The Effect of Administration of an Emetic (PP796) on Paraquat Toxicity in Dog and Monkey

CTL/R/391

SUMMARY

(Only Dog Part)

Study design

In the case of dogs, the total dosing volume was 50 ml containing the required volume of "Gramoxone" (formulation no. JF1423/B) such that animals received either 20 mg or 30 mg paraquat cation/kg body weight and either 2 or 3 mg of emetic/kg body weight. Animals were dosed by stomach tube with paraquat either with emetic or without, and the animals were then observed and the time to vomit recorded. Blood samples (approximately 5 ml) were taken at intervals from a jugular vein. Paraquat was measured in these samples.Two experiments were carried out, each experiment involving 8 animals.

NOTE: It is not clear from the report which animals were dosed at 20 mg/kg and which at 30 mg/kg. The same is true for the emetic dose (2 vs. 3 mg)!

Formulation

Gramoxone (JF1423/B) with PP796

Results

Mortality

Without emetic:

Dog 1	died on day 4
Dog 2	died on day 4
Dog 3	died on day 7
Dog 9	died on day 8
Dog 10	died on day 6
Dog 11	died on day 5

No mortality in dogs treated with emetic.

Clinical Observations

None of the dogs given only paraquat vomited. In the dogs given the ematic together with the paraquat, emesis ocurred at 12 to 30 minutes (experiment 1, 2mg emetic/kg???) and 6 to 12 minutes (experiment 2, 3 mg/kg emetic???). Three out of four dogs died in the non-emetic groups of both experiments. In the emetic-dosed groups all dogs survived.

Toxicokinetics

The concentration of paraqbat in the plasma of dogs dosed with paraquat plus emetic was considerably less than that in the plasma of dogs dosed with paraquat alone

Conclusion

It seems clear that the inclusion of an emetic dose of PP796 to a formulation of paraquat reduces the absorption of paraquat and thus significantly reduces the toxicity of the formulation.

THE CONCENTRATION OF PP 796 REQUIRED TO PRODUCE EMESIS IN EXPERIMENTAL ANIMALS AND AN ESTIMATION OF THE EMETIC DOSE IN MAN

REPORT NO: CTL/R/390

SUMMARY

This report contains a summary of studies conducted in dogs, monkeys, pigs and men with PP796 and reports the emetic effect as a function of the concentration.
The author concludes:

"From the limited evidence of clinical trials and data from experimental animals, it is concluded that PP 796 should be added to paraquat formulations at a level of 5 mg in 10 ml (0.05%). It is estimated that the majority of those ingesting 10 ml of this formulation will vomit within an hour."

PARAQUAT : .COMPARISON OF THE ORAL TOXICITY TO THE DOG OF THE TWO FORMULATIONS JF 1682B AND JF 6043

Report No: CTL/P/362

SUMMARY

Study design

JF6043 is a formulation containing 200 g/l of the herbicide paraquat plus pyridine bases, 0.05% (w/v) PP796 (emetic) and 10% (w/v) wetters. This report describes an experiment carried out to compare to oral toxicity to dogs of JF6043 and the existing 20% (w/v) paraquat formulation 'Gramoxone^{T} Export **(JF1682B)**.
Groups of two dogs (one male and one female) were fasted for 24 hours, then given various doses (JF1682B: 20 mg/kg and 50 mg/kg; JF6043: 50 mg/kg and 100 mg/kg) of one or other of the undiluted formulations by gavage and observed for up to two weeks.

Formulation

JF6043: formulation containing 200 g of the herbicide paraquat (1,1'-dimethyl-4,4'' bipyridylium dichloride) per litre (ie. 20% w/v) plus 1% (w/v) pyridine bases, 0.05% (w/v) PP796 (emetic) and 10% (w/v) wetters.
JF1682B: the existing 20% (w/v) paraquat formulation 'Gramoxone^{T}Export .

Results

Mortality

JF 1682B:

20 mg paraquat ion/kg (0/2):	No mortality
50 mg paraquat ion/kg (2/2):	Dog 4 found dead on Day 1 Dog 3 sacrificed Day 4

JF 6043

50 mg paraquat ion/kg (0/2):	No mortality
100 mg paraquat ion/kg (1/2):	Dog 3 found dead on Day 5

Clinical Observations

JF 1682B:
Dog 4 which received 50 mg/kg vomited and showed signs of respiratory distress within an hour of dosing. None of the other dogs vomited. Dog 3 first showed signs of poisoning three days after dosing and was killed *in extremis* a day later when it was subdued, thin, showing some inco-cordination of its hind limbs, producing very dark faeces and had discharge around its eyes.

JF 6043
Both dogs on the lower dose and the female on the higher dose vomited within an hour of dosing. At the higher dose level the male was found dead five days after dosing although the only sign of toxicity prior to death was subdued behaviour. The female was subdued three to seven days after dosing but was otherwise normal.

Conclusion

The values obtained suggest that JF6043 is less hazardous when administered orally to dogs than JF1682B. It is reasonable to associate this reduction in hazard in the paraquat formulation directly with the presence of an emetic which helped induce vomiting in three of the four animals which received JF6043,thereby reducing the total amount of paraquat cation available for absorption into the animal. In this instance the reduction in hazard is of the order of two to five times.

PP796: Emetic Study in Paraquat Treated Dogs

CTL/T/2471

SUMMARY

Study Design

A previous study showed that different doses of the emetic PP796 in dogs induced different times to initiation of vomiting and different degrees of emetic response. This study was performed in order to investigate the effects, in dogs, of different doses of PP796 when administered simultaneously with paraquat.
Groups of 3 male dogs were dosed orally with 20mg/kg paraquat ion (as the dichloride) and with 0, 0.5, 3.0 or 20mg/kg of the emetic PP796.
The effects of paraquat administration were assessed in the 4 groups by: i) peak plasma paraquat concentration, ii) area under the plasma paraquat concentration/time curve and iii) grossly observable paraquat-related lung lesions at necropsy 8 days after dosing.

Formulation
Paraquat dicloride (Y0061/066/001), containing 33.07% paraquat ion.
Emetic PP796 (Y00706/016/002)

Results
Mortalities
20 mg paraquat ion/kg& 20 mg/kg PP796 Dog 10 sacrificed Day 4

Clinical Signs
All dogs dosed with PP796 showed a decreased time to first vomit after dosing when compared with those dosed with paraquat alone. Dogs dosed with 20mg/kg PP796 + paraquat showed a shorter mean time to first vomit than those dosed with 3mg/kg PP796 + paraquat. Clinical signs were severe in those dogs dosed with 20mg/kg PP796 + paraquat. Vomiting was accompanied by prolonged episodes of marked retching and subdued behaviour, restlessness and recumbency, and these effects lasted for about 5 hours after dosing. Dog 10 struggled violently during the dosing procedure and vomited soon after dosing (1.5 minutes).
At 3mg/kg PP796 + paraquat, subdued behaviour, recumbency and panting accompanied vomiting but these effects were apparent only during the initial 1-2 hours post dosing.
0.5mg/kg PP796 + paraquat produced mild clinical effects. Slight hypoactivity was apparent in the first 1-2 hours and vomiting occurred in the first hour after dosing.
Paraquat alone (20mg/kg) was tolerated well. Slight hypoactivity was seen within the first 1.5 hours of dosing and the time to vomiting was extremely variable.

Body Weight
With the exception of dog 10, only minor effects on body weight were observed. Dog 10 had lost over 14% of his body weight at day 4 post dosing.
Toxicokinetics
There was a marked decrease in the peak plasma paraquat concentration, area under the curve and the severity of the paraquat-related lung lesions of dogs dosed with 0.5mg/kg or 3.0mg/kg PP796 + paraquat when compared with dogs dosed with paraquat alone. These reductions were dose related. The response in dogs dosed with 20mg/kg PP796 + paraquat was variable, some dogs showing a reduction in the effects of paraquat whilst others showed no decrease
The AUCs of dogs dosed with 0.5 or 3.0mg/kg PP796 + paraquat were also markedly lower than those of dogs dosed with paraquat alone. The values in dogs dosed with 20mg/kg PP796 + paraquat, with the exception of male 10, were lower than in dogs dosed with paraquat alone.

Terminal Studies

A number of treated dogs showed gross lung lesions which were considered to be related to paraquat toxicity. These lesions took the form of dark red areas, some of which were consolidated. There was a decrease, supported statistically, in the severity of the lung lesion in dogs dosed with 0.5 and 3.0mg/kg PP796 + paraquat when compared with dogs dosed with paraquat alone. There was considerable variation in the severity of paraquat-related lung lesions in dogs dosed with 20mg/kg PP796 + paraquat

Conclusion

The emetic PP796 reduced the effects of paraquat when administered simultaneously. In this study, increased doses of PP796 progressively reduced the effects of oral paraquat administration up to a maximum dose of 3.0mg/kg PP796. Large doses of PP796 (20mg/kg) resulted in a marked variability in the effects of paraquat, some dogs showing further reductions in the effects of paraquat whilst others showed a reversal of this trend.

Gramoxone
Single Dose Oral Toxicity Study in Dogs

CTL/C/2103

CTL/02103/Research/Report

SUMMARY

Study Design

This study was undertaken to investigate the toxic response to a single oral administration of GRAMOXONE in Beagle dogs and to monitor subsequent blood levels. The GRAMOXONE formulation (CTL reference No. Y00061/052/001) used in this study contained 20.5% w/v paraquat ion. Groups of 2 male and 2 female dogs were treated with dose levels of 2.5, 5, 10 or 20 mg of paraquat ion/kg

The animals were observed daily for 14 days for any signs of ill health or reaction to treatment. Food consumption and body weight were measured daily. Serial blood samples were taken from each of the animals over the 24 h period following dose administration. Individual plasma samples prepared from the aliquots of heparinised blood were despatched to the Sponsor for blood level analyses.

All animals were subjected to detailed gross pathological examination.
The results obtained may be summarised as follows.

Formulation

GRAMOXONE formulation (Y00061/052/001),20.5% w/v paraquat ion.

Results

Mortalities

2.5 mg paraquat ion.kg (0/4):	No mortalities
5 mg paraquat ion.kg' (0/4):	No mortalities

10 mg paraquat ion.kg' (1/4):	Dog 11found dead Day 7
20 mg paraquat ion.kg' (4/4):	Dog13 found dead day 7 Dog 15 sacrificed Day 7 Dog 16 sacrificed Day 7 Dog 14 sacrificed Day 8

Clinical Signs

Emesis was observed within 24 h of dosing in one animal receiving 10 mg paraquat ion/kg (10) and 2 receiving 20 mg paraquat ion/kg (15 and 16). The majority of animals also appeared subdued after dosing with 3 animals exhibiting laboured breathing (5 - 5 mg paraquat ion/kg and 14, 16 - 20 mg paraquat ion/kg). Encrustation of the palpebral openings was observed following dosing at 20 mg pq ion/kg and in isolated cases following doses of 5 or 10 pq ion/kg.

Toxicokinetics

The plasma paraquat profiles showed a dose-dependent increase in plasma paraquat absorption. Gramoxone mean peak plasma paraquat levels were observed between 0.5 hours and 2 hours post-dose. No sex difference in paraquat absorption was observed at any dose level administered. The kinetics show that peak plasma paraquat levels approaching 10g/ml or 24h AUC of approximately 40 µg/ml.h produce significant toxicity necessitating removal of the dog from the study

Body Weight

The premature decedents lost between 1.2 and 2.0 kg before death. The remaining animals showed only minor body weight losses or moderate weight gains.

Food Consumption

The premature decedents exhibited severe anorexia following dosing. Dog 12 (10 mg paraquat [HYPERLINK http://ion.kg]) showed temporary inappetance in Week 1. The remaining dogs had normal food consumption values.

Terminal Studies

Discolouration of the pleura and inflammation of the gastro-intestinal mucosa characterised the gross necropsy findings of all groups. There was some discolouration of the kidneys of Dogs l, 5 and 11 (2.5, 5.0 and 10 mg paraquat ion/kg respectively)

Conclusion

Single doses of 2.5, 5, 10 and 20 mg paraquat ion./kgin the GRAMOXONE formulation administered to groups of 2 male and 2 female Beagle dogs resulted in an incidence of lethality which corresponded to an LD50 value of 11.9 mg paraquat.

Gramoxone L
SINGLE DOSE ORAL TOXICITY STUDY IN DOGS
CTL/C/2104
02104-RES

SUMMARY

Study Design

This study was undertaken to investigate the toxic response to a single oral administration of Gramoxone L in Beagle dogs and to monitor subsequent blood levels. The Gramoxone L formulation (Y00061/106/001) used in this study contained 9.17% w/v paraquat ion. Groups of 2 male and 2 female dogs were treated with dose levels of 5, 10 or 20 mg Paraquat ion/kg. The animals were observed daily for 14 days for signs of ill health or reaction to treatment. Food consumption and body weight were measured daily. Serial blood samples were taken from each of the animals over the 24 h period following dose administration. Individual plasma samples prepared from the aliquots of heparinized blood were dispatched to the Sponsor for blood level analyses. All animals were subjected to detailed gross pathological examination.

Formulation

Gramoxone L (Y00061/106/001)

9.17% paraquat ion w/v

Results

Mortalities

5 mg paraquat ion/kg (1/4):	Dog 1 sacrificed Day 6
10 mg paraquat ion/kg (2/4):	Dog 7 sacrificed Day 7 Dog 8 sacrificed Day 8
20 mg paraquat ion/kg (4/4):	Dog 10 sacrificed Day 6 Dog 9 sacrificed Day 7 Dog 12 sacrificed Day 7 Dog 11found dead Day 11

Clinical Signs

Emesis occurred after dosing in all groups to varying degrees and likewise each group demonstrated subdued behaviour, laboured breathing and dehydration as reactions to treatment.

