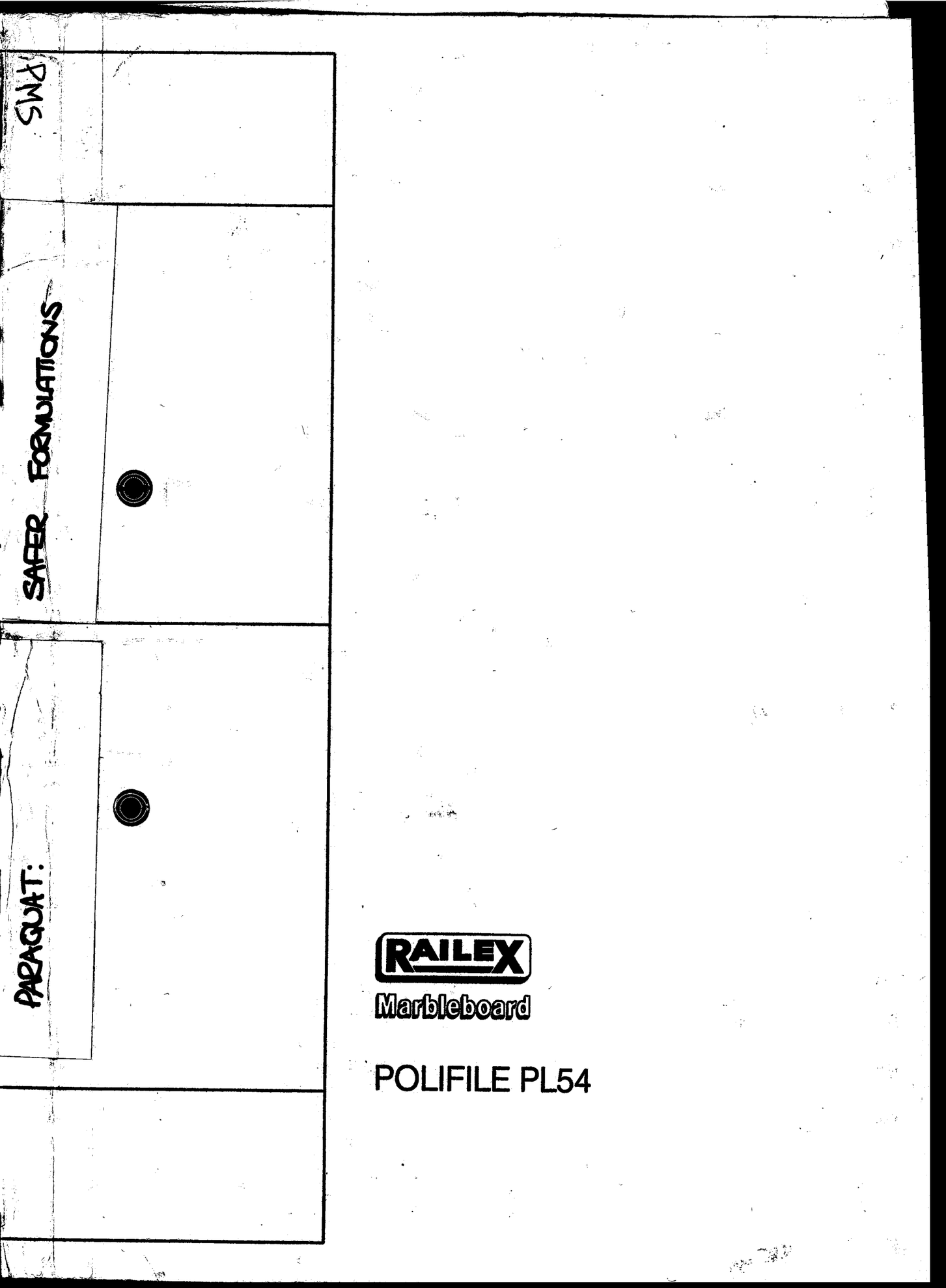

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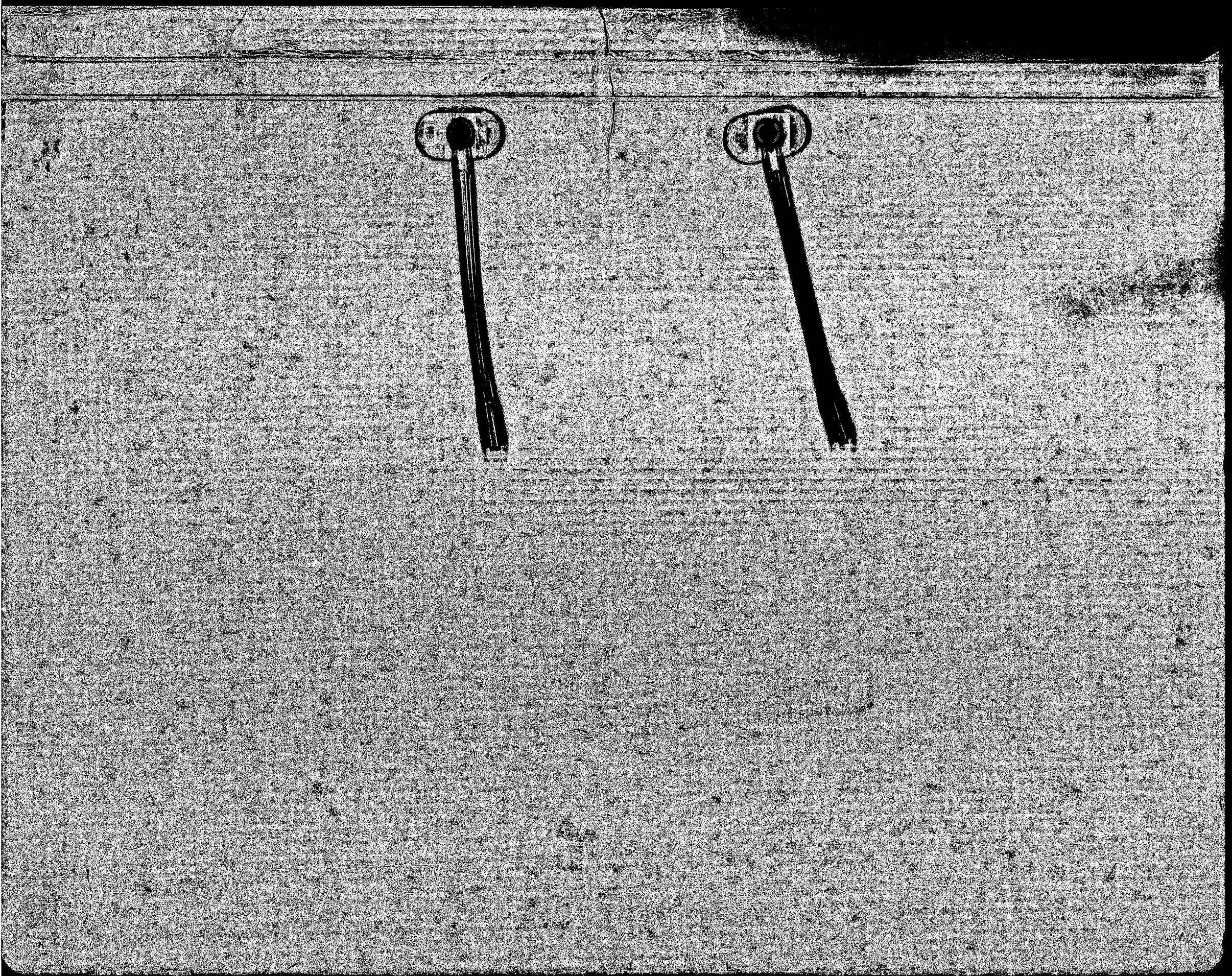


From
Dr J R Heylings
Biochemical Toxicology

ICI Central Toxicology Laboratory

Alderley Park
Macclesfield Cheshire SK10 4TJ

Tel: 0625 582711
Telex: 669095/669388
Fax: 0625 582897

To
Dr D C Taylor
Formulation Research Section
ICI Pharmaceuticals

Copies to
Dr E J Pywell, Pharms
Mr J R Evans, Pharms
Dr F J T Fildes, Pharms
Dr A Garner, Pharms
Dr R C Scott, M & P
Dr S E Jaggers, Exec
Dr R S Morrod, Exec

Your ref	Our ref	Direct line	Tel ext	Date
	GI001/LCM	Redacted - EU PII	Redacted - EU PII	1 July 1991

FORMULATION ADDITIVES AND GASTROINTESTINAL TOXICITY

Over the past few years, as you know, my group at CTL have been working on new formulations of Paraquat which have reduced oral toxicity in experimental animals (primarily the dog) with a view to developing a new paraquat product which has lower oral toxicity in man. The scientific approach we have been following centres around adding various chemicals which have known pharmacological effects on the gastrointestinal tract, to paraquat liquid concentrates. This research programme ran in parallel with an alternative project which involved a slow release formulation of paraquat prepared as a Multiple Emulsion. Although paraquat emulsions were shown to be considerably less toxic than aqueous formulations the Emulsion programme was stopped in 1990 principally on the grounds of formulation cost. Over the last two years we have now developed the idea of adding chemicals which have direct effects on gastic pH and motility to paraquat concentrates. I would like to share some of this information with you in the hope that this approach has value in the formulation of some pharmaceutical products.

Magnesium Trisilicate

We have tested many different antacids as additives to the commercial formulation of paraquat 'Gramoxone' in various animal models. These animal models included a Calcium/Paraquat-induced gastric mucosal lesion assay where both histological damage, together with the absorption of paraquat and permeability markers were measured in the rat in vivo. Magnesium based antacids were most effective in this test and in particular the longer acting antacids such as Magnesium trisilicate could prevent GI tract damage in the presence of the corrosive agent paraquat. Consequently, the increase in passive permeability of paraquat following damage was prevented.

