.5	0.24	0.93	1.24	0.99
4	2.7	0.1	2.6	1.1	0.78	2.4	0.36	0.81	1.36	1.05
6	2.1	0.2	2.3	1.5	0.35	2.2	0.32	0.67	1.21	0.92
8	2.5	0.1	1.6	1.5	0.36	3.0	0.14	0.34	1.19	1.13
10	1.6	0.6	0.2	0.7	0.24	1.8	0.05	0.31	0.69	0.66

- indicates sample lost

TABLE III

EXPERIMENT 7 - THE TOXICITY OF THREE FORMULATIONS OF PARAQUAT BASED ON 'STENCHED' GRAMOXONE W

FORMULATION 1 - containing 200 mg PQ^{2+}/ml

Group	Dose (mg/kg) PQ^{2+}	Dose (mg/kg) PP796	Animal No.	Weight (kg)	Time to first vomit Hours	Time to first vomit Minutes	Time to death	Deaths	Date dosed
1	70	0	407	3.9	0	23	SURVIVED	0/2	2 November 1976
			409	4.3	-	-	SURVIVED		2 November 1976
2	100	0	343	3.6	0	19	SURVIVED	2/4	5 October 1976
			345	4.65	0	17	SURVIVED		5 October 1976
			369	2.85	0	04	48 hours		12 October 1976
			371	3.35	0	08	72 hours		12 October 1976
3	250	0	355	4.35	0	10	24 hours	2/2	7 October 1976
			357	4.25	0	13	48 hours		7 October 1976

FORMULATION 2 - containing 200 mg PQ^{2+}/ml + 0.5 mg PP796/ml

Group	Dose (mg/kg) PQ^{2+}	Dose (mg/kg) PP796	Animal No.	Weight (kg)	Time to first vomit Hours	Time to first vomit Minutes	Time to death	Deaths	Date dosed
1	40	0.1	373	2.3	-	-	SURVIVED	5/8	12 October 1976
			375	2.1	-	-	13 days		12 October 1976
			399	3.85	4	53	6 days		1 November 1976
			401	3.4	-	-	33 days		1 November 1976
			541	2.85	6	35	10 days		18 April 1977
			583	3.5	8	00	SURVIVED		18 April 1977
			589	2.65	8	00	19 days		18 April 1977
			593	2.65	6	43	SURVIVED		18 April 1977
2	70	0.175	359	3.7	0	14	SURVIVED	1/2	7 October 1976
			361	3.25	0	15	72 hours		7 October 1976
3	100	0.25	347	4.3	0	36	48 hours	2/2	5 October 1976
			349	5.25	1	30	48 hours		5 October 1976

FORMULATION 3 - containing 200 mg PQ^{2+}/ml + 4 mg PP796/ml

Group	Dose (mg/kg) PQ^{2+}	Dose (mg/kg) PP796	Animal No.	Weight (kg)	Time to first vomit Hours	Time to first vomit Minutes	Time to death	Deaths	Date dosed
1	100	2	351	4.85	0	11	SURVIVED	0/2	8 October 1976
			353	4.75	0	09	SURVIVED		8 October 1976
2	250	5	363	4.1	0	07	SURVIVED	0/2	7 October 1976
			365	3.75*	0	04	SURVIVED		7 October 1976
3	350	7	403	3.65	0	10	24 hours	2/2	1 November 1976
			405	3.8	0	10	11 days		1 November 1976
4	500	10	377	2.2	-	-	4.5 hours	2/2	12 October 1976
			379	2.65	0	14	24 hours		12 October 1976

* In the records of weekly bodyweights, the weight of this animal appears as 4.85 kg 7 days before dosing, and 4.80 kg the day after dosing. It is therefore probable that the weight of 3.75 kg recorded on the day of dosing and from which the dose was calculated, was erroneous. The true dose was therefore probably 200 mg PQ^{2+}/kg rather than the intended 250 mg PQ^{2+}/kg.

APPENDIX 1

DETAILS OF DOSE PREPARATION

SECTION I

THE TOXICITY AND MODE OF ACTION OF THE EMETIC PP796 ALONE

EXPERIMENT 1 - To find the dose of PP796 which would induce vomiting within 1 hour.

Emetic dose: 1 and 2 mg/kg

Volume: 20 ml with water containing 'Complan' 1 g in 1 ml

Group	Dose mg PP796/kg	Animal No.	Bodyweight (kg)	Volume of PP796 soln. (ml)	Date dosed
1	1	281	3.90	3.9	5 May 1976
		283	3.50	3.5	5 May 1976
2	2	281	4.18	8.4	25 May 1976
		283	3.70	7.4	25 May 1976

A solution of PP796 containing 1 mg/ml was prepared by dissolving 100 mg PP796 in 100 ml of deionised water.

The volumes of PP796 solution shown above were made up to 20 ml with water containing 'Complan' 1 g in 1 ml.

EXPERIMENT 2 - To investigate the toxicity of PP796.