Toxicokinetics

Administration of Gramoxone L resulted in a dose-dependent increase in plasma paraquat absorption. Peak plasma paraquat levels were observed at 30 minutes and one hour post-dose. The peak levels were observed to increase with increasing doses of paraquat ion. This dose- dependant absorption of paraquat is reflected in the AUC values at 1, 4 and 24h with increased doses of paraquat resulting in increased systemic paraquat absorption. The final 24h AUC value increases proportionally with the dose of paraquat administered

Body Weight

The premature decedents showed severe anorexia prior to death while those animals

surviving showed only minor or transient loss of appetite. Body weight losses of between 1.5 and 2.6 kg were sustained by the premature decedents while the survivors displayed only minor fluctations in body weight.

Terminal Studies

The gross findings which characterised the premature decedents comprised discolouration of the pleura, particularly around the margins and often associated with a firmness of the tissue and varying degrees of inflammation of the gastro-intestinal tract. Three of the premature decedents (Nos. 7 and 8 - 10 mg pq ion/kg and 12-20 mg pq ion/kg.day) exhibited some discolouration of the renal surfaces.

Conclusion

Administration of increasing doses of Gramoxone L (5-20mg paraquat ion/kg) to adult dogs resulted in a dose-dependent increase in paraquat absorption. No sex differences were observed with paraquat absorption at the dose levels administered during this study. The median lethal dose of Gramoxone L was determined to be 8.2mg paraquat ion/kg. The plasma kinetics show that peak plasma paraquat levels approaching 10μg/m1 and/or 24 hour area under the curve values of approximately 20 μg/ml.h or greater produced significant toxicity necessitating removal of the dog from the study.

Preeglox L
Single Dose Oral Toxicity Study in Dogs

CTL/C/2105
02105/Research/Report

SUMMARY

Study Design

This study was undertaken to investigate the toxic response to a single oral administration of PREEGLOX L in Beagle dogs and to monitor subsequent blood levels. The PREEGLOX L formulation (Y00061/107/001) used in this study contained 4.31% w/v paraquat ion and 4.11% w/v diquat ion. Groups of 2 male and 2 female dogs were treated with dose levels of 5, 10 or 20 mg of paraquat ion/kg. The animals were observed daily for 14 days for any signs of ill health or reaction to treatment. Food consumption and body weight were measured daily. Serial blood samples were taken from each of the animals over the 24 h period following dose administration. Individual plasma samples prepared from the aliquots of heparinised blood were dispatched to the Sponsor for blood level analysis. All animals were subjected to detailed gross pathological examination

Results

Mortalities

5 mg paraquat ion/kg (0/4):	No mortalities.
10 mg paraquat ion/kg (2/4):	Dog 8 sacrificed Day 3

	Dog 7 sacrificed Day 4
20 mg paraquat ion/kg (3/4)	Dog 9 found dead Day 4 Dog 10 sacrificed Day 6 Dog 11 sacrificed Day 4

Clinical Signs

Emesis was observed within 24 h of dosing in all groups. Laboured breathing was observed prior to sacrifice in one animal (7) which received 10 mg pq ion/kg and in 2 animals (10 and 11) which received 20 mg pq ion/kg. The female dogs which received either 10 or 20 mg pq ion/kg showed evidence of dehydration as did one male dog (10) which received 20 mg pq ion/kg.

Toxicokinetics

The plasma paraquat profiles showed a dose-dependant increase in plasma paraquat absorption and mean peak plasma levels were observed at 30 or 60 minutes post-dose. The absorption of diquat was similar to that observed for paraquat although lower levels of diquat were absorbed. At most doses where animals were terminated, these individuals had shown peak plasma paraquat levels of 4 μg/ml or greater and/or 24h area under the curve values of approximately 24 mg/ml.h or greater.

Body Weight

Body body weight losses were sustained by animals given 10 or 20 mg pq ion/kg. Premature decedents lost between 1.4 and 1.8 kg while the surviving dogs lost 0.2-0.9 kg in the 7 days following dose administration and gained between 0.2 and 0.6 kg over the second 7 days. Animals treated with 5 mg pq.ion/kg exhibited smaller fluctations in body weight.

Food Consumption

The premature decedents showed severe reductions in food intake while the survivors showed only minor or transient inappetance.

Terminal Studies

The lungs of all dogs given 10 or 20 mg pq ion/kg appeared darkened in colour typically along the lobe margins. The lungs of Dog 4 (5 mg pq ion/kg) were similarly affected. The lung tissue of Dogs 4, 5, 6, 10 and 12 appeared firm in texture.
Half of the dogs treated with either 10 or 20 mg pq ion/kg exhibited varying degrees of inflammatory change in the mucosal surfaces of the gastro-intestinal tract and one animal (7 - 10 mg pq ion/kg) had discoloured and enlarged kidneys.

Conclusion

Single doses of 5, 10 and 20 mg pq ion/kg in the PREEGLOX L formulation administered to groups of 2 male and 2 female Beagle dogs resulted in an incidence of lethality which corresponded to an LD50 value of 12.2 mg pq ion/kg.

PREEGLOX L (WITHOUT SURFACTANTS)
SINGLE DOSE ORAL TOXICITY STUDY IN DOGS

CTL/C/2106
02106-RES

SUMMARY

Study Design

This study was undertaken to investigate the toxic response to a single oral administration of PREEGLOX L (without surfactants) in Beagle dogs and to monitor subsequent blood levels. The PREEGLOX L formulation (CTL reference No. Y00061/108/001) used in this study contained 3.92% w/v paraquat ion and 3.69% w/v diquat ion. Groups of 2 male and 2 female dogs were treated with dose levels of 10, 20 or 40 mg Paraquat ion/kg.
The animals were observed daily for 14 days for signs of ill health or reaction to treatment. Food consumption and body weight were measured daily. Serial blood samples were taken from each of the animals over the 24 h period following dose administration. Individual plasma samples prepared from the aliquots of heparinized blood were dispatched to the Sponsor for blood level analyses. All animals were subjected to detailed gross pathological examination.

Formulation

Preglox L, (Y00061/108/001), 3.92% w/v paraquat ion, 3.69% w/v diquat ion

Results

Mortalities

10 mg paraquat ion/kg (2/4):	Dog 3 sacrificed Day 9 Dog 2 found dead Day 10
20 mg paraquat ion/kg (2/4):	Dog 8 sacrificed Day 5 Dog 5 sacrificed Day 8
40 mg paraquat ion/kg (3/4):	Dog 11 sacrificed Day 3 Dog 9 sacrificed Day 4 Dog 12 sacrificed Day 8

Clinical Signs

Emesis was observed within 24 h of dosing in all groups. Green coloured faeces were also recorded for all groups typically between 1 and 3 h after dosing. Laboured breathing was observed in all groups and Dogs 5 (20 mg paraquat ion/kg') and 12 (40 mg paraquat ion/kg) appeared dehydrated prior to their humane sacrifice.

Toxicokinetics

The plasma paraquat profiles showed a dose-dependant increase in plasma paraquat absorption and mean peak plasma levels were observed at either 30 minutes or 1 h post-dose. The absorption of diquat was similar to that observed for paraquat although lower levels of diquat were absorbed. At all doses where animals were terminated, these individuals had shown peak plasma paraquat levels approaching a mean value of 11μg/m1 and/or 24h area under the curve values of approximately 16 μg/ml.h or greater. No sex differences were observed with paraquat or diquat absorption at the dose levels administered during this study

Body Weight

All premature decedents sustained body weight losses of between 1.3 and 2.7 kg. Dog 10 (40 mg paraquat ion/kg) lost 1.6 kg over the 3 days after dosing but regained 1.3 kg by the end of the 14 day observation period. The remainder of the animals exhibited smaller fluctuations in body weight.

Food Consumption

The premature decedents showed severe reductions in food intake while the survivors showed only minor or transient inappetance.

Terminal Studies

Dogs from all groups showed a dark discoloration of the lungs and varying degrees of inflammatory change in the mucosal surfaces of the gastro-intestinal tracts. Dog 8 (20 mg paraquat ion/kg) and Dog 10 (40 mg paraquat ion/kg) had discolored kidneys.

Conclusion

Single doses of 10, 20 and 40 mg paraquat ion/kg in the PREEGLOX L (without surfactants) formulation administered to groups of 2 male and 2 female Beagle dogs resulted in an incidence of lethality which corresponded to an LD50 value of 12.6 mg paraquat ion/kg. The plasma kinetics show that peak plasma paraquat levels approaching a mean value of 11μg/m1 and/or a 24 hour area under the curve value of approximately 16 μg/m1.h or greater produced significant toxicity necessitating removal of the dog from the study.

PARAQUAT: ABSORPTION AND ACUTE ORAL TOXICITY OF YF8004A IN THE DOG

REPORT NO: CTL/R/1115

SUMMARY

Study design

YF8004A, a liquid concentrate containing 200g/1 paraquat, was dosed orally to male dogs at 8, 16, 32, 64 and 128mg paraquat ion/kg. This was equivalent to 0.04 - 0.64 m1/kg of formulation. Clinical examinations were made prior to dosing and following dosing. Following dosing of the animals, each dog was monitored continuously for the first 2 hours. Records were made of all clinical signs. Close observation of each animal was continued

up to 12 hours and prior to each blood sampling time point. Animals were monitored closely at least twice daily thereafter with particular attention to food residues or prolonged GI tract symtoms. Care was also taken to identify any symptoms of pulmonary failure. Animals which did not fully recover from the initial symptoms on the day of dosing were humanely terminated.

Formulation

YF8004A (CTL 00061/170/001)
Like GRAMOXONE, contains 200g/1 paraquat ion (19.8% w/v),the same wetter system and alerting agents which include pyridine stench and blue dye and emetic agent (1.85g/1 PP796).

Mortality

128 mg paraquat ion/kg (1/3) **Dog 13 terminated Day 4**

Clinical Signs

As the dose level of formulation was increased from 8mg/kg to 64mg/kg there was a dose related reduction in the time taken to cause emesis due to the concomitant increase in the emetic dose. In all cases the first emesis contained the vast majority of the blue coloured formulation. The frequency of emesis reduced markedly about one hour after the onset of this symptom. On Day 2 there were significant food residues with the three dogs receiving the 128mg/kg dose. On day 3, dog 13 (128 mg/kg) was diagnosed with altered pulmonary function and persistent inappetance and was terminated on day 4.

Toxicokinetics

There was a dose related increase in both peak plasma paraquat concentration and area-under curve (AUC) from the 8 to 128mg/kg paraquat dose levels. The magnitude of change was however reduced by the fact that the time to emesis falls as the dose level of paraquat (and hence emetic), increases. This is substantiated by the fact that the time to peak plasma level falls from 120 min to 15 min between 16 and 128 mg/kg doses. The plasma profile at each dose level shows that paraquat reaches the bloodstream rapidly after oral dosing. However, it is particularly apparent at the higher dose levels that paraquat blood levels are relatively low due to a combination of reduced GI tract absorption and normal renal excretion. This results in very low plasma paraquat levels by 7 hours.

Conclusion

The absorption of paraquat from the GI tract was very low with YF8004A. This was directly a result of the formulation additives which accelerate the removal of paraquat from its site of absorption in the small intestine. This results in lower blood levels of paraquat.

PARAQUAT: ABSORPTION AND ACUTE ORAL TOXICITY OF YF9622 IN THE DOG

REPORT NO: CTL/R/1224

SUMMARY

Study design

The objective of this study was to assess the acute oral toxicity of a new MAGNOXONE formulation, YF9622. YF9622 was dosed to a groups of four dogs, at a concentration of 64 mg/kg. Blood samples were taken at frequent intervals during the 24 hour post-dose period and were analysed for plasma paraquat concentration. Plasma AUC was determined and the dogs were observed closely for clinical and behavioural abnormalities.

Formulation

Based on 200g/l formulation of Magnoxone. Paraquat ion 17.16% w/w (20.18% w/v). (CTL reference number Y00061/224). Contains magnesiumtrisilicate as gelling agent. YF9622 contains the same wetter system, alerting agents (pyridine stench and blue dye) and emetic agent (1.85 g/l PP796) as the previous MAGNOXONE formulation (YF8004A), although YF9622 as a lower pyridine base concentration (1g/l).

Results

Mortality

64 mg paraquat ion/kg (3/4)	**Dog 3 terminated Day 3**
	Dog 2 terminated Day 4
	Dog 4 terminated Day 4

Clinical Signs

Time to first emesis varied from 4 minutes (dog 3) to 28 minutes (dog 1). The majority of emesis occurred within the first hour after dosing and visual evidence of the formulation was confined to the first 20-40 minutes after dosing. Vomiting was observed on occasions in all four dogs up to day 3. Three of the four dogs showed adverse clinical signs of dehydration, subdued behaviour, decreased activity, inappetance and significant weight loss resulting in their termination for humane reasons on day 3 or 4.
Dog No.1 showed no significant clinical signs but was also killed on day 4.

Body Weight

Dogs 1, 2 and four lost between 0.5 and 2.7 kg body weight. (No information given on dog 3.)

Macroscopic and Microscopic Findings

Macroscopic Findings: Reddened, firm areas were present in the lung of all four dogs. Endocardial haemorrhage was present in the left ventricle of three out of four dogs (numbers 1, 2 and 4).
Microscopic Findings: Histological examination of the hearts showed that in dogs with macroscopic evidence of endocardial reddening , there was corresponding microscopic evidence of haemorrhage with or without inflammatory changes of the endocardial or subendocardial myocardium.

Toxicokinetics

Dog No 3 showed a very high peak plasma concentration within 15 minutes of dosing, despite of early initial emesis at 4 & 5 minutes after dosing. The blood paraquat levels remained very high over the early time period which suggests that some liquid vomit

containing the formulation had been breathed in during emesis.
The time to peak plasma concentration was 30 minutes for 3 of the 4 dogs and the peak plasma paraquat concentration for these 3 dogs (#1,3,4) were 6.9, 30.9 and 11.6 µg/ml, respectively.