As we are all aware the removal of gastric acid by oral antacids has proven therapeutic value in reducing mucosal damage caused by a variety of topical irritants. Magnesium trisilicate has several other unique properties which also indirectly reduce paraquat irritancy and absorption. On contact with gastric HCl, it is converted into silicon dioxide gel which adheres to the mucosa. This provides a physical barrier to the diffusion of the bipyridyl

continued....

cation. Also, trisilicate and SiO_2 adsorb paraquat via electrostatic interaction. The gelling effect increases the viscosity of the gastric contents and thereby slows gastric emptying. This is particularly effective when large volumes have been administered orally. We have shown this by tracer methods to measure gastric emptying using ^{14}C-paraquat and ^{3}H-mannitol. Slowing of gastric emptying has obvious beneficial effects with paraquat since a greater proportion of an ingested dose can be removed by gastric lavage or emesis. The optimum concentration of Magnesium trisilicate in both rat and dog is 100g/l in a 200g/l paraquat formulation.

Phosphodiesterase inhibitor PP796/M63197

Our new paraquat formulations contain 1.5g/l PP796, which in addition to its cardiovascular properties is also a potent emetic agent. At this concentration, a minimal lethal dose of 'Gramoxone' delivers an effective emetic dose to dogs, pigs and monkeys. Being a phosphodiesterase inhibitor, the mechanism of action of PP796 is via the CNS and not via irritation of the GI tract nerve endings as is brought about by other emetic drugs such as ipecac.

Magnesium sulphate

The third additive to our new paraquat formulation is $MgSO_4$. This osmotic purgative is present at 100g/l and further reduces the amount of paraquat absorbed into the blood. It does not interfere with the action of the trisilicate or emetic at this concentration. However, it brings about diahorrea after about 1-2 hours in rodents and dogs. Paraquat is absorbed in the small intestine, primarily by the jejunum and by a combination of active and passive mechanisms (Heylings, J.R., Toxicol Appl Pharmacol 107, 482-493, 1991). Flushing the small bowel with saline cathartics, such as $MgSO_4$ reduces the absorption of paraquat by virtue of its rapid removal from its site of absorption.

Xanthan Gum

A further additive to our paraquat concentrate alters the dispersion characteristics of the formulation following gavage dosing. Xanthan at 3g/l increases the viscosity of the formulation and also suspends the fine particles of trisilicate to give a uniform mixture. The thickening also helps to slow gastric emptying and therefore acts synergistically with the Magnesium trisilicate.

'Magnoxone' (YF7981)

The full paraquat formulation containing all the above pharmacological additives at their optimized concentrations ('Magnoxone') reduces the oral toxicity of 'Gramoxone' by some 15 times in the dog. We have demonstrated that the shift in toxicity is directly proportional to the shift in plasma paraquat AUC (0-24hrs) which confirms that the oral absorption of paraquat has been markedly reduced. The formulation is herbicidally equivalent to Gramoxone once diluted for use and can be formulated relatively simply on a small or large scale. A worldwide patent application has recently been filed for this new formulation of paraquat.

Continued....

Possible Application of Paraquat Formulation Technology to Pharmaceutical Products.

Following a discussion with our Regulatory Toxicology Manager, Stuart Jaggers, I would like to explore the idea of combining some of the additives described above as suspensions with certain types of drug. The emetic/purgative formulation may have uses in Veterinary practice for the treatment of animal poisoning. The Magnesium trisilicate, however, may reduce irritancy of the upper GI tract associated with specific drugs or may alter the absorption kinetics of the drug by virtue of its indirect effects on luminal pH and motility.

Magnesium trisilicate and GI irritancy

In the past various approaches have been used to reduce GI irritancy caused by non-steroidal anti-inflammatory drugs. For instance, aspirin can be enterically coated, buffered with sodium bicarbonate or simply avoided when the patient has a history of peptic ulceration. The use of non systemic antacids in formulations of non-steroidal anti-inflammatory drugs is surprisingly little. I have found one or two preparations in the Pharmacopoeia where aspirin has been tableted with aluminimum hydroxide and phenylbutazone with magnesium hydroxide. However, I am unaware of any liquid suspensions of magnesium trisilicate which contain NSAI drugs. MgTS is a very cheap commodity and has very low toxicity in man. Furthermore, a therapeutic antacid dose can be delivered in a 5ml volume of suspension. This would, by virtue of its dispersion, be a more effective formulation compared to a tablet. As well as reducing irritancy, such a formulation may alter the absorption profile of the drug in a favourable manner via effects on pH, ionization and lipophilicity, or indirectly via effects on GI motility.

I note that MgTS is still widely used in obstetric practice to prevent the acid aspiration syndrome. Therefore, in addition to situations where non-systemic antacids are favoured (eg. pregnancy), the cost factor of such an approach compares favourably with other expensive systemic antisecretory agents such as H_2 antagonists and omeprazole.