Emetic dose: 5, 10, 20 and 30 mg/kg

Volume: 100 ml with water

Group	Dose mg PP796/kg	Animal No.	Bodyweight (kg)	Volume of PP796 soln. (ml)	Date dosed
1	5	339	4.45	4.45	23 September 1976
		341	3.75	3.75	23 September 1976
2	10	339	4.75	9.5	5 October 1976
		341	3.70	7.4	5 October 1976
3	20	339	4.75	19.0	7 October 1976
		341	3.70	14.8	7 October 1976
4	30	339	5.00	30.0	13 October 1976
		341	3.60	21.6	13 October 1976

A solution of PP796 containing 5 mg/ml was prepared. The volumes shown above were made up to a total volume of 100 ml with water.

EXPERIMENT 3 - To investigate the effect of subemetic doses of PP796 on gastric retention time.

Preliminary

Emetic dose: 0.1, 0.2 and 0.4 mg/kg

Volume: 100 ml with water

Group	Dose mg PP796/kg	Animal No.	Bodyweight (kg)	Volume of PP796 soln. (ml)	Date dosed
1	0.1	533	4.20	0.084	18 March 1977
		535	4.00	0.080	18 March 1977
2	0.2	537	3.35	0.134	18 March 1977
		539	3.60	0.144	18 March 1977
3	0.4	541	3.00	0.240	18 March 1977
		543	2.50	0.200	18 March 1977

The volumes of PP796 solution shown above were taken from a solution containing 5 mg PP796/ml. The dose was made up to 100 ml with water.

Main experiment

Phenol red solution: 30 ml

Emetic dose: 0 and 0.2 mg/kg

Volume: 40 ml with water

Group	Dose	Animal No.	Bodyweight (kg)	Volume of PP796 soln. (ml)	Date dosed
1	30 ml phenol red 10 ml water	533	4.20	0	21 March 1977
		535	4.00	0	21 March 1977
		537	3.35	0	21 March 1977
		539	3.60	0	21 March 1977
2	30 ml phenol red 10 ml water 0.2 mg PP796/kg	541 **	3.00	1.2	21 March 1977
		543	2.50	1.0	21 March 1977
		437	2.50	1.0	21 March 1977
		279	3.83	1.5	21 March 1977
		583**	3.25	1.3	21 March 1977
		585	3.53	1.4	21 March 1977

** Animals spared

APPENDIX 1

(continued)

SECTION I

(continued)

The animals were dosed with 30 ml of a solution of phenol red (supplied by the ICI Central Toxicology Laboratory) followed by 10 ml of water.

Group 2 animals also received a dose of 0.2 mg PP796/kg in the test solution. The volumes of PP796 solution shown on page 31 were taken from a solution containing 0.5 mg PP796/ml.

APPENDIX 1

(continued)

SECTION II

THE EFFECT OF PP796 UPON THE TOXICITY OF A COMMERCIAL FORMULATION OF PARAQUAT (GRAMOXONE W), A 20% AQUEOUS SOLUTION OF PARAQUAT CONTAINING WETTING AGENTS

EXPERIMENT 4 - To find the lethal dose of Gramoxone W.

Paraquat dose: 85 mg/kg

Volume: 20 ml water containing 'Complan' 1 g in 1 ml

Dose mg PQ^{2+}/kg	Animal No.	Bodyweight (kg)	Volume PQ^{2+} soln.(ml)	Date dosed
87	285	4.40	4.40	12 May 1976
	287	3.35	3.35	12 May 1976
	289	4.05	4.05	12 May 1976
	291	3.90	3.90	12 May 1976

The sample of Gramoxone W was stated to contain 200 g PQ^{2+}/l. A paraquat dosing solution with a nominal concentration of 100 mg/ml was prepared by making 50 ml of Gramoxone W up to 100 ml with deionised water.

However, since the Gramoxone W sample actually contained only 174 g/l instead of 200 g/l, the concentration of the dosing solution was only 87 mg/ml.

The volumes of this solution shown above were made up to 20 ml with water containing 'Complan' 1 g in 1 ml.

EXPERIMENT 5 - To test the protective effect of PP796 at the lethal dose level of Gramoxone W.

Paraquat dose: 100 mg/kg

Emetic dose: 0 and 2 mg/kg

Volume: 20 ml with water containing 'Complan' 1 g in 1 ml.

APPENDIX 1

(continued)

SECTION II

(continued)

Group	Dose mg PQ^{2+}/kg	Dose mg PP796/kg	Animal No.	Bodyweight (kg)	Volume of test soln. (ml)	Date dosed
1	100	0	63	4.30	4.3	25 May 1976
			95	4.00	4.0	25 May 1976
			293	3.55	3.6	25 May 1976
			295	3.60	3.6	25 May 1976
			313	3.55	3.6	16 June 1976
			315	3.65	3.7	16 June 1976
			317	4.45	4.5	16 June 1976
			319	4.40	4.4	16 June 1976
2	100	2	297	3.95	4.0	1 June 1976
			299	3.85	3.9	1 June 1976
			301	4.25	4.3	1 June 1976
			303	3.85	3.9	1 June 1976
			305	4.90	4.9	16 June 1976
			307	4.20	4.2	16 June 1976
			309	5.10	5.1	16 June 1976
			311	3.35	3.4	16 June 1976

The paraquat solution containing 100 mg PQ^{2+}/ml was prepared by making 57.5 ml Gramoxone W up to 100 ml with deionised water. Half of this solution was used to make up the doses for the animals in Group 1. For the animals in Group 2, 100 mg PP796 was added to 50 ml of the above solution to give an emetic concentration of 2 mg/ml.