Conclusion

This study has demonstrated that YF9622 is more toxic than previous versions of MAGNOXONE such as YF8004A. AUCs indicative of potential lethal effects were observed at 64mg/kg which is only a five-fold increase above the Median Lethal Dose for GRAMOXONE.

PARAQUAT: ABSORPTION AND ACUTE ORAL TOXICITY OF YF9677 IN THE DOG

REPORT NO: CTL/R/1225

SUMMARY

Study design

The objective of this study was to assess the acute oral toxicity of a new MAGNOXONE formulation, YF9677. Paraquat formulation YF9677 was dosed to groups of dogs, in a step-wise approach, at 32, 64 or 128mg/kg. Blood samples were taken at frequent intervals during the 24 hour post-dose period and were analysed for plasma paraquat concentration. Plasma AUC was determined and the dogs were observed closely for clinical and behavioural abnormalities. Both dogs of the 32 mg/kg dose group were terminated 3 days post dosing and the 64 mg/kg dose group was terminated 4 hours post dosing in order to provide prognostic AUC values and to minimize any adverse clinical signs. The dogs of the highest dose group were observed for 10 days post dosing.

Formulation

200g/l, paraquat ion 17.16% w/w (20.18% w/v).
(CTL reference number Y00061/225) Contains magnesiumtrisilicate as geling agent. YF9677 contains the same wetter system, alerting agents (pyridine stench and blue dye) and emetic agent (1.85 g/l PP796) as the previous MAGNOXONE formulation (YF8004A), although YF9677 has a higher pyridine base concentration .

Results

Mortality

32 mg paraquat ion/kg (1/2):	**Dog 1 terminated Day 3**
128 mg paraquat ion/kg (3/5)	**Dog 7 terminated Day 1**
	Dog 10 terminated Day 3
	Dog 6 terminated Day 4

Clinical Signs

Time to first emesis varied from 4 minutes (dog 1) to 22 minutes (dog 4). Dogs dosed with 64 or 128 mg/kg appeared to vomit more frequently and for a more prolonged period of

time than those dosed with 32mg/kg

Of the two dogs dosed with 32mg/kg of the formulation, one (dog 1) was subdued and inappetent on day 2 and by day 3 this dog was dehydrated, hypothermic, had lost weight.

The dogs dosed with 64mg/kg were killed after the 4 hour blood sample. There were no clinical abnormalities noted within this time period. Of the dogs dosed at 128mg/kg of the formulation, two (Nos. 8 & 9) survived to scheduled termination on day 10. Both of these dogs left some food, vomited and lost some weight in the initial 3-4 day period after dosing. Dog No.7 was killed for humane reasons 7 hours after dosing on day 1, due to adverse clinical signs. Dog No.6 and No.10, presented similar clinical signs of inappetence, vomiting, decreased activity, subdued behaviour and extreme dehydration. Dog No.10 was killed for humane reasons on day 3 and was observed to be thin, hunched and had a loud and forceful heart beat. Dog No.6 was killed for humane reasons on day 4 due to adverse clinical signs.

Body Weight

In the 32 mg/kg dose grou,p dog 1 had lost 1.3 kg weight by day 3, while dog 2 showed only minimal weight loss (0.1 kg). In the high dose group, dogs 6, 8 and 10 had lost between 0.8 and 1.8 kg of their weight by day 10 post dosing.

Macroscopic and Microscopic Findings

Macroscopic Findings: Reddened firm areas were present in the lung of all dogs dosed with 32 or 128mg/kg of the formulation, but were not evident in the dogs killed 4 hours after dosing with 64mg/kg. These findings are considered to be treatment related. Endocardial haemorrhage was present in the left ventricle of 1 of the 2 dogs dosed with 32mg/kg and 2 out of 5 dogs dosed with 128mg/kg and is considered to be treatment related. Pallor of the liver was observed in 3 or the 5 dogs dosed with 128 mg/kg and is considered to be treatment related. Fluid stomach and/or gut contents were present in a number of dogs in all 3 groups and small areas of mucosa reddening were present in two dogs.
Microscopic Findings: Histological examination of the hearts showed that in dogs with macroscopic evidence of endocardial reddening, there was corresponding microscopic evidence of haemorrhage with or without inflammatory changes of the endocardium or subendocardial myocardium.

Toxicokinetics

Dog No.1 (32mg/kg) showed high plasma profile over the first two hours post-dose, with a peak plasma level of 11.0µg /m1 at 15 minutes and a secondary peak of 10µg /m1 at 2 hours. The pharmacokinetic profile of this dog was considered to be abnormal and may be due to inhalation of vomit (containing the dose) or abnormally high gut permeability. Dog No.2 had extremely low plasma paraquat concentrations throughout with a peak of 3.2 µg/m1 at 30 minutes.

The time to peak plasma concentration was 15-30 minutes for all 3 dose levels of paraquat and the peak plasma paraquat concentrations ranged from 3.2 to 11.0 µg/ml, from 7.5 to 12.7 µg/ml and from 6.6 to 17.6 µg/ml for the 32, 64 and 128 mg/kg dose groups, respectively.

The two dogs that survived at 128mg/kg had the lowest peak plasma values and the lowest AUC values within their treatment group.

Conclusion

Paraquat formulation YF9677 has been dosed to dogs at 32, 64 and 128mg/kg. Based on plasma AUC and clinical observations, it would appear that YF9677 is at least ten-times less toxic than GRAMOXONE

Paraquat 200g/1 SL Formulation (A3879BU)
Toxokinetic Study in the Dog

CTL/XD7201

SUMMARY

Study design

A group of three male beagle dogs received oral doses (by capsule) of Paraquat 200g/1 SL formulation (A3879BU), on 5 occasions at monthly intervals. The nominal dose levels used were 8, 16, 32, 64 and 128mg paraquat ion/kg. These doses were equivalent to emetic dose levels of 0.06, 0.12, 0.25, 0.49 and 0.98 mg/kg PP796. Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis were recorded. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat and PP796 (the emetic included in the formulation) to be determined. Veterinary examinations (including cardiac and pulmonary auscultation) were made prior to each dose and prior to termination. General clinical observations, bodyweights and food consumption were measured at weekly intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examined *post mortem*. Specified tissues were taken for subsequent histopathological examination.

Formulation:

A3879: Global 200g/l product. Inteon , (203 g/l paraquat ion, 1.56 g/l PP796)

A3879BU	
	g/l
PQ ion (100%, emetic free)	200.00
DQ ion (100%)	-
PP796 (100%)	1.50
DEP ion	-
Manutex RM	9.00
Mag Sulphate	123.74
Antifoam DB-100	0.50

Antifoam MSA	-
Silcolapse 5020	
Toximul TA-1020/B	42.00
Agnique PG-8105 / Al2575	-
BioSoft SDBS 30LA	46.65
Ethomeen C/25	-
Aerosol OT-75E	21.05
Synprolam 35X15	-
Sulfacid blue 5J	2.50
F,D & C, blue no.1	-
Pyridine bases H	0.10
cis-3-hexenol	-
alpha-picoline	
Valeric Acid	-
Acetic acid	
Sodium Hydroxide	
Bitrex	-

Results

At dose levels of 46, 92, 184 and 368mg A3879BU formulation/kg (equivalent to 8, 16, 32 and 64mg paraquat ion/kg), plasma profiles of both paraquat and emetic were consistent with time to emesis and absence of paraquat toxicity. At each of these dose levels, plasma paraquat profiles were similar with increasing absorption of emetic at increasing doses. High plasma paraquat levels were not observed due to progressively earlier emesis with increasing dose. Peak plasma paraquat levels were observed after 1 hour at for the 8 and 16 mg/kg doses and at 30 minets for the 64 and 128 mg/kg doses. All dogs showed recovery from emesis within 2 hours of dosing, at these levels.
When dogs were exposed to 736mg A3879BU formulation/kg (equivalent to 128mg paraquat ion/kg), the initial plasma paraquat levels were higher than at the previous doses. This was only transient and plasma levels of paraquat dropped to that of lower dose levels by 4 hours.
However at this dose level, clinical signs including prolonged retching, abdominal discomfort and decreased activity were observed for up to 3 hours after dosing. Male 3, which had the highest peak plasma paraquat level, showed additional signs of inappetance, weight loss and decreased activity for several days following this last dose and slight lung lesions, similar to those seen with paraquat toxicity, were evident *post mortem* in this dog. This suggests that for this dog at least, 736mg A3879BU formulation/kg (128 mg paraquqt ion/kg) was close to a maximum tolerated dose level. The lung pathology of the other two dogs was normal.

Conclusion

Doses of 46-736mg A3879BU formulation/kg, equivalent to 8-128mg paraquat ion/kg, were well tolerated in the dog. The highest dose used represents more than ten times the toxic dose of Gramoxone, approximately 55mg formulation/kg (equivalent to 10mg paraquat ion/kg). This demonstrates in the dog, a vomiting species, a substantial improvement in

the safety of the A3879BU formulation, compared with the standard Gramoxone formulation.

PARAQUAT 200 G/L SL FORMULATION (A3879BU): FURTHER ASSESSMENT OF TOXICOKINETICS IN DOGS

CTL/XD7273/TECHNICAL TOXICOLOGY/REPORT

SUMMARY

Study design

Four groups of three male beagle dogs received oral doses (by capsule) of the Paraquat 200 g/L SL formulation (A3879BU), on 2 or 3 occasions at monthly intervals. The different groups were dosed with A3879BU formulations which had undergone changes in manufacture necessary for large-scale formulation (to improve storage) and inclusion of a biomarker. The different formulations tested were:

a) a batch that had been stored at 50°C for 10 weeks,

b) the pilot manufacturing batch,

c) a batch manufactured for Sri Lanka and,

d) the poured-off upper layer from Sri Lankan packs (some phase separation had been observed during storage of the Sri Lankan batch).

The nominal dose levels used were 32 and/or 64 mg paraquat ion/kg, plus 128 mg paraquat ion/kg used only for the Sri Lankan batch.
The dogs were observed continuously for 4 hours after each dose and then frequently during the remainder of the day. All incidences of emesis were recorded and vomit and faeces were removed immediately to prevent possible re-ingestion. Blood samples were taken at intervals following dosing to enable a plasma profile of paraquat and PP796 to be determined. Veterinary examinations were made prior to each dose and prior to termination. General clinical observations, bodyweights and food consumption were measured at weekly intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examined *post mortem*. Specified tissues, including lung and kidney, were taken for subsequent histopathological examination

Formulations

High Temperature Stored A3879BU:
(Y00061/998) 20.1% w/w paraquat ion and 0.10% w/w PP796

Pilot manufacture A3879BU:
(Y00061/999) 18.2% w/w paraquat ion and 0.13% w/w PP796

Sri-Lankan Batch A3879BU (A14380A):
(Y12693/062) 18.0% w/w paraquat ion, 0.13% w/w PP796 & 0.051% w/w diquat (biomarker)

A14380A Poured Upper Layer:
(Y12693/089) 16% w/w paraquat ion, 0.17% w/w PP796 & 0.043% w/w diquat (biomarker)

A3879BU

PQ ion (100%, emetic free)	200.00
DQ ion (100%)	-
PP796 (100%)	1.50
DEP ion	-
Manutex RM	9.00
Mag Sulphate	123.74
Antifoam DB-100	0.50
Antifoam MSA	-
Silcolapse 5020	
Toximul TA-1020/B	42.00
Agnique PG-8105 / AI2575	-
BioSoft SDBS 30LA	46.65
Ethomeen C/25	-
Aerosol OT-75E	21.05
Synprolam 35X15	-
Sulfacid blue 5J	2.50
F,D & C, blue no.1	-
Pyridine bases H	0.10
cis-3-hexenol	-
alpha-picoline	
Valeric Acid	-
Acetic acid	
Sodium Hydroxide	
Bitrex	-

Results

Mortality

64 mg paraquat ion/kg (Pilot Manufacture): Dog 5 terminated day 6

64 mg paraquat ion/kg (Upper Layer): Dog 11 terminated day 1

1280 mg paraquat ion/kg (Sri-Lanka Batch): Dog 7 terminated day 2

Clinical Observations

High Temperature Stored A3879BU:

With a higher dose of the paraquat formulation (and hence, increased dose of emetic), the time to first emesis was 12-20 minutes post-dosing for each of the 3 dogs, as opposed to 28-37 minutes at the lower dose. The frequency of emesis was also greater at the higher dose and the duration of emesis was longer in 2 of the 3 dogs. There were no additional clinical signs of significance following dosing and, once the

emetic effects had passed, the dogs were clinically normal.

Pilot manufacture A3879BU
There was no difference in the time to first emesis following the two doses, the time to first emesis ranging from 20-32 minutes post-dosing for each of the 3 dogs, on each occasion. There was also no difference in the frequency and duration of emesis following the different doses. There were no additional clinical signs of significance following dosing and, once the emetic effects had passed, the dogs were clinically normal.

Sri-Lankan Batch A3879BU (A14380A)
There was no real difference in the time to first emesis following increasing doses of the paraquat formulation, with the exception of Male 7 following the 128 mg/kg dose. The time to first emesis for this dog on this occasion was only 7 minutes (other dogs 16-28 minutes).

A14380A Poured Upper Layer
The time to first emesis was as expected, within 15 - 20 minutes of dosing, in two of the dogs. For Male 11, emesis occurred earlier than expected (at 12 minutes post dose). For all dogs, once the emetic effects had passed, the dogs were clinically normal

Body Weight and Food Consumption

Dogs 5, 7 and 11 (which were all terminated early) lost approximately 1 kg bodyweight, due to inappetance, following doses at 64 mg paraquat ion/kg of the pilot manufacture A3879BU, 128 mg paraquat ion/kg Sri Lankan batch (A14380A) or 64 mg paraquat ion/kg A14380A poured upper layer, respectively.

Bood Clinical Chemistry

Changes were generally confined to those dogs which were killed intercurrently (increased plasma alanine transaminase, alkaline phosphatase, glutamate dehydrogenase and /or creatine kinase activities and /or high plasma urea and/or bilirubin).