Magnesium ion and GI absorption

The qualification of MgTS as the favoured antacid in our research with Paraquat is not entirely due to its chemical properties as an antacid gelling agent. Only trisilicate and silicates of magnesium effectively reduced mucosal damage and permeability. We are currently investigating this at CTL in order to understand the mechanism by which luminal divalent cations affect GI absorption of divalent bipyridyl herbicides. In the case of paraquat, Ca^{2+} stimulates paraquat uptake both *in vitro* and *in vivo* in rat and dog. This effect is blocked by the Ca-chelator EGTA or by high levels of luminal Mg^{2+}. Although calcium channel blockers such as verapamil are anti-ulcer, this is not a Ca^{2+} channel effect. Luminal Mg^{2+} (1-100mg/ml) reduces GI damage caused by paraquat + calcium in a dose related manner.

Continued....

Finally I would be interested to hear from you or your colleagues if any of the above approaches could have implications in drug formulation or whether the idea of a "MAGNESIUM TRISILICATE ASPIRIN SUSPENSION" has been tried before.

Regards

DR J R HEYLINGS
Biochemical Toxicology

From
Dr J R Heylings
Biochemical Toxicology

ICI Central Toxicology Laboratory

Alderley Park
Macclesfield Cheshire SK10 4TJ

Tel: 0625 582711
Telex: 669095/669388
Fax: 0625 582897

To
G A Willis - PSS, Fernhurst
A J Starling - Dev "
S R Shields - Products "

Copies to
R C Scott
T Green
G J Oliver
J D Pidgeon, Dev, Fernhurst

Your ref	Our ref	Direct line	Tel ext	Date
	PQ002/JJB	Redacted - EU PII	Redacted - EU PII	23 July 1991

HUNGARIAN FORMULATIONS OF PARAQUAT

We have examined the acute toxicity of the thickened 'Hungarian' paraquat formulation (YF7970) with various concentrations of emetic PP796 in dogs. The formulations all contained 200g/l paraquat ion and were dosed as neat concentrates to three adult male dogs over the paraquat dose range 8-64mg/kg.

Formulation	Paraquat g/l	PP796 g/l	Code
YF7970*	200	0.5	H1
YF7970	200	3.0	H2
YF7970	200	1.5	H3

*YF7970 (H1) contains 0.5g/l PP796. H2 and H3 were prepared by adding extra PP796 up to a final concentration of 3.0 or 1.5 g/l respectively.

Low emetic Formulation (H1)

Formulation H1 containing a standard concentration of PP796 (0.5g/l) was originally tested in April 1991 at dose levels of 8 and 16mg/kg paraquat. This formulation did not produce early vomiting in dogs and consequently confirmed our previous findings with Gramoxone in dogs that 0.5g/l is an ineffective concentration of PP796 in this species. As shown in Figure 1, the time to emesis was 44±4 and 33±12 min for 8 and 16mg/kg paraquat. Consequently the paraquat AUC values of 15±4 and 22±4μg/ml suggest that formulation H1 only has an intrinsic 2x safety factor compared to Gramoxone.

continued.....

High Emetic Formulation (H2)

Formulation H2 contained six times the standard concentration of PP796, ie 3g/l. This is equivalent to the French formulation AV8700169 tested last October which is a 100g/l paraquat product containing 1.5g/l PP796. Thus, animals received the same dose of paraquat and emetic in mg/kg when they received H2 or AV8700169. Dogs were dosed in July 1991 at 32 and 64mg/kg paraquat. As shown in Figure 2, relative low paraquat AUC values were obtained with H2. At 32mg/kg the extra emetic gave an AUC of 9±2μg/ml.hr and all animals remain healthy. However, the time to emesis was 21±5 min which is still what we would regard as delayed. At 64mg/kg, the paraquat AUC rose to 17±10μg/ml.h with time to emesis still longer than predicted at 22±5 min. The result at 64mg/kg was variable with AUC values of 15, 29 and 37μg/ml.h for each dog. The dog with the AUC value of 37μg/ml.h was terminated on Day 8 due to inappetence and suspected pulmonary dysfunction. The other five dogs dosed with H2 remain healthy. Thus, formulation H2 is likely to have an LD50 around 60-70mg/kg paraquat which suggests about a 5x safety factor compared to Gramoxone.

Medium Emetic Formulation (H3)

Formulation H3 contained only three times the standard concentration of PP796, ie 1.5g/l. This formulation was tested in July 1991 at a dose level of 32mg/kg paraquat. This dose of paraquat was chosen since it was predicted that this level of emetic would by virtue of early emesis would allow higher doses of paraquat to be used than in H1. As shown in Figure 2, formulation H3 gave a higher paraquat AUC than predicted, 27±6μg/ml.h and a later time to emesis than predicted at 24±5min. One out of the three animals was terminated on Day 11 with suspected pulmonary fibrosis. This dog had an AUC of 35.3μg/ml.h. The LD50 for formulation H3 is likely to be between 30-40mg/kg paraquat or three times higher than Gramoxone. Thus, formulation H3 probably has a 3x safety factor compared to Gramoxone.

continued.....