The appropriate volumes of these solutions were made up to a total volume of 20 ml with water containing 'Complan' 1 g in 1 ml.

EXPERIMENT 6 - To find the upper limit of paraquat dose against which PP796 can provide protection.

a. Using a low (20 ml) total volume of dosing solution.

Paraquat dose: 250 and 500 mg/kg

Emetic dose: 2.5 and 5 mg/kg

Volume: 20 ml water

Group	Dose mg PQ^{2+}/kg	Dose mg PP796/kg	Animal No.	Bodyweight (kg)	Volume PQ^{2+} soln. (ml)	Volume PP796 soln. (ml)	Date dosed
1	250	2.5	329	3.8	9.5	3.80	7 July 1976
			331	4.65	11.6	4.65	7 July 1976
2	500	5.0	321	5.05	25.25	12.60	21 June 1976
			323	3.85	19.25	9.60	21 June 1976

Dosing procedure

A paraquat solution containing approximately 100 mg PQ^{2+}/ml was prepared by making 55 ml Gramoxone W up to 100 ml with deionised water (actual concentration 96 mg/ml).

A solution of PP796 containing 2.5 mg/ml was prepared by dissolving 0.25 g PP796 in 100 ml of deionised water. This was used for dosing the animals in Group 1.

A solution of PP796 containing 2 mg/ml was used for dosing the animals in Group 2.

The appropriate volumes of each solution shown in the table were made up to a total volume of 20 ml with deionised water.

b. Using a high (100 ml) total volume of dosing solution.

Paraquat dose: 250 and 500 mg/kg

Emetic dose: 2.5 and 5.0 mg/kg

Volume: 100 ml water

Group	Dose mg PQ^{2+}/kg	Dose mg PP796/kg	Animal No.	Bodyweight (kg)	Volume PQ^{2+} soln. (ml)	Volume PP796 soln. (ml)	Date dosed
1	250	2.5	325	4.55	11.38	4.55	15 July 1976
			327	4.25	10.63	4.25	15 July 1976
2	500	5.0	333	5.05	25.25	10.1	6 August 1976
			335	4.35	21.75	8.7	6 August 1976

The dosing solutions were the same as those used in EXPERIMENT 6a:

paraquat 100 mg PQ^{2+}/ml
PP796 2.5 mg/ml

The volumes of each solution shown in the table were made up to a total volume of 100 ml with deionised water.

APPENDIX 1

(continued)

THE TOXICITY OF THREE COMMERCIAL FORMULATIONS OF PARAQUAT BASED ON 'STENCHED' GRAMOXONE

EXPERIMENT 7 - To assess the toxicity of three commercial formulations of paraquat.

Formulation 1

Paraquat dose: 70, 100 and 250 mg PQ^{2+}/kg

Emetic dose: 0

Volume: 100 ml with water

Group	Dose mg PQ^{2+}/kg	Dose mg PP796/kg	Animal No.	Bodyweight (kg)	Volume of test soln. (ml)	Date dosed
1	70	0	407	3.90	1.37	2 November 1976
			409	4.30	1.50	2 November 1976
2	100	0	343	3.60	1.80	5 October 1976
			345	4.65	2.33	5 October 1976
			369	2.85	1.43	12 October 1976
			371	3.35	1.68	12 October 1976
3	250	0	355	4.35	5.44	7 October 1976
			357	4.25	5.31	7 October 1976

Formulation 2

Paraquat dose: 40, 70 and 100 mg PQ^{2+}/kg

Emetic dose: 0.1, 0.175 and 0.25 mg PP796/kg

Volume: 100 ml with water

Group	Dose mg PQ^{2+}/kg	Dose mg PP796/kg	Animal No.	Bodyweight (kg)	Volume of test soln. (ml)	Date dosed
1	40	0.1	373	2.3	0.46	12 October 1976
			375	2.1	0.42	12 October 1976
			399	3.85	0.77	1 November 1976
			401	3.4	0.68	1 November 1976
			541	2.85	0.57	18 April 1977
			583	3.5	0.70	18 April 1977
			589	2.65	0.53	18 April 1977
			593	2.65	0.53	18 April 1977
2	70	0.175	359	3.70	1.29	7 October 1976
			361	3.25	1.14	7 October 1976
3	100	0.25	347	4.30	2.15	5 October 1976
			349	5.25	2.63	5 October 1976

APPENDIX 1

(continued)

SECTION III

(continued)

Formulation 3

Paraquat dose: 100, 250, 350 and 500 mg PQ^{2+}/kg

Emetic dose: 2, 5, 7 and 10 mg PP796/kg

Volume: 100 ml with water

Group	Dose mg PQ^{2+}/kg	mg P	96/kg	Animal No.	Bodyweight (kg)	Volume of test soln. (ml)	Date dosed
1	100			351	4.85	2.43	5 October 1976
				353	4.75	2.38	5 October 1976
2	250			363	4.10	5.12	7 October 1976
				365	4.80*	4.68	7 October 1976
3	350			403	3.65	6.38	1 November 1976
				405	3.80	6.65	1 November 1976
4	500	1		377	2.20	5.50	12 October 1976
				379	2.65	6.63	12 October 1976

The animals were dosed with appropriate volumes of the test formulations taken from the bulk samples.