Macroscopic and Microscopic Findings

Microscopically, multifocal areas of erosion/ulceration were seen within the gastric mucosa and minimal proximal tubular degeneration/necrosis was present in the renal tubules, in two of the dogs killed intercurrently. These changes were consistent with an acute insult and are therefore considered treatment related. The changes within the lungs seen in several dogs, including those killed intercurrently and at scheduled termination were similar (comprising minimal/slight interstitial fibrosis, alveolar macrophages, pneumonocyte hypertrophy and interstitial pneumonitis) and occupied small areas of the lung parenchyma. These slight lung lesions are consistent with paraquat toxicity. Given the general lack of clinical signs referable to the lungs, they are unlikely to be of any pathological significance. One dog (5 - pilot manufacture) did have increased breathing rate and audible lung sounds but the lung pathology was no different to that seen in dogs without these signs; these lung changes are considered to be sub-acute to chronic in nature. They may have resulted from aspiration of small quantities of vomit at previous, lower doses of paraquat formulation.

Toxicokinetics

High Temperature Stored A3879BU

Following a dose of 32 mg paraquat ion/kg, there was variability seen in the time to reach peak plasma concentrations, which ranged from 0.5 to 2 hours in individual animals with a peak concentrations between 1.45 ug/m1 and 3.5 ug/m1. When the same animals received a dose of 64mg paraquat ion/kg there was less variability in response between animals. In 2 out of 3 animals peak plasma levels of approximately 10 ug/m1 were observed 15 minutes after dosing, whilst in the third animal paraquat absorption was delayed with a peak of 5.6 ug/m1 occurring at 30 minutes after dosing. Overall 24 hour AUC values were between 4.25 and 8.61 ug/ml.h following a dose of 32 mg and 64 mg paraquat ion/kg, respectively .

Following administration of 32 mg paraquat ion/kg, the plasma emetic levels were consistent across the group with the peak plasma emetic concentration of 8.1 ng/ml observed at 1 hour and then slowly eliminated from the plasma so that at 12 hours it was back at baseline levels. When the same animals received the higher dose of 64 mg paraquat ion/kg, the peak plasma levels occurred at 30 minutes, but there was considerable variability in values between the three animals with levels ranging from 3.9-12.2 ng/ml. With increasing dose of formulation, the initial rate of emetic absorption increased from 0.11 to 0.28 ng/ml/min and this was accompanied by faster elimination of emetic from the plasma therefore resulting in lower AUC values at the higher dose.

Pilot manufacture A3879BU

Following administration of 32 mg paraquat ion/kg, peak plasma paraquat levels were observed at 30 minutes with values ranging from 2.2-6.6 ug/m1. When the dose was increased to 64 mg paraquat ion/kg, there was no alteration in the peak plasma concentration observed, the main difference observed with this increased dose was a slower elimination of paraquat from the plasma between 0.5 and 4 hours post dose. This resulted in similar 1 hour AUC values for the two doses but at 4 and 24 hours, the AUC values from the higher dose were approximately 25% greater.

At both 32 and 64 mg paraquat ion/kg, dog 5 had lower plasma emetic levels than the other two animals in this group. The peak plasma emetic levels were observed at 30 minutes post dose. With this batch of A3879BU, more emetic was absorbed during the first 15 minutes after the 32 mg paraquat ion/kg dose than the 64 mg paraquat ion/kg dose. The absorption and elimination of emetic followed the same pattern, however the plasma emetic levels following the 64 mg paraquat ion/kg dose always remained higher than those observed with the lower dose and therefore resulted in higher AUC values throughout.

Sri-Lankan Batch A3879BU (A14380A)

There was evidence of a dose response following doses of 32, 64 and 128 mg paraquat ion/kg . At the lower doses, the peak plasma paraquat levels were observed at 0.5 and 1 hour post dose, with levels of 0.95 – 1.52 ug/m1 and 1.86 – 3.6 ug/m1 following a 32 and 64 mg paraquat ion/kg dose, respectively. At the highest dose level, there was variability in the plasma paraquat response between the three animals; dog 7 showed a much higher peak plasma level of 13.33 ug/m1 at 15 minutes post dose whilst dogs 8 and 9 showed peak levels of 2.51 and 3.99 ug/m1 at l hour and 30minutes post-dose,

respectively. Despite the levels of paraquat in the plasma reaching such high levels in dog 7, it was rapidly eliminated from the plasma and, at 7 hours post dose, all animals showed similar low paraquat levels

The peak plasma emetic levels were observed at 30 mins after dosing. At the low dose levels there was no evidence of a dose response, with the higher dose of 64 mg paraquat ion/kg resulting in a lower initial rate of absorption than the 32 mg paraquat ion/kg dose; however there was a greater than 4-fold increase when the dose level was increased to 128 mg paraquat ion/kg. The early phase of the absorption profile was affected by considerable animal variability. Following each dose, there was always one animal that had absorbed very little emetic at 15 minutes, which may have affected the dose response.

Poured Upper Layer

At 64 mg paraquat ion/kg, the peak plasma levels were observed at 30 minutes post-dose. There was considerable variability seen between the three animals; the peak levels ranged from 2.08 to 16.97 ug/m1 for dogs 10 and 11 respectively, whilst the peak level for dog 12 was 7.17 ug/m1. Although the peak plasma concentration was high in dog 11, the paraquat was rapidly eliminated from the plasma and, at 7 hours post dose, all dogs showed similar plasma paraquat levels. This variability in plasma paraquat levels during the early stages of exposure also had an impact on the overall AUC values.

Following a dose of 64 mg paraquat ion/kg, peak plasma emetic levels were observed at 30 minutes post dose. Emetic absorption was rapid with slow, steady elimination that was essentially complete by 7 hours post dose. Dog 11 showed peak plasma emetic levels almost 2-fold higher than the other 2 dogs, with a peak plasma value of 27.40 ng/ml (compared with approximately 12.3 and 13.5 ng/ml) and an initial rate of absorption of 1.827 ng/ml/min compared with 0.28 and 0.68 ng/ml/min in the other 2 dogs. The plasma profiles from dogs 10 and 12 were almost identical, whilst dog 11 showed much higher levels up to 7 hours post dose, after which point the plasma emetic levels in all 3 dogs were similar.

Conclusion

Oral administration to dogs of Paraquat 200 g/L SL formulation (A3879BU)/kg, at dose levels of 32, 64 or 128 mg paraquat ion/kg to dogs, produced effective emesis which generally prevented any increased exposure to paraquat with increased doses of formulation.

Higher paraquat absorption was seen following storage of the A3879BU formulation at high temperature and from the poured-off upper layer of the Sri Lankan batch. Although the pilot manufacture showed increased paraquat absorption, the Sri Lankan batch gave a very similar toxicokinetic profile compared to the lab-scale A3879BU.

However, these data demonstrate that despite storage at a high temperature, alterations in manufacturing scale or formulation separation following storage, in a vomiting species namely the dog, this alginate-based formulation still shows a considerable improvement in oral toxicity over Gramoxone

GRAMOXONE 200 G/L SL FORMULATION (A3879D): TOXICOKINETIC STUDY IN THE DOG

CTL/XD7388/Regulatory Report

SUMMARY

Study design

A group of three male beagle dogs received a single oral dose (by capsule) of a 200 g/L paraquat formulation, Gramoxone (A3879D), at a nominal dose level of 8 mg paraquat ion/kg. Allowing for specific gravity and purity, this dose was equivalent to 43 mg Gramoxone (A3879D)/kg. This paraquat formulation is equivalent to the commercial product, which was used in the early kinetic studies conducted in CTL in the 1980s and 1990s. The purpose of the present study was to compare the kinetics for this product with the historical data. The same protocol, as far as possible, was used.

The dogs were observed continuously for 4 hours after dosing and then frequently during the remainder of the day. All incidences of emesis were recorded and vomit and faeces were removed immediately to prevent possible re-ingestion. Blood samples were taken at intervals following dosing to enable a plasma profile of paraquat and PP796 (the emetic included in the formulation) to be determined. Veterinary examinations were made prior to dosing and two weeks after dosing. General clinical observations, bodyweights and food consumption were measured at weekly intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and 24 hours after dosing and two weeks after dosing.

Formulation

Gamoxone 200 g/l SL formulation (A3879D), NON-INTEON, (Y12693/170)

Results

Mortality

No mortality occured.

Clinical Observations

Vomiting was only observed in one dog (3) at 95 minutes post-dose and again at 3 hrs 20 minutes. No other clinical findings. No signs of toxicity

Body Weight and Food consumption

No effects on body weight and food consumption

Bood Clinical Chemistry

No effects on blood clinical chemistry

Toxicokinetics

There was considerable variation in the plasma profile in the three individual animals with peak plasma paraquat levels occurring between 1 and 2 hours post dose. Paraquat was steadily eliminated from the plasma and was only detectable at very low levels at 24 hours. The plasma emetic profile varied between the individual animals with peak levels occurring between 30 minutes and 2 hours post dose, with values ranging from 0.65-1.12 ng/ml. There was a rapid absorption of emetic up to 30 minutes post dose, with a further gradual increase at 2 hours post dose. Following this, emetic levels fell quite rapidly until 4 hours post dose and elimination then continued at a slower rate, and at 24 hours post dose no emetic was detectable.

Conclusion

There were no clinical signs of paraquat toxicity at this dose level, which concurs with previous studies in this Laboratory (Heylings, 2004). A comparison of emetic kinetics cannot be made since the emetic, PP796, was not measured in the previous studies. The incidence of emesis, in only 1 of the 3 animals, was also consistent with the previous observations and indicates that the systemic concentrations of PP796 are insufficient to cause prompt emesis in the dog at this exposure level. There was no association between emetic levels and vomiting in this study, since emesis was observed in a dog with relatively low levels of emetic in plasma over the first few hours following dosing.

PARAQUAT 240 G/L SL FORMULATION (A7813K): TOX1COKINETIC STUDY IN THE DOG

CTL/XD7355/Regulatory/Report

SUMMARY

Study design

A group of three male beagle dogs received oral doses (by capsule) of paraquat 240 g/L SL formulation (A78 13K), on 3 occasions at monthly intervals. The nominal dose levels used were 32, 64 and 128 mg paraquat ion/kg. Allowing for specific gravity and purity, these doses were equivalent to achieved dose levels of 150, 302 and 602 mg A7813K formulation/kg.

Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis were recorded. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat and PP796 to be determined. Veterinary examinations were made prior to each dose and prior to scheduled termination. General clinical observations, bodyweights and food consumption were measured at weekly intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examined *post mortem.* Specified tissues were taken for subsequent histopathological examination.

Formulation

240 g/l paraquat formulation (A7813K), (Y12693/044) (252 g/l paraquat ion, 1,5 g/l PP796)

A7813K	g/l
PQ ion (100%, emetic free)	240.00
DQ ion (100%)	-
PP796 (100%)	1.50
DEP ion	-
Manutex RM	9.00
Mag Sulphate	61.87
Antifoam DB-100	-
Antifoam MSA	0.25
Silcolapse 5020	
Toximul TA-1020/B	-
Agnique PG-8105 / AI2575	10.00
BioSoft SDBS 30LA	-
Ethomeen C/25	-
Aerosol OT-75E	-
Synprolam 35X15	-
Sulfacid blue 5J	-
F,D & C, blue no.1	3.85
Pyridine bases H	-
cis-3-hexenol	2.00
alpha-picoline	
Valeric Acid	-
Acetic acid	
Sodium Hydroxide	
Bitrex	-

Results

Mortality

No mortality occured.

Clinical Observations

No signs of toxicity.

Body Weight and Food consumption

Dog 2 lost 300 g body weight in week 11, following the 128 mg dose. No other effects on body weight and food consumption.

Bood Clinical Chemistry

No effects on blood clinical chemistry.

Macroscopic and Microscopic Findings

Small, discolored areas were present in the left and right apical lung lobes of dog 2. These small lung lesion corresponded to areas of minimal interstitial fibrosis, interstitial pneumonia, alveolar macrophage infiltration and pneumonocyte hypertrophy. The lungs of the other two dogs were normal. Minimal medullary calcification was present in the kidney of all 3 dogs; this is a common finding in dogs of this age and strain.

Toxicokinetics

There was no clear evidence of a dose response between the two lower dose levels (32 and 64 mg), as shown by the relative consistent AUC values. The peak plasma concentrations occured at a little more than 1 hour on average. When the dose was increased to 128 mg paraquat ion/kg, the peak plasma paraquat concentration still occurred close to 1 hour post dose but was about 2-fold higher. At dose levels above 64 mg paraquat ion/kg, there was more variability in the plasma paraquat levels between the three individual animals; dog 1 absorbed the least paraquat, dog 2 absorbed the most paraquat and, generally, dog 3 was in between.
The emetic was generally rapidly absorpted after dosing (peak concentrations occured close to 30 minutes post dose), which was followed by a gradual elimination of the emetic. At the lower dose levels, elimination of emetic from the plasma was essentially complete at 7 hours.
At the highest dose, elimination was slower and was essentially complete by 12 hours after dosing.

Conclusion

This study has demonstrated that dogs will tolerate high doses of up to 602 mg A7813K formulation/kg (equivalent to 128 mg paraquat ion/kg). Even at this high dose, peak plasma paraquat levels only reached maximal 5.16 μg/m1 and returned rapidly to almost baseline within 4 hours, demonstrating effective clearance into urine. The clinical signs following administration of A78 13K were minimal; the emetic response was prompt and did not last more than about 2 hours.