Summary

Formulation	Paraquat Dose mg/kg	Paraquat AUC µg/ml.h	PP796 Conc g/l	PP796 Time to emesis min	Estimated Safety Factor
YF7970 H1 (low emetic)	8	15	0.5	44	2x
	16	22	0.5	33	
YF7970 H2 (high emetic)	32	9	3.0	21	5x
	64	17	3.0	22	
YF7970 H3 (medium emetic)	32	27	1.5	24	3x

Discussion

There are several interesting features which have emerged with these Hungarian thickened formulations of paraquat. First of all there is very little intrinsic safening at 0.5g/l PP796. I would suggest that this is no more than 2x over Gramoxone. At very high emetic levels (pro rata with the French system AVO 8700169) the safety factor can be shifted only to 5x. Surprisingly, the time to emesis was only shortened to 20min, despite the very high dose of PP796. Such a delay in emesis has not been observed before in high emetic formulations which include: Multiple Emulsions, Gramoxone, the French system or Magnoxone. Thus, the thickened Hungarian formulation appears to be unique in its ability to delay the absorption of PP796.

continued.....

A direct comparison can be made between YF7981A (Magnoxone) and YF7970 (H3). Both contain 1.5g/l PP796 and both have been dosed at 32mg/kg paraquat. As shown in Figure 3, Magnoxone gives a reproducible AUC of 9±4μg/ml.h compared to the more variable AUC of 27±6μg/ml.h for H3. Further, the mean time to emesis occurs at 15min with Magnoxone which is 9 min earlier than H3. The predicted safety factors for the two formulations are thus very different with 15x for Magnoxone (YF7981A) and 3x for H3 (YF7970).

As shown in Figure 4 a similar difference in AUC occurs with 0.5g/l PP796 when Magnoxone is compared with H1.

A further comparison can be made between a high emetic Gramoxone formulation and the high emetic Hungarian H2 formulation. We found in 1990 that increasing the concentration of emetic to 2.4g/l in Gramoxone gives a 5x safety factor. This is as effective as a 3.0g/l version of the Hungarian formulation (H2).

In conclusion, a 200g/l paraquat formulation based on the Hungarian thickened system (YF7970) is increasingly less toxic to dogs by a factor of 2x, 3x and 5x for 0.5, 1.5 and 3.0g/l PP796 respectively. However, simple addition of these concentrations of emetic to Gramoxone would probably provide an equivalent shift in oral toxicity. This prediction is based on the extensive database we have for plasma paraquat AUC, time to emesis and lethality in dogs.

Dr J R Heylings
Biochemical Toxicology
CTL

Figure 1.