Formulation 3 was prepared by adding 350 mg PP796 to a 100 ml sample of Formulation 2 to give an emetic concentration of 2 mg for every 100 mg PQ^{2+}.

The volumes of each solution shown in the tables were made up to 100 ml with tap water.

* See page 29

APPENDIX 2

TIMES OF VOMITING FOLLOWING DOSING

SECTION I

THE TOXICITY AND MODE OF ACTION OF THE EMETIC PP796 ALONE

EXPERIMENT 1 - To find a dose which would produce vomiting within 1 hour.

Dose: PP796/kg	1 mg						2 mg			
Animal No. / No. of times of vomiting	281		283				281		283	
	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.
1	0	16	1	00 to	1	45	0	22	0	23
2	0	16.5	1	45 to	2	17	0	25	0	27
3	0	17					0	32	1	20
4	0	22					0	39		
5	0	29					1	45		
6	0	40					2	23		
7	0	41					3	40		
8							5	25		

EXPERIMENT 2 - To investigate the toxicity of PP796.

Dose: PP796/kg	5 mg				10 mg				20 mg				30 mg			
Animal No. / No. of times of vomiting	339		341		339		341		339		341		339		341	
	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.
1	0	03	0	11	0	05	1	37	0	16	0	14	0	06	0	19
					no further record of vomiting											

APPENDIX 2

(continued)

SECTION II

THE EFFECT OF PP796 UPON THE TOXICITY OF A COMMERCIAL FORMULATION OF PARAQUAT (GRAMOXONE W), A 20% AQUEOUS SOLUTION OF PARAQUAT CATION CONTAINING WETTING AGENTS

EXPERIMENT 4 - To find the lethal dose of Gramoxone W.

Dose: PQ^{2+}/kg	85 mg							
Animal No. / No. of times of vomiting	285		287		289		291	
	hr.	min.	hr.	min.	hr.	min.	hr.	min.
1	5	30	9	00	3	49	5	45
2	9	30			5	30	5	59
3					9	00	8	15

EXPERIMENT 5 - To test the protective effect of PP796 at the lethal dose level of Gramoxone W.

Group 1

Dose: PQ^{2+}/kg	100 mg															
Animal No. / No. of times of vomiting	63		95		293		295		313		315		317		319	
	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.
1	3	45	4	57	4	58	4	22	4	44	4	57	4	44	5	00
2	4	36	5	20			4	42	5	06			4	52	5	40
3	4	40					4	45								
4	4	57					4	57								
5	7	35					5	10								

Group 2

Dose: PQ^{2+}/kg + PP796/kg	100 mg + 2 mg															
Animal No. / No. of times of vomiting	297		299		301		303		305		307		309		311	
	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.	hr.	min.
1	0	55	0	31	0	25	7	35	0	20	4	50	0 20 x 3		0	26
2	(vomited several times						9	00	0	22	6	10	0	22	0	27
3	during first hour)								0	24			0	23	0	28
4									0	37			0	28	1	21

APPENDIX 2

(continued)

SECTION II

(continued)

EXPERIMENT 6 - To find the upper limit of paraquat dose against which PP796 can provide protection.

a. Using a low volume of dosing solution.

Group 1

Dose: 250 mg PQ^{2+}/kg + 2.5 mg PP796/kg				
Animal No. / No. of times of vomiting	329		331	
	hr.	min.	hr.	min.
1	0	23	0	37
2	0	39	1	02
3			2	07

Group 2

Dose: 500 mg PQ^{2+}/kg + 5.0 mg PP796/kg				
Animal No. / No. of time of vomiting	321		323	
	hr.	min.	hr.	min.
1	0	15	0	20
2	0	25	0	30
3	1	06		

b. Using a high volume dosing solution.

Group 1

Dose: 250 mg PQ^{2+}/kg + 2.5 mg PP796/kg				
Animal No. / No. of times of vomiting	325		327	
	hr.	min.	hr.	min.
1	0	10	1	47
2	0	40		

Group 2

Dose: 500 mg PQ^{2+}/kg + 5 mg PP796/kg				
Animal No. / No. of times of vomiting	333		335	
	hr.	min.	hr.	min.
1	0	20	0	20
2	0	35	1	05
3	2	28	1	40
4	3	00	2	00
5	3	17	2	14
6			2	53
7			3	01
8			4	02
9			5	08
10			6	10

APPENDIX 2

(continued)

SECTION III

THE TOXICITY OF THREE COMMERCIAL FORMULATIONS OF PARAQUAT BASED ON 'STENCHED' GRAMOXONE

EXPERIMENT 7 - To assess the toxicity of three commercial formulations of paraquat.