PARAQUAT 100 G/L SL FORMULATION (A9409AL): TOXICO1CINETIC STUDY IN DOGS

CTL/XD7396/Regulatory/Report

and

PARAQUAT 100G/L SL FORMULATION (A9409AL): PATHOLOGICAL EXAMINATION OF STORED TISSUES

CTL/XD7511/REGULATORY/REPORT

SUMMARY

Study design

A group of three female beagle dogs received oral doses (by capsule) of paraquat 100 g/L SL formulation (A9409AL), on 3 occasions at monthly intervals. This formulation contains an acid- triggered gel , emetic (PP796) and purgative (magnesium sulphate). The nominal dose levels used were 32, 64 and 128 mg paraquat ion/kg. Allowing for specific gravity and purity, these doses were equivalent to dose levels of 354, 707 and 1414 mg A9409AL formulation/kg.

Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis and defecation were recorded. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat and PP796 to be determined. Veterinary examinations were made prior to each dose and prior to scheduled termination (2 weeks after the final dose) . General clinical observations, bodyweights and food consumption were measured at weekly intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examined *post mortem.* Specified tissues were taken for subsequent histopathological examination.

Formulation

Paraquat 100 g/L SL formulation (A9409AL), (Y12693/100)
(101 g/L paraquat ion, 2.2 g/L PP796)

A9409AL	g/l
PQ ion (100%, emetic free)	100.00
DQ ion (100%)	-
PP796 (100%)	2.00
DEP ion	-
Manutex RM	12.00
Mag Sulphate	123.70
Antifoam DB-100	0.50
Antifoam MSA	-
Silcolapse 5020	
Toximul TA-1020/B	15.50
Agnique PG-8105 / AI2575	31.70
BioSoft SDBS 30LA	-
Ethomeen C/25	-
Aerosol OT-75E	-
Synprolam 35X15	-
Sulfacid blue 5J	2.50
F,D & C, blue no.1	-
Pyridine bases H	-
cis-3-hexenol	-
alpha-picoline	10.00
Valeric Acid	-
Acetic acid	
Sodium Hydroxide	
Bitrex	-

Results

Mortality

No mortality occured.

Clinical Observations

With increasing doses of the paraquat formulation (and hence, increasing dose of emetic), there was little change in the time to first emesis, which was generally approximately 15 minutes post- dosing. There was also little change in the duration and frequency of emesis with increasing doses of the paraquat formulation. There were no additional clinical signs of significance following dosing and, once the emetic effects had passed, the dogs were clinically normal.

Body Weight and Food consumption

There were no effects on bodyweights and food consumption.

Bood Clinical Chemistry

No effects on blood clinical chemistry.

Macroscopic and Microscopic Findings

There were no treatment related macroscopic and microscopic findings in lung, kidney liver, heart, oesophagus, stomach, duodenum, jejunum or ileum.

Toxicokinetics

The plasma paraquat profile was similar at all doses with peak concentrations occurring mostly at 15 and 30 minutes post-dose. There is no evidence of a dose response to the administration of A9409AL and this is reflected by the consistent AUC values at 1, 4 and 24 hours. Despite a 4-fold increase in dose volume the initial rate of paraquat absorption, determined over the first 15 minutes, was consistently low, at approximately 110 ng/ml/min. The plasma profiles for emetic were similar at all dose levels. At all dose levels the peak plasma concentration was observed between 15 and 30 minutes post dose. There are slight differences in the initial rate of absorption between the doses but this is not related to the amount of emetic given. However, there is a clear association between the initial rate of absorption and the time to first emesis, such that the higher the initial rate of absorption the earlier the time to first emesis. Elimination of both paraquat and emetic from the plasma was essentially complete by 24 hours.

Conclusion

Oral administration of up 1414mg A9409AL formulation/kg, a 100 g/L SL paraquat formulation equivalent to 128mg paraquat ion/kg, was well tolerated in the dog.

PARAQUAT A14380A: FURTHER TOXICOKINETIC ASSESSMENT OF A SEPARATED FORMULATION (126 g PARAQUAT ION/L) IN DOGS

SUMMARY

Study design

A separated paraquat formulation A14380A, which had been manufactured for Sri Lanka, was given orally (by capsule) to a group of three female beagle dogs on 2 occasions, at monthly intervals. This formulation had undergone phase separation during storage, resulting in a lower analysed paraquat concentration (126 g paraquat ion/l) and a higher emetic concentration (3.4 g/l) at the top of the drum compared with the nominal concentration of 200 g paraquat ion/1 and 1.5 g emetic/1 in the original formulation. The safety profile of this separated upper layer of this formulation has been assessed in this study. This formulation also contains an acid-triggered gel, emetic (PP796), a traceable biomarker (diquat) and purgative (magnesium sulphate). The nominal dose levels used were 5.05 and 10.1 mg paraquat ion/kg. Allowing for specific gravity and purity, these doses were equivalent to dose levels of 44 and 88 mg A14380A formulation/kg.

Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis and defecation were recorded. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat and PP796 to be determined. Veterinary examinations were made prior to each dose and prior to scheduled termination (2 weeks after the final dose). General clinical observations, bodyweights and food consumption were measured at weekly intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examinedpost *mortem.* Specified tissues were taken for subsequent histopathological examination.

Formulation

Separated paraquat formulation (A14380A), 126 g paraquat ion/l, 3.4 g/l PP796, diquat (conc. unknown) , magnesium sulphate (conc. unknown)

Results

Mortality

44 mg paraquat ion/kg (1/3): Dog 3 sacrificed 3 days after dosing

Clinical Observations

This formulation caused prompt and effective emesis (within 25-45 minutes of dosing), with no clinical signs of toxicity.

Body Weight and Food consumption

There were no treatment related effects on bodyweight and food consumption.

Bood Clinical Chemistry

There were no treatment related changes

Macroscopic and Microscopic Findings

Dog 3, killed intercurrently on day 4, had a subcutaneous haematoma in the ventral neck region, at *post-mortem* examination. The lungs of this animal were mottled red, localized to the diaphragmatic, left middle and left cranial lobes. The spleen was considered to be reduced in size.
Females 1, 2 and 4, killed at scheduled termination, had no abnormal macroscopic findings
Histological evaluation of dog 3 revealed slight congestion/haemorrhage and a minimal alveolitis within the lungs. However, the degree of histological change within the lungs is unlikely to account for the respiratory distress described clinically. A slight arteritis was found within an artery adjacent to the stomach. There was also a minimal vacuolar degeneration of the proximal tubules within the kidneys of this animal.
Animals 1, 2 and 4 had no abnormal microscopic findings.

Toxicokinetics

The plasma paraquat profile was similar at both doses with peak concentrations occurring at 0.5 and 1 hour post-dose. Following administration of A14380A, the peak levels observed were between 0.67 anf 1.92 μg/ml. There was no evidence of a dose response following administration of A14380A and this is reflected by the consistent AUC values at 1,4 and 24 hours. The initial rate of absorption was lower following the higher dose of A14380A with values of 0, 18 and 21 ng/ml/mim for the 5.05 mg/kg dose group and 0.67, 2.26 and 3.16 ng/ml/mim for the 10.1 mg/kg dose group.
Unlike the plasma paraquat profiles, the plasma emetic levels showed evidence of a dose response. In both dose groups the peak emetic levels occurred at 30 minutes post-dose with emetic levels between 4.76 and 5.8 μg/ml following the 44 A14380A/kg dose, and levels between 7.65 and 9.61 following the higher dose. The initial rate of emetic absorption was higher following administration of 44 mg A14380A/kg than the 88 mg/kg dose with mean values of 0.187 and 0.032 ng/ml/min, respectively

Conclusion

A separated paraquat formulation A14380A was well tolerated following oral administration to dogs at doses of up to 88 mg formulation/kg, equivalent to 10 mg paraquat ion/kg. Since there were no signs of paraquat toxicity at this dose, it is concluded that A14380A is less toxic than the equivalent dose of Gramoxone (a 200 g paraquat ion/1 formulation).

The cause of death of Female 3 is considered to be unrelated to treatment with the Sri Lankan batch A14380A. T he plasma paraquat levels were considered to be too low to produce paraquat toxicity. The clinical and macroscopic findings of subcutaneous swelling around the ventral neck region, haematoma formation around the jugular furrow and gasping and respiratory distress, suggest an acute haemorrhage from the jugular vein (possibly as a consequence of repeated venepuncture in this area on day 1) which then subsequently obstructed the airway.

Paraquat((135 g/l), Diquat (115 g/l)SL Formulation (A12984D): Toxokinetic Study in Dogs

XD7489/Regulatory/Report

SUMMARY

Study design

A group of three female beagle dogs received oral doses (by capsule) of the paraquat / diquat formulation Al2984D, on 3 occasions at monthly intervals. This formulation is based on Inteon technology and also contains an emetic (PP796) and purgative (magnesium sulphate). The nominal dose levels used were 32, 64 and 128 mg paraquat ion/kg. Allowing for specific gravity and purity, these doses were equivalent to dose levels of 280, 559 or 1119 mg Al2984D formulation/kg.

Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis and defecation were recorded. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat, diquat and PP796 to be determined. Veterinary examinations were made prior to each dose and prior to scheduled termination (2 weeks after the final dose). General clinical observations, bodyweights and food consumption were measured at weekly intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examinedpost *mortern.* Specified tissues were taken for subsequent histopathological examination.

Formulation

Paraquat (135g/1), Diquat (115g/1) SL formulation (Al2984D) (Y12693/131)

(14% w/v Paraquat cation, 11.4% w/v Diquat cation ,0.15% w/v PP796)

A12948D	g/l
PQ ion (100%, emetic free)	135.00
DQ ion (100%)	115.00
PP796 (100%)	1.50
DEP ion	-
Manutex RM	9.00
Mag Sulphate	65.00
Antifoam DB-100	0.50
Antifoam MSA	-
Silcolapse 5020	
Toximul TA-1020/B	-
Agnique PG-8105 / Al2575	
BioSoft SDBS 30LA	-
Ethomeen C/25	-
Aerosol OT-75E	-
Synprolam 35X15	-

Sulfacid blue 5J	2.50
F,D & C, blue no.1	-
Pyridine bases H	-
cis-3-hexenol	2.00
alpha-picoline	
Valeric Acid	-
Acetic acid	
Sodium Hydroxide	
Bitrex	

Results

Mortality

64 mg paraquat ion/kg (1/3): Dog 3 sacrificed the day of the 64 mg/kg dosing

Clinical Observations

With the increased dose of the paraquat formulation (and hence, increased dose of diquat and emetic), there was a reduction in the time to first emesis from approximately on average 29 minutes post- dosing to 14 minutes. There was little change in the duration of emesis with the increased dose of the paraquat formulation. However, the frequency of emesis was increased with increasing dose, and signs of purgation, abdominal discomfort and decreased activity were observed, for up to 4 hours after dosing, at 128 mg paraquat ion/kg. Following the second dose of 64 mg paraquat ion/kg, dog No. 3 vomited thick blue/green vomit with a gel mass at 13 minutes after dosing and continued to vomit and or retch occasionally until 40 minutes after dosing. From 1 hour post dosing, this dog showed signs of decreased activity, restlessness and was groaning. At approximately 5 hours post dose the dog vomited again and was sacrifieced for humane reasons at approximately 6 hours post dose, showing tremors, reduced stability, pallor, hunched posture and salivation.
In all other dogs, there were no additional clinical signs of significance following dosing and, once the emetic effects had passed, the dogs were clinically normal.

Body Weight and Food consumption

There was a slight bodyweight loss following both the 64 and 128 mg/kg doses in all of the dogs a slight reduction in food consumption over several days was evident after dosing at both the 64 and 128 mg/kg doses, in all dogs.

Bood Clinical Chemistry

No effects on blood clinical chemistry.

Macroscopic and Microscopic Findings

Dog 3, killed intercurrently in week 6, had pale, multifocal areas within the left and right caudal lobes of the lung, red areas in the fundic stomach and in the colon.

Histological evaluation of dog 3 revealed a minimal neutrophil infiltration within both the ileum and colon, as well as a minimal alveolitis and minimal interstitial fibrosis within the lungs.

Toxicokinetics

When animals received doses of 32 and 64 mg paraquat ion/kg, peak plasma levels were observed at 1 hour post-dose. This is reflected in the reduced AUC values at 1, 4 and 24 hours and is consistent with the earlier times to emesis. Following an oral dose of 64 mg paraquat ion/kg, dog 3 was observed to have a peak plasma paraquat concentration of 36.3 µg/m1 and was removed from the study at approximately 6 hours post-dose. The other two animals receiving this dose showed similar and much lower plasma paraquat levels. When the dose was increased to 128 mg paraquat ion/kg, the peak plasma concentration occurred at 30 minutes post-dose. The paraquat was cleared rapidly from the plasma and was almost completely eliminated by 7 hours post-dose. The higher paraquat exposure resulted in increased AUC values at 1, 4 and 24 hours compared with the previous doses. The emetic plasma profile showed a dose response, with increasing doses either the peak occurred earlier or the mean plasma emetic levels increased. After administration of 32 mg paraquat ion/kg, the peak plasma emetic level occurred at 0.5 and 1 hour post-dose. When the dose was increased to 64 and 128 mg paraquat ion/kg, the peak plasma emetic levels occurred at 15 and 30 minutes post-dose. The emetic was rapidly absorbed into the plasma following dosing. After reaching peak levels the emetic was steadily eliminated so that, at 7 hours post-dose, elimination was essentially complete. With increasing doses of Al2984D, there was an increase in the initial rate of emetic absorption over the first 15 minutes, which is consistent with earlier times to emesis as the dose level increased.

Discussion

Dog 3, killed intercurrently following the 64 mg/kg dose, showed a significantly increased absorption of paraquat, diquat and emetic, compared to the other 2 dogs on study, with plasma levels in excess of those than seen at the 128 mg/kg dose. The peak plasma paraquat level of 36.3ug/m1 and 4 hour AUC value of 82.01gg/ml.h were far in excess of any values seen with the Inteon paraquat formulations and are considered not to be representative of the true toxicity of this formulation. The most likely explanation is that one of the capsules did not dissolve within the stomach and passed directly into the intestine, where absorption of paraquat is much more rapid. This would explain the presence of the green-coloured, slightly mucoid contents within the colon, which resembled the test substance. The mild inflammation within the ileum and colon is consistent with local irritation. Therefore the plasma profile at 64 mg/kg in this dog is believed to be atypical.