FORMULATION STUDIES XD1328 E50

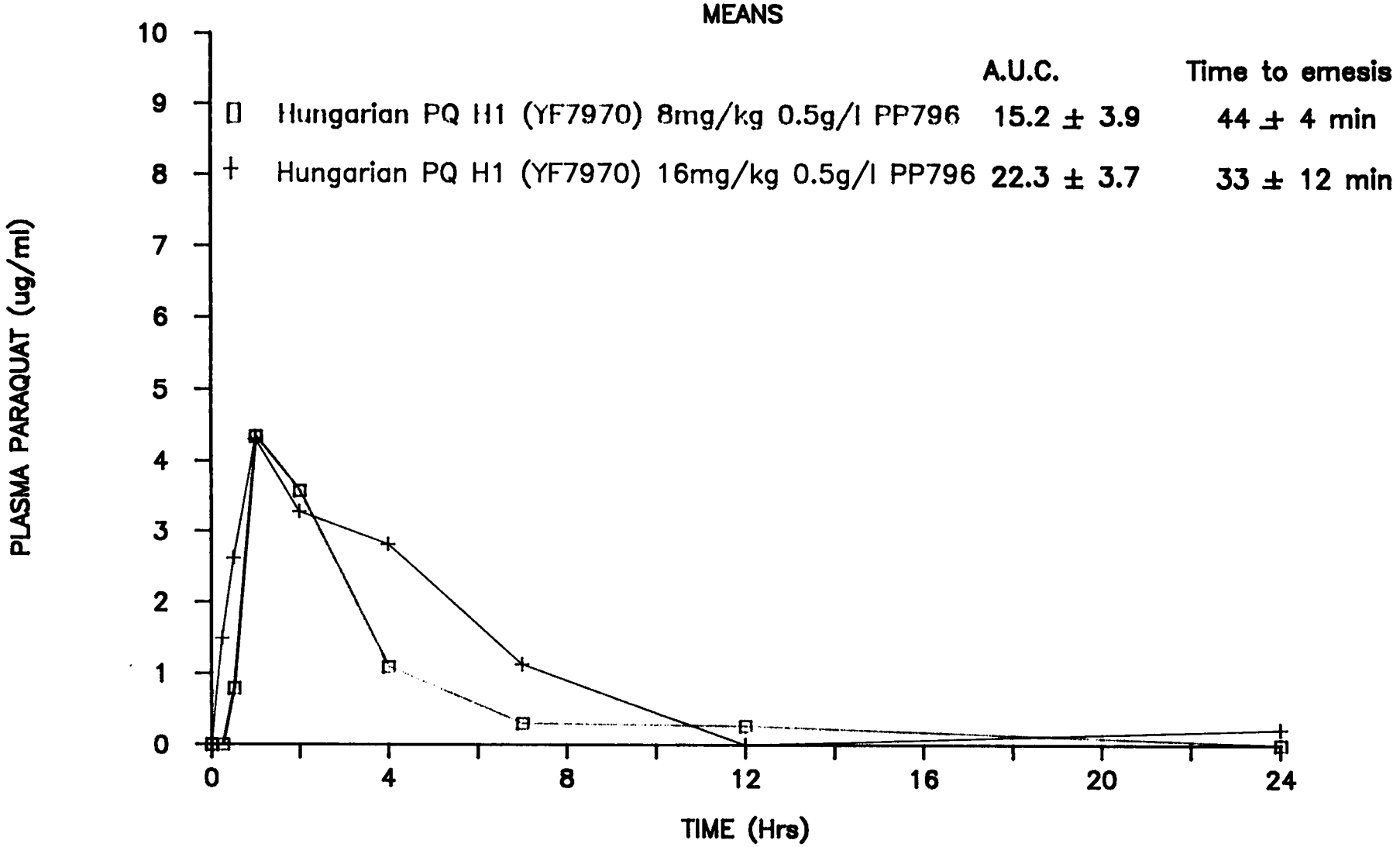


Figure 2.

FORMULATION STUDIES XD1328 E53

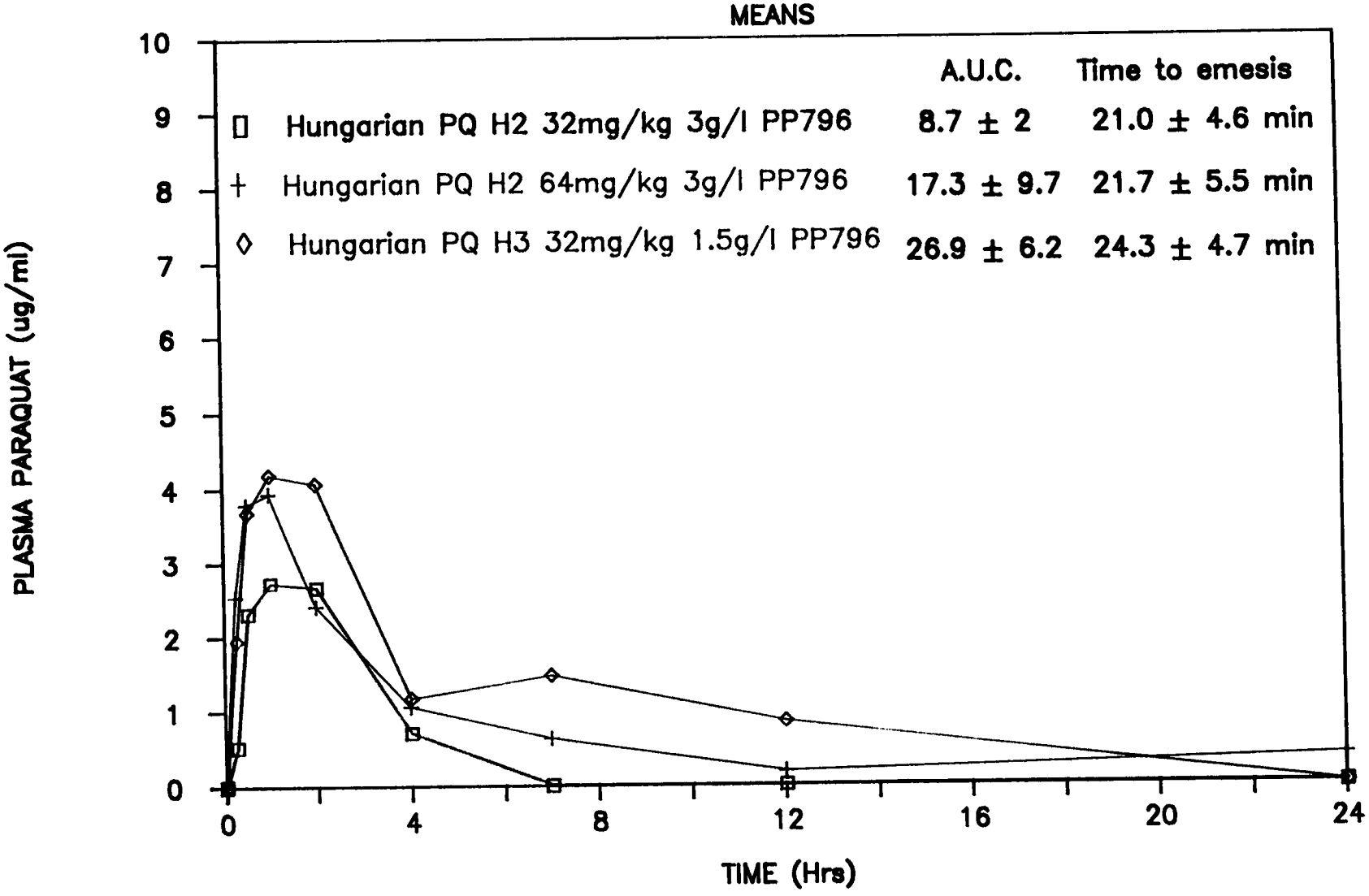


Figure 3.