Formulation 1

Group 1

Dose 70 mg PQ^{2+}/kg				
Animal No. / No. of times of vomiting	407		409	
	hr.	min.	hr.	min.
1	0	23	-	-
2	0	29		
3	0	43		

Group 2

Dose 100 mg PQ^{2+}/kg								
Animal No. / No. of times of vomiting	343		345		369		371	
	hr.	min.	hr.	min.	hr.	min.	hr.	min.
1	0	19	0	17	0	04	0	08
2			2	46				

Group 3

Dose 250 mg PQ^{2+}/kg				
Animal No. / No. of times of vomiting	355		357	
	hr.	min.	hr.	min.
1	0	10	0	13
2	0	15	5	00
3	0	34		
4	5	09		

APPENDIX 2

(continued)

SECTION III

(continued)

Formulation 2

Group 1

Dose 40 mg PQ^{2+}/kg + 0.1 mg PP796/kg								
Animal No. / No. of times of vomiting	373	375	399	401	541	583	589	593
	hr. min.	hr. min.	hr. min.	hr. min.	hr. min.	hr. min.	hr. min.	hr. min.
1	- -	- -	5 07	- -	6 35	8 00	8 00	6 43
2					7 05			
3					8 00			

Group 2

Dose 70 mg PQ^{2+}/kg + 0.175 mg PP796/kg				
Animal No. / No. of times of vomiting	359		361	
	hr.	min.	hr.	min.
1	0	14	0	15
2			5	12

Group 3

Dose 100 mg PQ^{2+}/kg + 0.25 mg PP796/kg				
Animal No. / No. of times of vomiting	347		349	
	hr.	min.	hr.	min.
1	0	36	1	30
2			2	39

APPENDIX 2

(continued)

SECTION III

(continued)

Formulation 3

Group 1

Dose 100 mg PQ^{2+}/kg + 2 mg PP796/kg				
Animal No. / No. of times of vomiting	351		353	
	hr.	min.	hr.	min.
1	0	11	0	09

Group 2

Dose 250 mg PQ^{2+}/kg + 5 mg PP796/kg				
Animal No. / No. of times of vomiting	363		365	
	hr.	min.	hr.	min.
1	0	07	0	04 x 4
2			0	05
3			0	17

Group 3

Dose 350 mg PQ^{2+}/kg + 7 mg PP796/kg				
Animal No. / No. of times of vomiting	403		405	
	hr.	min.	hr.	min.
1	0	10	0	10
2	1	44	0	20
3	2	04	0	22
4	3	00	1	22
5	3	18	2	00
6	3	30	2	20
7			3	15

APPENDIX 2

(continued)

SECTION III

(continued)

Formulation 3

Group 4

Dose 500 mg PQ^{2+}/kg + 10 mg PP796/kg				
Animal No. / No. of times of vomiting	377		379	
	hr.	min.	hr.	min.
1	-	-	0	14

APPENDIX 3

FOOD CONSUMPTION

SECTION I

THE TOXICITY AND MODE OF ACTION OF THE EMETIC PP796 ALONE

EXPERIMENT 1 - To find the dose of PP796 which would induce vomiting within 1 hour

Animals dosed day 1 (5 May 1976)																							
Group	Dose	Animal No.	Pre-dose (days)							Observation period (days)													
			-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	1 mg PP796/kg	281	35	0	30	80	95	110	15	150	35	130	145	150	180	175	180	180	120	170	160	170	90
		283	40	70	80	115	100	50	75	45	95	105	115	125	125	75	80	110	115	130	150	130	125

Animals dosed day 1 (25 May 1976)																							
Group	Dose	Animal No.	Pre-dose (days)							Observation period (days)													
			-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	2 mg PP796/kg	281	90	150	150	140	180	175	175	90	115	155	170	175	150	165	155	140	145	145	150	180	105
		283	125	130	125	130	145	130	135	105	180	130	155	135	120	150	120	120	120	170	180	140	0

0 represents no food eaten on that day

APPENDIX 3

(continued)

SECTION I

(continued)

EXPERIMENT 2 - To investigate the toxicity of PP796

Group	Dose	Day/date dosed	Animal No.	Pre-dose (days)							Observation period (days)												
				-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	5 mg PP796/kg	1 (23/Sep/76)	339	175	170	175	180	175	165	125	85	50	170	190	185	185	190	190	190	180	180	105		
			341	150	80	145	135	110	45	55	20	140	65	155	135	95	90	65	90	170	125	0		

Group	Dose	Day/date dosed	Animal No.	Pre-dose							Observation period (days)												
				-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	10 mg PP796/kg	1 (5/Oct/76)	339								95	35											
			341								20	25											

APPENDIX 3

(continued)

SECTION I

(continued)

EXPERIMENT 2 - (continued)

Group	Dose	Day/date dosed	Animal No.	Pre-dose (days)							Observation period (days)												
				-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
3	20 mg PP796/kg	1 (7/Oct/76)	339								75	170	170	180	160	80							
			341								0	20	170	175	65	20							

Group	Dose	Day/date dosed	Animal No.	Pre-dose (days)							Observation period (days)												
				-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
4	30 mg PP796/kg	1 (13/Oct/76)	339								85	95	135	175	180	130	140	160	150	130	160	170	175	185
			341								80	120	115	165	160	85	135	110	125	115	160	175	125	160

APPENDIX 3

(continued)

SECTION II

THE EFFECT OF PP796 UPON THE TOXICITY OF A COMMERCIAL FORMULATION OF PARAQUAT (GRAMOXONE W), A 20% AQUEOUS SOLUTION OF PARAQUAT CONTAINING WETTING AGENTS

EXPERIMENT 4 - To find the lethal dose of Gramoxone W

Group	Dose	Day/date dosed	Animal No.	Pre-dose (days)							Observation period (days)													
				-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	87 mg PQ^{2+}/kg	1 (12/May/76)	285	175	190	180	175	180	180	175	65	50	0	0	Dead									
			287	105	105	25	135	170	105	110	5	80	0	0	110	0	10	140	60	40	105	130	125	180
			289	130	105	115	125	165	155	115	125	0	0	Dead										
			291	90	165	55	85	110	100	60	30	50	0	10	0	0	Dead							