NOTE PAGE 17 OF THE REPORT: "Female No. 3 vomited thick blue/green vomit with a gel mass at 13 minutes after dosing..." Doesn't this show that the capsule was disolved in the stomach?

Conclusion

The plasma profiles of paraquat, diquat and emetic were consistent with the clinical observations within this study. The active ingredients were rapidly absorbed but not sustained at high concentrations due to emesis.

Paraquat 100g/l Formulation (A9409B)
Toxicokinetic Study in Dogs
XD7502/Regulatory/Report

SUMMARY

Study design

A group of three female beagle dogs received oral doses (by capsule) of the 100g/1 R-Bix paraquat formulation, A9409B, on 3 occasions at monthly intervals. This formulation contains an emetic (PP796), a thickening agent (Kelzan) and a stenching agent (methyl pyridine). The nominal dose levels used were 32, 64 and 128 mg paraquat ion/kg. Allowing for specific gravity and purity, these doses were equivalent to dose levels of 333, 666 and 1332 mg A9409B formulation/kg.

Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis and defecation were recorded. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat and PP796 to be determined. Veterinary examinations were made prior to each dose and prior to scheduled termination (2 weeks after the final dose). General clinical observations, bodyweights and food consumption were measured at weekly intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examined *post mortem.* Specified tissues were taken for subsequent histopathological examination.

Formulation

Paraquat 100 g/l SL formulation (A9409B) (Y12963/171)
((9.6% w/v paraquat ion, 0.2% w/v PP796

A9409B	
PQ [g/l]	100
Inteon	no
Emetic [g/l)	2
Olfactoric. Alert Agent	Alpha picoline
OAA [g/l]	10
Alginate[g/l]	0
Purgative [g/l]	0
Bitrex [g/l]	0

Results

Mortality

No mortality occured.

Clinical Observations

Administration of the R-Bix paraquat 100 g/1 SL formulation A9409B was generally well tolerated by dogs at dose levels up to 128 mg paraquat ion/kg. With increasing doses of the paraquat formulation (and hence, increasing dose of emetic), there was little change in the time to first emesis, which was generally approximately 15 minutes post- dosing. The frequency of emesis was increased with increasing dose and signs indicative of abdominal discomfort were observed, for up to 4 hours after dosing at 128 mg paraquat ion/kg, in all 3 females. No signs of Toxicity.

Body Weight and Food consumption

There were no treatment related effects on bodyweight

Blood Clinical Chemistry

There were no treatment related effects on blood chemistry

Macroscopic and Microscopic Findings

At *post-mortem,* dog 2 had a well circumscribed pale area within the right cranial lobe of the lungs, 1.0cm in diameter, and a well circumscribed dark spot within the right caudal lobe, 0.5cm in diameter. Histological evaluation revealed minimal haemorrhage, interstitial fibrosis and alveolar macrophage infiltration within the lungs.
Dog 3 had a well circumscribed pale spot within the pleura of the right cranial lobe of the lungs, 1.0cm in diameter.
There were no other significant macroscopic findings.

Toxicokinetics

When animals received a dose of 32 mg paraquat ion/kg, peak plasma levels were observed between 30 minutes and 1 hour post dose. When the dose level was increased to 128 mg paraquat ion/kg, peak plasma levels were observed at 30 minutes post-dose. Elimination of paraquat from the plasma was rapid following a dose of 32 mg/kg or 128 mg/kg, whilst following administration of 64 mg/kg, the elimination was slower with plasma levels between 30 minutes and 2 hours remaining relatively constant prior to a true elimination phase. Elimination of paraquat from the plasma was essentially complete by 12 hours post-dose at all dose levels. The initial rate of paraquat absorption following an oral dose of 32 mg or 128 mg/kg were comparable at 64.6 ng/ml/min and both had consistent times to emesis of about 15 minutes, however, the mid-dose of 64 mg/kg had reduced paraquat absorption at 15 minutes with a rate of 38.3 ng/ml/min and this coincided with a later time to first emesis of 23 minutes

Peak plasma emetic levels were observed at 15 to 30 minutes post-dose following

administration of all doses of A9409B. The initial rate of emetic absorption was highest following administration of the lowest dose of 32 mg/kg which also had the fastest time to first emesis of 15 minutes, whilst the next dose of 62 mg/kg had the lowest rate and the slowest time to first emesis of 23 minutes. The 64mg/kg dose resulted in decreased absorption of the emetic across the full exposure period whilst the lowest and highest dose both showed very similar plasma kinetics.

Conclusion

This formulation caused prompt and effective emesis (from approximately 15-23 minutes up to 1 hour after dosing). However, each dog showed some clinical signs of abdominal discomfort for up to 3 hours following the cessation of emesis following the 128mg/kg dose. There were no effects on food consumption or bodyweights and there were no abnormalities detected at veterinary examination.

PARAQUAT 200G/L SL FORMULATION A3879EZ: TOXICOKINETIC STUDY IN DOGS

XD7532/REGULATORY/REPORT

SUMMARY

Study design

A group of three male beagle dogs received oral doses (by capsule) of the 200g/L paraquat formulation A3879EZ on 3 occasions at monthly intervals. This formulation is based on Inteon technology and also contains an emetic (PP796), a purgative (magnesium sulphate) and a stenching agent (cis-hexanol) . The formulation contains built-in wetters and was similar to a previous formulation, A3879BU. The nominal dose levels used were 32, 64 and 128 mg paraquat ion/kg. Allowing for specific gravity and purity, these doses were equivalent to dose levels of 184, 368 and 736 mg A3879EZ formulation/kg.

Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis and defecation were recorded. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat and PP796 to be determined. Veterinary examinations were made prior to each dose and prior to scheduled termination (2 weeks after the final dose). General clinical observations, bodyweights and food consumption were measured at intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examined *post mortem*. Specified tissues were taken for subsequent histopathological examination.

Formulation

Paraquat 200g/L SL formulation A3879EZ (20.9% w/v paraquat cation, 0.16%w/v PP796)

(Y12693/167)
cis-hexanol : 2 g/L
magnesium sulphate: 123.74 g/L
wetters: monoethanolamine: 62.5 g/L
GERONOL-CF-AR: 25 g/L
ETHOMEEN C/25: 10 g/L

Results

Mortality

128 mg paraquat ion/kg (2/3): Dog 3: terminated 6 hrs after dosing

Dog 2: terminated day 2

Clinical Observations

With the increased dose of the paraquat formulation (and hence, increased dose of emetic), there was a reduction in the time to first emesis from approximately 25 minutes post-dosing to 17 minutes. The duration of emesis also increased with the increased dose of the paraquat formulation. The frequency of emesis was increased with increasing dose, and signs of purgation, abdominal discomfort and decreased activity were observed, for up to 4 hours after dosing, at 64 and 128 mg paraquat ion/kg.

Following the third dose of 128 mg/kg, dog 3 showed moderate decreased activity, was moderately subdued and was salivating excessively at 6 hours after dosing. Due to the much higher plasma concentrations seen in this dog, compared to the other two, at all time points following dose, this dog was terminated for humane reasons.

Two days following the third dose of 128 mg/kg, dog 2 showed decreased activity, tremors, hunched posture, subdued behaviour, was visibly thin and had lost weight. This dog was terminated for humane reasons.

There were several observations of green coloured faeces or mucus, seen at all dose levels of A3879EZ, indicating that at least some of this formulation is not held within the stomach prior to emesis.

Body Weight and Food consumption

Dog 2 showed a significant reduction in food consumption and a significant weight loss of 1.1 kg 2 days following the 128 mg/kg dose. There were no other treatment related changes.

Bood Clinical Chemistry

No effects on blood clinical chemistry.

Macroscopic and Microscopic Findings

Dog 2 showed pale areas, located on the left and right peripheral apical lobes of the lung. This animal also revealed a diffuse area of reddening, within the duodenal mucosa and an area of reddening on the pyloric stomach mucosa. Dog 3 showed a red area, on the edge

of the right cardiac lobe of the lungs and red areas along the length of the jejunal mucosa and within the pyloric stomach mucosa.
There were no other significant macroscopic findings.

Dog 2 had a minimal alveolar macrophage infiltration and a minimal interstitial fibrosis within the lungs. This animal also showed a slight area of erosion/ulceration within the stomach, slight mucosal congestion and a slight inflammatory cell infiltration. Dog 3 had a minimal alveolar macrophage infiltration within the lungs.

Toxicokinetics

When animals received a dose of 32 and 64 mg paraquat ion/kg, peak plasma paraquat levels were observed mostly at 1 h post-dose with levels between 2.75 and 8.26 µg/ml and between 4.15 and 8.98 µg/ml, respectively. When the dose was increased to 128 mg paraquat ion/kg, peak plasma paraquat levels were observed at mainly at 30 minutes post-dose with levels between 7.14 and 13.98 µg/ml.
With increasing doses of A3879EZ the initial rate of paraquat absorption increased, however, this was not proportional to the dose of paraquat ion received. As the dose of paraquat ion increased the plasma paraquat profile altered with a broader peak shape, probably indicating that the elimination of paraquat from the plasma was taking longer to occur. Following the 32 and 64 mg/kg doses the 4 and 24h AUC values are similar. However, at the 128 mg A3879EZ/kg dose the 4 and 24h AUC values were approximately 2-fold higher than those seen at the lower doses.

Peak plasma emetic levels were observed at 30 minutes post-dose. With increasing dose the peak plasma emetic level increased. The initial rate of emetic absorption, determined over the initial 15 minutes, also increased with increasing dose and this was corresponding with earlier times to first emesis. The emetic was rapidly absorbed into the plasma, its elimination was gradual and essentially complete by 7h post-dose.

Conclusion

This 200g/L Inteon formulation of paraquat, A3879EZ, does not appear to offer the improved safety profile at all doses. The reason why A3879EZ was more toxic may relate to a poorer gelling effect in the stomach. The surfactant systems are different between the formulations and may have interfered with the acid-triggered gelling. Evidence for this in the present study relates to the observed green colour in the faeces of some animals and higher plasma paraquat levels.

Gramoxone
Effects of Increased Emetic Levels on Toxokinetics in the dog
026698-RES

SUMMARY

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. 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.**

Study Design

The dose levels of the Gramoxone formulation with increased emetic were selected as 16, 32 and 48mg paraquat ion/kg. The formulation was dosed to groups of 3 male dogs on three occasions by capsule. The same three dogs were use for the 16 mg/kg and the 48 mg/kg dosing.

Plasma samples were collected from the dogs during the 24h period after dosing and the concentration of paraquat in these plasma samples was determined. The toxicokinetic parameters AUC (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.

Formulation

Gramoxone Export, Y00061/131
200g/L paraquat ion, 0.5g/L PP796 **fortified to 1.2g emetic/L.**

Mortalities

16 mg paraquqt ion/kg	no mortalities
32 mg paraquqt ion/kg	no mortalities
48 mg paraquat ion/kg	dog 1 sacrificed on day 1 dog 3 sacrificed on day 8

Clinical Observations

Times to the first emesis varied from 12 to 23 minutes for the 16 mg dose group to 6 to 10 minutes for the medium dose group and 1 to 6 minutes for the highest dose group. The were no real differences in the number of episodes across the dose levels withe the exeption of dog 1 at the 48 mg/kg dose where emesis was still occurring more than 6 hours post-dose.

Toxicokinetics

Following the 16mg paraquat ion/kg dose the peak plasma level occurred at lh and 2hrs post-dose with levels between 4.29 μg/ml and 6.82 μg/ml detected, the level remained constant until 2h after which paraquat was steadily eliminated and by 24h no paraquat was detected in the plasma.

When the dose was increased to 32mg paraquat ion/kg the peak plasma level occurred earlier at 30 minutes and 1 h post-dose with levels between 2.79 μg/ml and 5.63 μg/ml detected, elimination of parquet from the plasma was steady until 7h 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 15 minutes, 1 and 3hrs post-dose with levels between 3.36 μg/ml and 8.06 μg/ml The elimination of paraquat from the plasma was slower at this dose. There was more variability in the individual responses at this dose level with dog 1 having a much greater absorption of paraquat across the 24h period than the other 2 animals.

Emetic values have not been determined in this study.

Body Weight

Dog 4 lost 0.7kg following the 32mg paraquat ion/kg dose and dog 3 lost 2.8kg following the 48mg paraquat ion/kg dose.. There we no other major effects on bodyweights over the duration of the study.

Food consumption

Dog 3 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.

Conclusion

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.

Paraquat 100 g/L SL formulation (A9409AM)
Preliminary Investigative Study In Dogs

XD7551/Technical Toxicology/Report

SUMMARY

Study design

A group of three male beagle dogs received oral doses (by capsule) of the 100g/L paraquat formulation A9409AM on six occasions at approximately monthly intervals. This formulation is based on INTEONS technology and also contains an emetic (PP796), a purgative (magnesium sulphate), stenching agent (pyridine bases) and a bittering agent (Bitrex). The dose levels used were 32, 64 and 128 mg paraquat ion/kg. Due to unexpected results with the initial dose of the first batch of formulation (J4669/49), a further batch of A9409AM was formulated at Jealotts Hill (batch reference J4899/010) and both batches were tested at monthly intervals: Dose level 32mg/kg was done twice with batch J4669/49

and once with the new batch J4899/010. Dose level 64 mg/kg was done with once with each batch. Batch J4899/010 was used for dose level 128 mg/kg.

Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis and defecation were recorded. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat and PP796 to be determined. Veterinary examinations were made prior to each dose and prior to scheduled termination (2 weeks after the final dose). General clinical observations, bodyweights and food consumption were measured at intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examined *post mortem*. Specified tissues were taken and stored for subsequent histopathological examination.