FORMULATION STUDIES XD1328

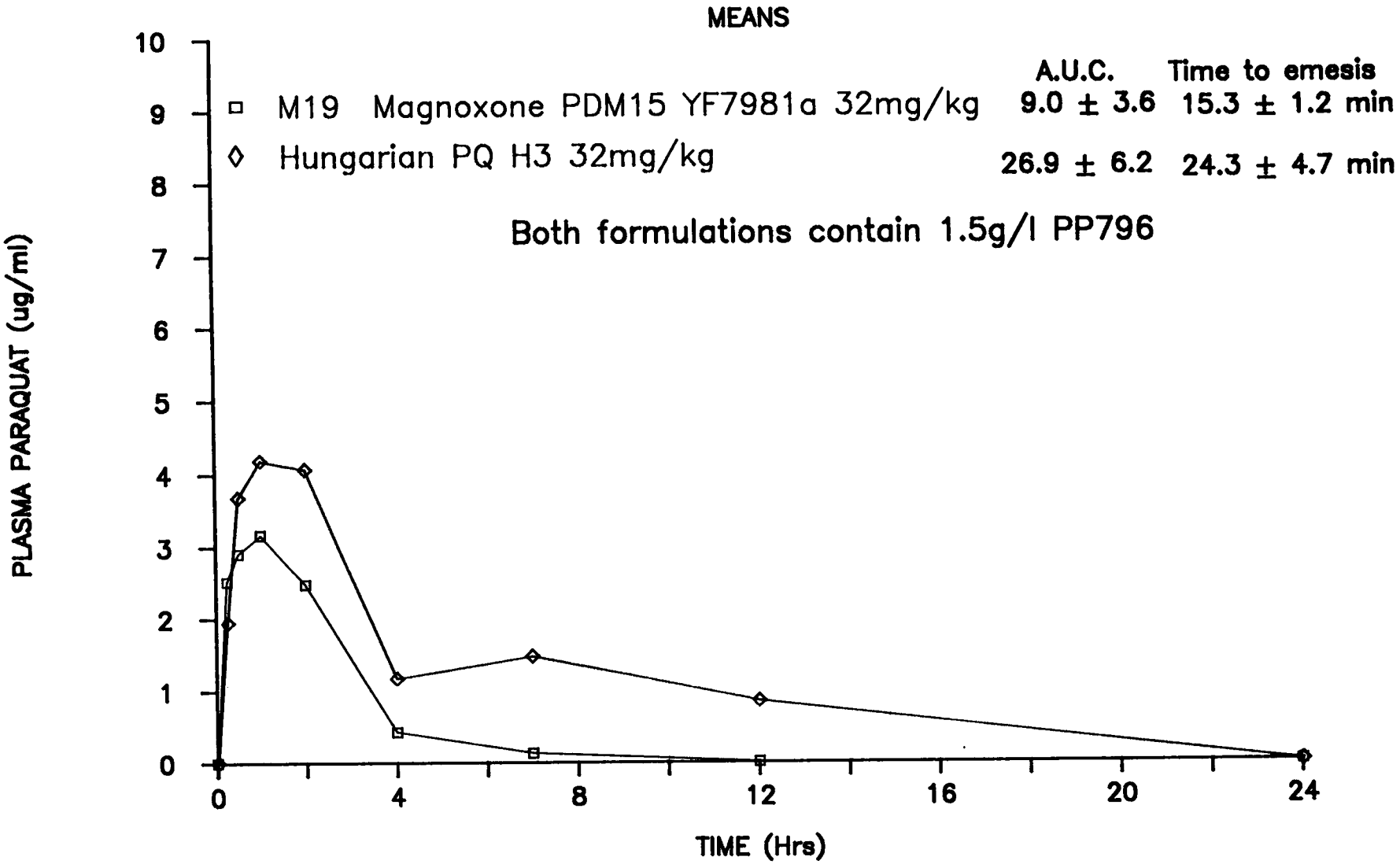
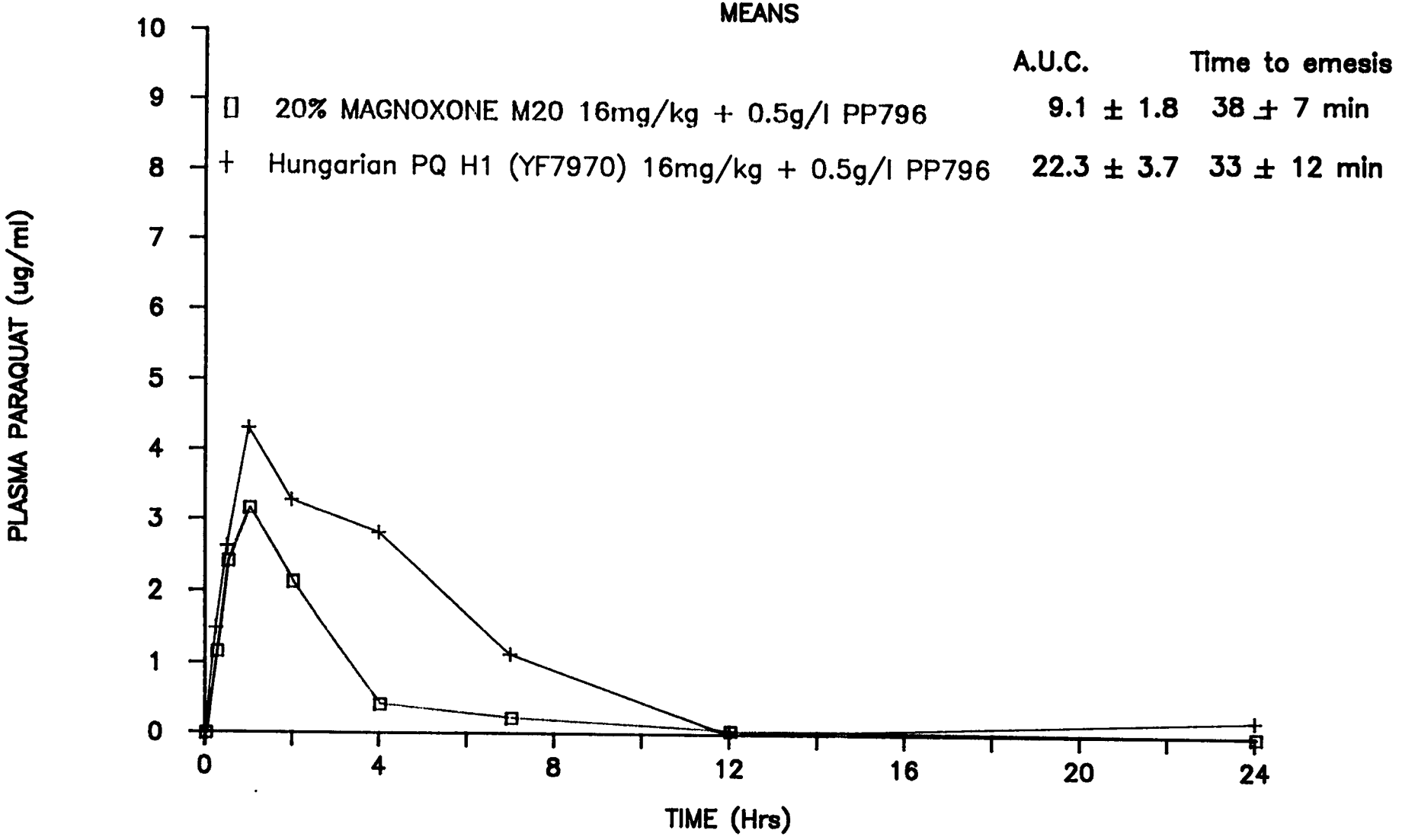