0 represents no food eaten on that day

APPENDIX 3

(continued)

SECTION II

(continued)

EXPERIMENT 5 - To test the protective effect of PP796 at the lethal dose level of Gramoxone W

Group	Dose PQ^{2+}	Dose PP796	Day/date dosed	Animal No.	Pre-dose (days)							Observation period (days)													
					-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
			1 (25/May/76)	63	65	110	120	160	140	130	135	45	0	Dead											
				95	130	125	95	100	110	115	125	Dead													
				293	140	120	110	120	145	155	55	0	Dead												
				295	145	105	155	160	170	170	165	75	75	Dead											
	mg/kg	mg/kg	1 (16/June/76)	313	60	60	155	145	150	145	110	Dead													
				315	165	165	170	180	160	155	165	20	Dead												
				317	130	130	145	175	165	175	115	60	Dead												
				319	180	185	185	175	160	165	125	60	0	Dead											
2	100 mg/kg	2 mg/kg	1 (1/June/76)	297	190	175	175	175	175	180	170	90	90	120	110	160	165	135	170	150	145	175	185	170	160
				299	90	100	105	70	145	140	180	110	60	60	60	115	115	105	55	100	180	180	165	140	165
				301	105	115	135	130	120	125	15	0	0	0	0	100	10	10	110	60	100	85	90	150	50
				303	180	180	180	180	180	190	65	0	0	30	0	20	0	15	0	0	-	-	-	-	Dead
			1 (16/June/76)	305	75	90	100	150	110	120	115	40	50	55	65	60	60	95	195	175	165	90	145	85	55
				307	140	135	160	170	145	175	155	120	15	Dead											
				309	170	185	150	170	170	175	175	100	145	155	175	125	150	135	185	185	160	170	180	120	135
				311	150	165	150	165	155	170	125	20	30	175	175	185	175	180	175	175	165	180	125	185	115

- no data

APPENDIX 3

(continued)

SECTION II

(continued)

EXPERIMENT 6 - To find the upper limit of paraquat dose against which PP796 can provide protection

(a) Using a low (20 ml) total volume of dosing solution

Group	Dose PQ^{2+}	Dose PP796	Day/date dosed	Animal No.	Pre-dose (days)							Observation period (days)												
					-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	250 mg/kg	2.5 mg/kg	1 7/July/76	329	50	100	45	60	100	120	50	10	0	0	0	Dead								
				331	165	150	170	180	175	180	100	0	Dead											
2	500 mg/kg	5 mg/kg	1 21/July/76	321	105	65	125	165	145	165	105	115	95	90	0	Dead								
				323	95	155	125	135	140	145	125	90	125	115	Dead									

APPENDIX 3

(continued)

SECTION II

(continued)

EXPERIMENT 6 (continued)

(b) Using a high (100 ml) total volume of dosing solution

Group	Dose PQ^{2+}	Dose PP796	Day/date dosed	Animal No.	Pre-dose (days)							Observation period (days)													
					-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	250 mg/kg	2.5 mg/kg	1 (15/July/76)	325	90	180	135	145	130	135	160	55	70	0	40	30	5	25	150	140	115	115	140	90	130
				327	145	190	175	175	165	175	140	0	35	0	35	40	0	15	20	50	25	70	130	60	130
2	500 mg/kg	5 mg/kg	1 (6/Aug/76)	333	105	85	105	100	80	105	100	0	Dead												
				335	120	180	160	50	110	130	110	0	Dead												

APPENDIX 3

(continued)

SECTION III

THE TOXICITY OF THREE COMMERCIAL FORMULATIONS OF PARAQUAT
BASED ON 'STENCHED' GRAMOXONE

EXPERIMENT 7 - To assess the toxicity of three commercial formulations of paraquat

Formulation (1) containing no PP796

Group	Dose PQ^{2+}	Dose PP796	Day/date dosed	Animal No.	Pre-dose (days)							Observation period (days)												
					-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	70 mg/kg	0 mg/kg	1 (2/Nov/76)	407	90	90	170	85	160	175	50	50	90	100	80	140	125	85	95	125	140	115	110	140	75
				409	125	165	80	130	150	170	5	55	65	120	105	120	160	175	150	175	180	160	135	120	150
2	100 mg/kg	0 mg/kg	1 (5/Oct/76)	343	180	165	175	165	180	160	170	75	150	150	120	65	35	145	140	170	175	160	160	175	150
				345	170	170	165	160	165	150	155	155	150	160	160	155	160	130	155	165	170	170	180	160	170
				369	105	115	100	140	160	130	80	0	10	Dead											
				371	125	0	95	100	95	110	70	0	30	0	Dead										
3	250 mg/kg	0 mg/kg	1 (7/Oct/76)	355	100	130	160	150	140	110	50	30	Dead												
				357	70	60	130	115	60	115	40	20	0	Dead											

APPENDIX 3

(continued)

SECTION III

(continued)

EXPERIMENT 7 (continued)