Formulation

Paraquat 100g/L SL formulation (A9409AM)
Batch reference number: J4669/49
CTL test substance reference number: Y12693/178
Purity: 10.2% w/v paraquat, 0.21% w/v PP796

Batch reference number: J4899/10
CTL test substance reference number: Y12693/209
Purity: 10.1% w/v paraquat cation, 0.21% w/v PP796 cation

A9409AM	g/l
PQ ion (100%, emetic free)	100.00
DQ ion (100%)	-
PP796 (100%)	2.00
DEP ion	-
Manutex RM	9.00
Mag Sulphate	123.74
Antifoam DB-100	0.50
Antifoam MSA	-
Silcolapse 5020	
Toximul TA-1020/B	15.50
Agnique PG-8105 / AI2575	31.70
BioSoft SDBS 30LA	-
Ethomeen C/25	-
Aerosol OT-75E	-
Synprolam 35X15	-
Sulfacid blue 5J	2.50
F,D & C, blue no.1	-
Pyridine bases H	1.00
cis-3-hexenol	-
alpha-picoline	
Valeric Acid	-
Acetic acid	

Sodium Hydroxide	
Bitrex	0.05

Results

Mortality

No mortality

Clinical Observations

The first dose of the A9409AM formulation (batch J4669/49) caused early emesis in two of the dogs and and a greater incidence of emetic episodes than considered usual for a dose equivalent to 32 mg paraquat ion/kg, based on similar INTEON® formulations (Brammer *et al*, 2005). Consequently, another batch (J4899/010) was dosed at 32 mg paraquat ion/kg and then the first batch (J4669/49) was repeated at this same dose level. There were no major differences in time to emesis or other clinical observations between the two batches at 32 mg paraquat ion/kg, although the duration of emesis was longer following the first dose of J4669/49 at 32 mg paraquat ion/kg than was seen following the repeat doses at this dose level. With the increased dose of the paraquat formulation (and hence, increased dose of emetic), from 32 to 64 mg paraquat ion/kg, there was a slight reduction in the time to first emesis from approximately 16 minutes post-dosing to approximately 10-14 minutes. Following the final dose of A9409AM, J4899/010 at 128 mg paraquat ion/kg ,the time to first emesis was slightly longer than at 64 mg paraquat ion/kg. There was little difference in the duration of emesis associated with increasing doses of the paraquat formulation.

Once the emetic effects had passed (generally 2 hours post dose), the dogs were clinically normal

Body Weight and Food consumption

There were no treatment related effects on bodyweight and food consumption.

Bood Clinical Chemistry

There were no treatment related changes

Macroscopic and Microscopic Findings

There were no macroscopic and microscopic abnormalities

Toxicokinetics

When animals initially received a dose of J4669/49 at 32 mg paraquat ion/kg the peak plasma paraquat levels were observed at 15 and 30 minutes post-dose with peak plasma levels between 7.89 µg/ml and 9.14 µg/ml. Paraquat was readily eliminated from the plasma, being largely cleared by 4 hours, with a mean 24h AUC value of 17.02 ± 1.75 µg /ml.h. When the same animals were administered J4899/010 at 32mg paraquat ion/kg, peak plasma paraquat levels occurred at 30 minutes and 1 hour post-dose with peak plasma levels between 0.69 µg/ml and 2.53 µg/ml and, again, paraquat was readily eliminated from the plasma with a mean 24 hour AUC value of 5.01 ± 1.58 µg /ml.h.

When the same animals were re-administered formulation from the original batch of J4669/49 at 32 mg paraquat ion/kg, a different plasma profile was observed to that seen after the first dose of this formulation. Peak plasma levels were observed at 30 minutes and1 hour post-dose with levels between 0.69 μg/ml and 1.84 μg/ml, and again, paraquat was readily eliminated from the plasma with a 24 hour AUC value of 4.26 ± 1.04 / μg /ml.h. Following administration of J4899/010 at 64 mg paraquat ion/kg, the peak plasma paraquat levels were observed at 30 minutes and 1 hour post-dose with values between 0.92 μg/ml and 1.97 μg/ml, and a 24 hour AUC value of 3.56 ± 0.83 μg/ml.h. The profile following 64 mg paraquat ion/kg J4899/010 was very similar to that observed following the lower dose of the same batch at 32 mg paraquat ion/kg. Following administration of J4669/49 at 64 mg paraquat ion/kg, the plasma profile was similar to that observed following re-administration of the same batch at 32 mg paraquat ion/kg. Peak plasma paraquat levels were observed at 30 minutes and 1 hour post-dose with values between 1.02 μg/ml and 3.41 μg/ml with a 24 hour AUC value of 5.94± 1.71 μg/ml.h.
Following the final dose of J4899/010 at 128 mg paraquat ion/kg , the plasma paraquat concentrations were increased with peak plasma level between 2.7 μg/ml and 7.3 μg/ml observed at 30 minutes and 1 hour post-dose and a fmal 24 hour AUC of 10.03 ± 1.10gg/ml.h.

Following administration of J4669/49 or J4899/010 at all the dose levels, the emetic was rapidly absorbed into the plasma and its elimination was gradual and essentially complete by 7 hours post-dose. Peak plasma levels were generally observed at 15 and 30 minutes post-dose. The initial rate of emetic absorption, determined over the initial 15 minutes, was found to increase with increasing doses of formulation. The exception to this was following the first administration of J4669/49 at 32 mg paraquat ion/kg , which had the highest rate of absorption.

Conclusion

Doses of A9409AM formulation, equivalent to 128 mg paraquat ion/kg, were well tolerated in the dog.

The authors further conclude:

"In view of the reproducible profiles observed with a repeat dose of J4669/49 and then further doses with this batch and a newly formulated batch, it is concluded that this first data set is anomalous. The cause of the increased paraquat and emetic absorption following the first dose of A9409AM (J4669/49) at 32 mg paraquat ion/kg is unknown."

REFERENCES

Brammer A, Heylings J and Swain C (2005) Paraquat 100 g/L SL formulation (A9409AL) : Toxicokinetic study in the dog. CTL Report no. CTL/XD7396/Regulatory Report.

Toxicokinetic Study In Dogs

XD7593/Regulatory/Report

SUMMARY

Study design

A group of three female beagle dogs received oral doses (by capsule) of the 100g/L paraquat formulation A9409AM on 3 occasions at approximately monthly intervals. This formulation is based on INTEON® technology and also contains an emetic (PP796), a purgative (magnesium sulphate), a stenching agent (pyridine bases) and a bittering agent (Bitrex). The dose levels used were 32, 64 and 128 mg paraquat ion/kg. Allowing for specific gravity and formulation strength, these doses were equivalent to dose levels of 352, 704 and 1408mg A9409AM formulation/kg.

Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis and defecation were recorded. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat and PP796 to be determined. Veterinary examinations were made prior to each dose and prior to scheduled termination (2 weeks after the final dose). General clinical observations, bodyweights and food consumption were measured at intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examined *post mortem*. Specified tissues were taken for subsequent histopathological examination.

Formulation

Paraquat 100 g/L SL formulation A9409AM (Y12693/238)
9.8% w/v paraquat cation, 0.2% w/v PP796

A9409AM	g/l
PQ ion (100%, emetic free)	100.00
DQ ion (100%)	-
PP796 (100%)	2.00
DEP ion	-
Manutex RM	9.00
Mag Sulphate	123.74
Antifoam DB-100	0.50
Antifoam MSA	-
Silcolapse 5020	
Toximul TA-1020/B	15.50
Agnique PG-8105 / AI2575	31.70
BioSoft SDBS 30LA	-
Ethomeen C/25	-
Aerosol OT-75E	-
Synprolam 35X15	-
Sulfacid blue 5J	2.50

F,D & C, blue no.1	-
Pyridine bases H	1.00
cis-3-hexenol	-
alpha-picoline	
Valeric Acid	-
Acetic acid	
Sodium Hydroxide	
Bitrex	0.05

Results

Mortality

No mortality

Clinical Observations

With the increased dose of the paraquat formulation (and hence, increased dose of emetic), there was a reduction in the time to first emesis from approximately on average18 minutes post dosing to approximately on average 11 minutes. There was little difference in the duration of emesis associated with increasing doses of the paraquat formulation. Additional clinical signs, including slightly hunched posture, decreased activity and/or fine tremors, were observed transiently in all 3 dogs following dosing at 64 or 128 mg/kg. Once the emetic effects had passed (generally from 2 hours post dose), the dogs were clinically normal

Body Weight and Food consumption

There were no treatment related effects on bodyweight and food consumption.

Bood Clinical Chemistry

There were no treatment related changes

Macroscopic and Microscopic Findings

There were no macroscopic and microscopic abnormalities

Toxicokinetics

As the dose of paraquat ion increased the plasma paraquat profile remained similar, indicating that elimination of paraquat from the plasma was similar at all doses. This is also reflected in the similar and low AUC values at 1, 4 and 24 hours across the three dose levels . Paraquat was essentially cleared from the plasma by approximately 7 hours post dosing.

When animals received a dose of 32 mg paraquat ion/kg mean peak plasma paraquat levels were observed at 1 hour post-dose . When the dose was increased to 64 or 128 mg paraquat ion/kg, mean peak plasma paraquat levels were observed at 30 minutes post-dose.

The initial rate of emetic absorption, determined over the initial 15 minutes, increased between the 32 and the 64 mg/kg doses but there was little difference between the 64 and 128 mg/kg doses. These values corresponded with slightly earlier times to first emesis at the 64 and 128 mg/kg doses, compared to 32 mg/kg. The emetic was rapidly absorbed into the plasma, its elimination was gradual and essentially complete by 7 hours post-dose.

Conclusion

Administration of the 100 g/L paraquat formulation, A9409AM, was tolerated well by dogs at all dose levels up to 1408 mg A9409AM formulation/kg (equivalent to 128 mg paraquat ion/kg). This formulation contained an emetic and caused prompt and effective emesis (from 8 minutes up to 1-2 hours after dosing), with no clinical signs of toxicity. There were no effects on food consumption or bodyweights and there were no abnormalities detected at veterinary examination.

Paraquat 200 g/l SL Formulation A3879FM
Toxokinetic Study in Dogs
XD7594/Regulatory/Report

SUMMARY

Study design

A group of three female beagle dogs received oral doses (by capsule) of the 200g/L paraquat formulation A3879FM on 3 occasions at approximately monthly intervals. This formulation is based on INTEON® technology and also contains an emetic (PP796), a purgative (magnesium sulphate), a stenching agent (pyridine bases) and the biomarker diethyl paraquat. The dose levels used were 32, 64 and 128 mg paraquat ion/kg. Allowing for specific gravity and formulation strength, these doses were equivalent to dose levels of 181, 362 and 723 mg A3879FM formulation/kg.

Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis and defecation were recorded. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat and PP796 to be determined. Veterinary examinations were made prior to each dose and prior to scheduled termination (2 weeks after the final dose). General clinical observations, bodyweights and food consumption were measured at intervals throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examined *post mortem*. Specified tissues were taken for subsequent histopathological examination.

Formulation

Paraquat 200 g/l SL formulation (A3879FM) (Y12693/239)

20% w/v paraquat ion, 0,16% w/v PP796

A3879FM	g/l
PQ ion (100%, emetic free)	200.00
DQ ion (100%)	-
PP796 (100%)	1.50
DEP ion	100ppm

Manutex RM	9.00
Mag Sulphate	123.74
Antifoam DB-100	0.50
Antifoam MSA	-
Silcolapse 5020	
Toximul TA-1020/B	0.42
Agnique PG-8105 / Al2575	-
BioSoft SDBS 30LA	0.47
Ethomeen C/25	-
Aerosol OT-75E	0.21
Synprolam 35X15	-
Sulfacid blue 5J	2.50
F,D & C, blue no.1	-
Pyridine bases H	0.10
cis-3-hexenol	-
alpha-picoline	
Valeric Acid	-
Acetic acid	
Sodium Hydroxide	
Bitrex	-

Results

Mortality

No mortality occured.

Clinical Observations

With the increased dose of the paraquat formulation (and hence, increased dose of emetic), there was a reduction in the time to first emesis from approximately 25/26 minutes post dosing to approximately 18 minutes. The duration of emesis increased with increasing doses of the paraquat formulation.
Additional clinical signs, including slightly hunched posture, restlessness and/or decreased activity, were observed transiently in all 3 dogs following dosing at 64 or 128 mg/kg. Slightly hunched posture and fine tremors were also observed in one dog following dosing at 32 mg/kg (dog 1). Once the emetic effects had passed (generally from 2 hours post dose), the dogs were clinically normal.

Body Weight and Food consumption

No effects observed

Bood Clinical Chemistry

No effects on blood clinical chemistry.

Macroscopic and Microscopic Findings

There were no macroscopic abnormalities. Two dogs had minor lung lesions which

were graded as minimal or slight: Dog1 had alveolar haemorrhage and dog 2 had multifocal subpleural fibrosis and multifocal macrophage infiltration in the lungs. Dog 3 had a unilateral inflammation of the renal pelvis but no lung lesions.

Toxicokinetics

Administration of the 200 g/L paraquat formulation, A3879FM, was tolerated well by dogs at all dose levels up to 128 mg paraquat ion/kg. The initial rate of paraquat absorption increased progressively between the increasing doses of A3879FM. As the dose of paraquat ion increased the plasma paraquat profile remained broadly similar, indicating that elimination of paraquat from the plasma was similar at all doses. Paraquat was essentially cleared from the plasma by approximately 7 hours post dosing at all doses. Peak plasma paraquat concentrations were obtained approximately 30 minutes post dose at the two higher dose levels and between 1 and 2 hours post dose at 32 mg/kg.

Peak plasma emetic concentrations occurred at 30 minutes post dose at all dose levels. There was little difference in the peak plasma emetic levels with mean values of 6.31, 8.63 and 6.90 ng/ml following administration of 32, 64 and 128 mg paraquat ion/kg, respectively. Elimination of emetic from the plasma was essentially complete by 7 hours.