Formulation (2) containing subemetic doses of PP796

Group	Dose PQ2+	Dose PP796	Day/date dosed	Animal No.	Pre-dose (days)							Observation period (days)													
					-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	40 mg/kg	0.1 mg/kg	1 (12/Oct/76)	373	135	55	100	140	165	125	70	70	95	80	40	60	55	55	60	110	140	140	155	170	170
				375	175	160	150	165	170	175	125	120	40	50	65	50	45	5	30	15	35	0	75	0	Dead
			1 (1/Nov/76)	399	60	80	110	75	85	110	30	5	5	20	20	0	10	Dead							
				401	105	85	145	130	160	130	160	50	25	95	0	0	0	0	0	0	10	0	15	5	5
			1 (18/Apr/77)	541	145	180	105	180	165	80	80	100	55	115	135	65	25	40	30	45	0	Dead			
				583	165	245	200	260	255	165	120	215	80	105	95	115	25	15	30	25	110	65	185	100	0
				589	150	225	200	205	220	165	95	130	60	70	140	90	15	35	0	0	85	45	0	10	20
				593	160	225	140	265	255	175	130	135	0	60	65	120	175	165	180	165	240	250	230	150	170
2	70 mg/kg	0.175 mg/kg	1 (7/Oct/76)	359	170	170	190	155	175	185	60	75	160	165	185	170	170	160	160	170	165	170	160	160	155
				361	60	60	175	160	140	180	40	50	0	0	Dead										
3	100 mg/kg	0.25 mg/kg	1 (5/Oct/76)	347	45	75	50	50	100	90	15	0	0	Dead											
				349	115	90	55	115	180	120	35	45	0	Dead											

APPENDIX 3

(continued)

SECTION III

(continued)

EXPERIMENT 7 (continued)

Formulation (3) containing emetic doses of PP796

Group	Dose PQ^{2+}	Dose PP796	Day/date dosed	Animal No.	Pre-dose (days)							Observation period (days)												
					-7	-6	-5	-4	-3	-2	-1	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	100 mg/kg	2 mg/kg	1 (5/Oct/76)	351	135	120	115	175	130	170	100	170	150	150	160	165	170	150	170	165	165	150	165	160	100
				353	125	130	130	125	140	155	105	30	90	65	50	125	145	125	90	115	180	55	150	165	75
2	250 mg/kg	5 mg/kg	1 (7/Oct/76)	363	15	60	80	165	135	130	0	0	0	5	25	85	20	135	0	100	95	100	70	45	40
				365	140	110	180	130	115	145	0	70	90	90	115	120	100	120	130	155	145	180	75	130	60
3	350 mg/kg	7 mg/kg	1 (1/Nov/76)	403	70	115	100	10	65	90	55	0	Dead												
				405	80	80	65	65	75	75	50	5	50	15	10	15	25	30	5	5	10	0	Dead		
4	500 mg/kg	10 mg/kg	1 (12/Oct/76)	377	115	55	70	85	145	175	75	Dead													
				379	130	75	65	85	105	90	55	0	Dead												

APPENDIX 4

BODYWEIGHTS

SECTION 1

THE TOXICITY AND MODE OF ACTION OF THE EMETIC PP796 ALONE

EXPERIMENT 1 - To find the dose of PP796 which would induce vomiting within 1 hour

Day	Dose
1	1 mg PP796/kg
21	2 mg PP796/kg

Animal No.	DAY OF EXPERIMENT						
	-5	1	10	17	21	31	38
281	3.90	3.90	3.85	4.10	4.18	4.23	4.35
283	3.50	3.50	3.45	3.63	3.70	3.85	3.75

APPENDIX 4

(continued)

SECTION I

(continued)

EXPERIMENT 2 - To investigate the toxicity of PP796

Day	Dose
1	5 mg PP796/kg
13	10 mg PP796/kg
15	20 mg PP796/kg
21	30 mg PP796/kg

Animal No.	DAY OF EXPERIMENT								
	-9	1	6	13	15	20	21	27	34
339	4.30	4.45	4.40	4.75	4.75	4.65	5.00	5.00	5.00
341	3.70	3.75	3.80	3.70	3.70	3.55	3.60	3.70	3.75

APPENDIX 4

(continued)

SECTION II

THE EFFECT OF PP796 UPON THE TOXICITY OF A COMMERCIAL FORMULATION OF PARAQUAT (GRAMOXONE W), A 20% AQUEOUS SOLUTION OF PARAQUAT CONTAINING WETTING AGENTS

EXPERIMENT 4 - To find the lethal dose of Gramoxone W

Group	Dose PQ^{2+}	Day/date dosed	Animal No.	DAY OF EXPERIMENT			
				-5	1	10	16
1	87 mg/kg	1 (12/May/76)	285	4.25	4.40	Dead	
			287	3.25	3.35	2.95	3.05
			289	3.95	4.05	Dead	
			291	3.80	3.90	Dead	

APPENDIX 4
(continued)
SECTION II
(continued)

EXPERIMENT 5 - To assess the protective effect of PP796 at the lethal dose level of Gramoxone W