Conclusion

Doses of up to 723 mg A3879FM formulation/kg, equivalent to 128 mg paraquat ion/kg, were well tolerated clinically in the dog.

PARAQUAT: 100G/L PARAQUAT FORMULATION (AV00169) - TOXICOKINETICS IN THE DOG

CTL/026467/RESEARCH/REPORT

(Contains Results from Report CTL/XD/1328)

SUMMARY

Study design

Data has been extracted from a series of research studies in the dog, conducted at CTL between 1987 and 1992 (CTL study XD1328) which compared the absorption of paraquat from different novel paraquat formulations with that of commercially available products. The data extracted from these studies provide plasma paraquat profiles following administration of a 100g paraquat ion/L formulation (AV00169) at nominal doses of 32 and 64mg paraquat ion/kg, administered orally via a gelatine capsule. Allowing for specific gravity and purity, these doses were equivalent to dose levels of 333 and 666mg formulation/kg.

Plasma samples were collected from male dogs during the 24 hour period after dosing and the concentration of paraquat in these samples was determined. The toxicokinetic parameters AUC_{0-1}, AUC_{0-4} and AUC_{0-24} were calculated. Clinical observations, including time to emesis,

were made frequently during the 24h post-dose period and continued daily for the duration of the study

Formulation

Paraquat 100g/L formulation (AV00169), (Y00061)

		AV00169
Paraquat ion:		100
Emetic:		1.5
Alerting Agent:	Alpha picoline	10
Thickener:	Rhodopol 23D (Xanthan gum)	15
Antifoam:	Silcolapse M5000	0.5
Adjuvant:	Sulframine 6848	40
	Synperonic N12	8
	Aerosil 200 (Silica)	10
Dye:	Coumassie Green G	0.5
Opacifier/colourant:	Titanium dioxide	3
Biocide:	Proxel AB	1

Results

Mortality

No mortality occured.

Clinical Observations

With increasing doses of the paraquat formulation (and hence, increasing dose of emetic), there was little change in the time to first emesis, which occured between 9 minutes and 28 minutes post-dosing. There was also little change in the duration and frequency of emesis with increasing doses of the formulation, although the data was less variable at the higher dose.

There were no additional clinical signs of significance following dosing and, once the emetic effects had passed, the dogs were clinically normal.

Body Weight and Food consumption

Following the 32mg paraquat ion/kg dose two animals (1 and 3) were observed to have lost 0.4 kg in the week following dosing and they had not regained the starting weight by the end of the second week post-dosing. Following the 64mg paraquat ion/kg dose all animals were observed to lose weight in the 7 day period after dosing, but by the end of the second week post-dosing only dog 3 had not regained the weight.

Dog 5 had diminished food consumption the first 4 days following dosing.

Toxicokinetics

After the 32 mg/kg dose time to reach peak paraquat plasma concentrations varied widely between the dogs: 15 minutes, 30 minutes and 2 hours. Peak concentations ranged from 3.26 to 4.15 μg/ml. At the 64 mg/ml dose level, peak concentations were reached at 30 minutes and 1 hour, with concentrations between 3.91 and 5.37 μg/ml. Following administration of both dose levels elimination of paraquat from the plasma was almost complete by 7 hours after dosing.

SUMMARY OF EFFECT OF PP796 ON PARAQUAT TOXICITY

RIC4219

SUMMARY

In the first section, this document contains a summary of the of the "Effect of Administration of an Emetic (PP796) on Paraquat Toxicity in Dog and Monkey" report (CTL/R/391). (It does not provide any of the missing dosing information from the report.)

The second section contains selection criteria for an emetic and the reasons (the outcome of the above mentioned study), why PP796 was chosen.

PP796
A Potent Emetic

RIC 4220

SUMMARY

The document contains summaries of studies with PP796 in different species: pigs, monkeys and marmosets, dogs, men.

The dog study quoted is CTL/R/391. See folder for this study for summary and table.

PP796: Effect of Stomach Status on Emetic Efficiacy in Dogs

CTL/T/2452

SUMMARY

Study design

The purpose of this study was to investigate the effect of underlying stomach status on the emetic efficancy of PP796.

Food was withheld for 24 hours and water for 1 hours prior to dosing and for a further 6 hours after dosing. PP796 was administered by oral gavage to dogs, at a known emetic dose level (3mg/kg) following pre-treatments which provided differing stomach conditions for dosage of PP796. The pre-treatments were: feeding food to give a full stomach (dog 1); administering COMPLAN solution to provide a liquid bolus (dog 2); starvation for 24 hours to provide an empty stomach (dog 3). The dogs were then observed continuously for 1 hour after dosing and then regularly for the remainder of the working day.

Formulation

PP796 (Y00706/016/001)

Results

Clinical Observations

Dog 1: Dosed PP796 After LABORATORY DIET A

Four minutes after dosing licking of lips, indicating nausea, was apparent and 2 minutes later a small quantity of food was vomited. Another quantity of food was vomited 12 minutes after dosing. Viscid faeces were produced and vomiting became fairly regular between 16 and 45 minutes after dosing. The stomach contents were completely emptied 20 minutes after dosing, thereafter vomiting produced a white froth. The dog appeared subdued during this time but had improved by 1 hour after dosing and no further vomiting was seen.

Dog 2: Dosed PP796 After COMPLAN

Five minutes after dosing the dog vomited most of the COMPLAN feed and 3 minutes later vomited the residue after marked retching. Between 8-11 minutes after dosing, excessive salivation and marked retching, followed by yellow viscid vomit, occurred. The dog was subdued and the bowels had been emptied. Thirteen minutes after dosing the dog appeared brighter and marked retching (producingvomit), alternated with attempts to defaecate , continued every few minutes until 35 minutes after dosing. After this time no further effects were seen.

Dog : 3 PP796 on an Empty Stomach

Five minutes after dosing, continuous retching produced a frothy white vomit and the dog was very subdued and unsteady. Between 9 and 25 minutes after dosing, yellow frothy vomit was produced after episodes of prolonged and marked retching. The bowels were emptied. Between 26 and 36 minutes after dosing, the dog appeared to be very subdued and restless, trying to defaecate and retching markedly. White froth was vomited 42 minutes after dosing and by 50 minutes after dosing the dog had recovered. No further effects were seen.

Conclusion

PP796 caused emesis in dogs at a dose level of 3mg/kg, regardless of stomach state. In each case, vomiting occurred within 5-6 minutes of dosing but there were differences in severity and duration of effect between the different stomach states. It would appear that the presence of some food in the stomach enables the dog to vomit productively soon after

dosing and so avoids the episodes of prolonged and marked retching seen after administration on an empty stomach.

PP796: Emetic Dose Response Study in Dogs

CTL/T/2459

SUMMARY

Study design

The purpose of this study was two-fold: (1) to investigate the emetic response of dogs to different dose levels of PP796, in terms of onset, duration and productivity of vomiting. (2) to assess the acute toxicity following a single oral dose of PP796.
Seven male and 7 female beagle dogs were allocated to treatment groups which received a single dose of PP796 at one of the following dose levels: 0, 0.1, 0.5, 1.0, 3.0, 10.0 or 20.0mg/kg. Clinical observations, food consumption and faecal consistency were recorded daily during a 7 day observation period and the dogs were weighed and examined periodically during this time.

Formulation

PP796 (2-amino-6-methy1-4-propy1-4H-1,2,4-triazolo(2,3-A)pyrimidin-5-one), CTL reference number Y00706/016/001,

Results

Mortality

No mortality

Clinical Observations

No effects were seen at dose levels of 0 or 0.1mg PP796/kg. Dose levels of 0.5, 1.0, 3.0, 10.0 and 20.0mg PP796/kg caused emesis in a dose-related manner in terms of onset, duration and severity.
Dose levels of 0.5 and 1.0mg PP796/kg produced emesis within 12-30 mins of dosing. The effects seen at these dose levels were of a mild to moderate nature and the dogs had recovered within lhr-l.5hrs after dosing.
Dose levels of 3.0, 10.0 and 20.0mg PP796/kg caused a prompt emetic response; 7-8 mins after dosing at 3.0mg PP796/kg and 5 mins after dosing at 10 or 20mg PP796/kg. The severity and duration of effects was much greater at these dose levels; severe vomiting characterised by prolonged periods of marked retching, diarrhoea, subdued and restless behaviour were seen in most of these dogs.
Several treated dogs had diarrhoea on day 1 after dosing. Apart from the immediate effects of PP796 on the gastrointestinal tract there were no other adverse effects of treatment with PP796

Body Weight and Food consumption

There were no treatment related effects on bodyweight and food consumption.

Conclusion

It is considered that 0.5mg PP796/kg represents the minimal effective dose level and 20mg PP796/kg the maximum tolerated dose level (based on the severity of vomiting).

The Intravenous Administration of ICI 63,197 to Beagle Dogs

R015/77

SUMMARY

Rationale

Pharmacological studies with ICI 63,197 (**PP796)** led to the tentative observations that emesis in dogs might be related to the rate of absorption of ICI 63,197 following oral administration. A serum level of 0.4 μg/ml was selected as a level which appeared to be associated with oral doses at which vomiting in dogs could be expected to occur. The studies described herein were designed in order to test the hypothesis relating to rate of absorption, by setting up intravenous infusions which would lead to levels of 0.4 μg/m1 achieved at different rates.

NOTE: no clinical observations (incidence of emesis) are reported for the main study. This study was performed with a male Beagle dog. Appendix 2 reports "Clinical Observations in the Perfusion Study Dogs" This study was performed with 3 female dogs. It is not clear how the two studies are related.

Study design

Female beagle dogs were subjected to intravenous doses of ICI 63,197 at different dose rates and different administration volumes and time spans:

- 30 minutes infusion: **Dog 1** (12 kg): 4.25 mg ICI 63197 in 15 ml
- 60 minutes infusion: **Dog 2** (12 kg): 4.57 mg ICI 63197 in 22.5 ml
- 90 minutes infusion: **Dog 3** (13 kg): 5.76 mg ICI 63197 in 45 ml

Results

Result of the appendix 2 study:

Dog 1: 10 min. post dose excessive salivation; 15 min post-dose, dog became agitated and violent defaecation occurred. No emesis.

Dog 2: 30 min. post-dose, agitation; vomiting after 40 to 60 minutes, salivation increasd during post-dose period.

Dog 3: 60 min. post dose, agitation. No emesis.

The authors conclude that "the severity of the clinical observations seem to coincide with the drug blood levels" and refer to table 3 of the report. According to this table, (*NOTE: the calculations are based on a 17.5 kg dog!)* the ICI 63197 peak plasma level is reached after 30 minutes (0.52 μg/m1), after, 60 minutes (0.34 μg/m1) and after 90 minutes (0.42 μg/m1) for dogs 1, 2 and 3 , respectively.

Charcoal Haemoperfusion in the Treatment of Paraquat Poisonings

Widdop, B. *et al.* (1977). Proc Europ Soc Tox 18 156-159

SUMMARY

Study design

Control group: Six adult dogs received 10 mg/kg paraquat by gavage and urine and urine and plasma samples were obtained for periods of up to 7 days after dosing. Frequent biochemical and haematological investigations were performed.

Haemoperfusion Trials: Ten dogs received paraquat (10 mg/kg) orally followed by a 10-12 hr period of haemoperfusion. This treatment started at varying intervals after dosage. Thus, 5 animals were treated 3 hr after dosing, 2 at 6 hr, 3 at 12 hr and 2 at 18 hr. The animals were sedated. Blood and urine samples were collected for up to 7 days for biochemical and haematological analyses.

Results

Mortality

All the control animals died between 9 and 12 days after dosing.

In the haemoperfusion treated group, one dog of the 12 hour group (1/3) and both animals of the of the 18 hour group (2/2) died.

Clinical Observations

All animals in the control group showed signs of renal impairment and respiratory distress.

Toxicokinetics

In the control group, peak plasma paraquat levels ranged from 4.7 to 24.0 μg/ml (mean 9.6 μg/m1) and occurred between 0.5 and 2.0 hr after dosing. Thereafter, the plasma paraquat levels declined very rapidly at first, followed by a very slow elimination phase. The amount of paraquat recovered in the urine over 7 days ranged from 13 to 47% of the administered dose.

In the haemoperfusion-treated group, peak plasma paraquat concentrations were found to be of the same order as those seen in control animals, indicating that similar amounts of paraquat had been absorbed. The most significant finding was that the majority of animals who were perfused within 12 hr of dosing survived. The amount of paraquat removed was dependent from the time to the start of the perfusion. Significant amounts of paraquat were removed in animals perfused within 3 hr of dosing, but not in those perfused at later intervals. A reduction in paraquat excretion was observed in animals perfused within 3 hr of dosing (Table 1).

There was a clear difference between the mean plasma paraquat concentrations in control and perfusion-treated groups (survivors) 2 to 6 days after dosing, the perfusion-treated animals exhibiting much lower plasma concentrations. (Day 2, perfusion-treated group:~150 μg/l mean plasma paraquat.level ; control group: ~230 μg/l mean plasma PQ level. Day 6, perfusion-treated group:~30 μg/l mean plasma paraquat.level ; control group: ~90 μg/l mean plasma PQ level.)

Table 1: Haemoperfusion -treated Dogs

Time dosing to start perfusion (hrs)	Died (D) Survived (S)	Amt PQ removed by perfusion (% dose)	Urinary Excretion of PQ (% dose)	Combined PQ Elimination (% dose)
3	S	19	14	33
3	S	13	6	19
3	S	13	9	22
3*	S	16	7	23
6	S	5	26	62*(Sic!)*
6	S	2	60	25*(Sic!)*
12	S	1	24	25
12	S	1	22	23
12	D	6	45	51
18	D	1	81	82
	D	7	44	51

** NOTE: no word what happend to the 5. dog (see above). Combined PQ elimination for 6 hrs can't be right.*

NOTE:
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.