Group	Dose PQ^{2+}	Dose PP796	Day/date dosed	Animal No.	DAY OF EXPERIMENT							
					-5	-4	1	7	10	11	17	18
1	100 mg/kg	0 mg/kg	1 (25/May/76)	63		4.40	4.30	Dead				
				95		3.60	4.00	Dead				
				293		3.55	3.55	Dead				
				295		3.60	3.60	Dead				
			1 (16/June/76)	313	3.65		3.55	Dead				
				315	3.75		3.65	Dead				
				317	4.50		4.45	Dead				
				319	4.45		4.40	Dead				
2	100 mg/kg	2 mg/kg	1 (1/June/76)	297	4.00		3.95			4.00		4.00
				299	3.90		3.85			3.85		3.90
				301	4.35		4.25			3.95		3.90
				303	4.00		3.85			3.60		Dead
			1 (16/June/76)	305	4.95		4.90		4.90		5.00	
				307	4.20		4.20		Dead			
				309	5.15		5.10		5.20		5.20	
				311	3.40		3.35		3.55		3.70	

APPENDIX 4

(continued)

SECTION II

(continued)

EXPERIMENT 6 - To find the upper limit of paraquat dose against which PP796 can provide protection

(a) Using a low (20 ml) total volume of dosing solution

Group	Dose PQ^{2+}	Dose PP796	Day/date dosed	Animal No.	DAY OF EXPERIMENT			
					-5	-3	1	7
1	250 mg/kg	2.5 mg/kg	1 (7/July/76)	329	3.80		3.80	Dead
				331	4.65		4.65	Dead
2	500 mg/kg	5 mg/kg	1 (21/June/76)	321		4.95	5.05	Dead
				323		4.00	3.85	Dead

APPENDIX 4

(continued)

SECTION II

(continued)

EXPERIMENT 6 (continued)

(b) Using a high (100 ml) total volume of dosing solution

Group	Dose PQ^{2+}	Dose PP796	Day/date dosed	Animal No.	DAY OF EXPERIMENT				
					-7	-6	1	9	16
1	250 mg/kg	2.5 mg/kg	1 (15/July/76)	325		4.60	4.55	4.30	4.35
				327		4.20	4.25	3.85	3.85
2	500 mg/kg	5 mg/kg	1 (6/Aug/76)	333	4.98		5.05	Dead	
				335	4.23		4.35	Dead	

APPENDIX 4

(continued)

SECTION III

THE TOXICITY OF THREE COMMERCIAL FORMULATIONS OF PARAQUAT
BASED ON 'STENCHED' GRAMOXONE

EXPERIMENT 7 - To assess the toxicity of three commercial formulations of paraquat

Formulation (1) containing no PP796

Group	Dose PQ	Dose PP796	Day/date dosed	Animal No.	DAY OF EXPERIMENT							
					-7	-5	-4	1	8	10	15	17
1	70 mg/kg	0 mg/kg	1 (2/Nov/76)	407		4.00		3.90		4.05		4.05
				409		4.50		4.30		4.50		4.45
2	100 mg/kg	0 mg/kg	1 (5/Oct/76)	343	3.85			3.60	3.60		3.50	
				345	4.85			4.65	4.78		4.50	
				369			2.85	2.85	Dead			
				371			3.35	3.35	Dead			
3	250 mg/kg	0 mg/kg	1 (7/Oct/76)	355	4.35			4.35	Dead			
				357	4.30			4.25	Dead			

APPENDIX 4

(continued)

SECTION III

(continued)

EXPERIMENT 7 (continued)

Formulation (2) containing subemetic doses of PP796

Group	Dose PQ^{2+}	Dose PP796	Day/date dosed	Animal No.	DAY OF EXPERIMENT											
					-10	-7	-5	-4	1	3	4	10	11	15	17	18
1	40 mg/kg	0.1 mg/kg	1 (12/Oct/76)	373				2.30	2.30				2.25			2.30
				375				2.10	2.10				1.85			Dead
			1 (1/Nov/76)	399				4.00	3.85				Dead			
				401				3.55	3.40				2.93			2.95
			1 (18/Apr/76)	541				2.85			2.35		Dead			
				583			3.50			3.30		3.00			2.80	
				589			2.65			2.43		3.20			1.95	
				593			2.65			2.20		2.30			2.45	
2	70 mg/kg	0.175 mg/kg	1 (7/Oct/76)	359	3.70				3.70			3.70		3.60		
				361	3.25				3.25			Dead				
3	100 mg/kg	0.25 mg/kg	1 (5/Oct/76)	347		4.55			4.30			Dead				
				349			5.30		5.25			Dead				

APPENDIX 4

(continued)

SECTION III

(continued)

EXPERIMENT 7 (continued)

Formulation (3) containing emetic doses of PP796

Group	Dose PQ^{2+}	Dose PP796	Day/date dosed	Animal No.	DAY OF EXPERIMENT							
					-7	-6	-4	1	9	11	16	18
1	100 mg/kg	2 mg/kg	1 (5/Oct/76)	351		4.95		4.85		4.70		4.70
				353		4.90		4.75		4.65		4.65
2	250 mg/kg	5 mg/kg	1 (7/Oct/76)	363	4.00			4.10	3.70		3.80	
				365	4.85			4.80	4.80		4.75	
3	350 mg/kg	7 mg/kg	1 (1/Nov/76)	403			3.80	3.65		Dead		
				405			3.95	3.80		3.38		Dead
4	500 mg/kg	10 mg/kg	1 (12/Oct/76)	377			2.20	2.20		Dead		
				379			2.65	2.65		Dead		