Figure 4.

FORMULATION STUDIES XD1328



From
Dr J R Heylings
Biochemical Toxicology

ICI Central Toxicology Laboratory

Alderley Park
Macclesfield Cheshire SK10 4TJ

Tel: 0625 582711
Telex: 669095/669388
Fax: 0625 582897

To
Dr L L Smith
Mr G A Willis
Dr N N Sabapathy

Copies to
Mr J D Pidgeon
Miss A J Starling
Dr S E Jaggers

Your ref	Our ref	Direct line	Tel ext	Date
	JRH076/LCM	Redacted - EU PII	Redacted - EU PII	26 October 90

FRENCH FORMULATION OF PARAQUAT

As a consequence of our recent findings with paraquat formulations containing a higher level of emetic PP796, we have examined the effect of the French formulation (AV 8700169) in the dog. This formulation contains 100g/l paraquat and 1.5g/l PP796 and was supplied by ICI Sopra, France.

This formulation was registered in France following CTL studies in 1986/7. These studies demonstrated that the acute oral LD50 in rats was similar to Gramoxone. However, as far as I am aware no dog studies were carried out on this formulation. Since we have identified that 1.5g/l PP796 effectively reduces the toxicity of Gramoxone in dogs by vitrue of causing emesis within 30 minutes, we have now examined the safening potential of the French formulation in six dogs.

The plasma paraquat AUC values are tabulated below and a full plasma profile is shown on the attached figure. The time to first emesis for the French formulation was 15 ± 6 min at 32mg/kg and 14 ± 2 min at 64mg/kg. The data fits very well with the predicted paraquat AUC versus time to emesis for the dose of PP796 given. This is based on a curve fit of more than 100 Gramoxone/ Magnoxone experiments with various levels of emetic.

FORMULATION	PARAQUAT g/l	PARAQUAT mg/kg	PP796 g/l	PP796 mg/kg	PQ AUC µg/ml.h	ESTIMATED SAFETY FACTOR
GRAMOXONE L	100	8	0.25	0.02	17	1X
	100	16	0.25	0.04	70	
GRAMOXONE L HIGH EMETIC	100	16	1.2	0.19	19	
	100	32	1.2	0.38	17	5X
	100	48	1.2	0.57	38	
FRENCH FORMULATION (AV 8700169)	100	32	1.5	0.48	9	
	100	64	1.5	0.96	13	(10X)

Cont...

The plasma AUC data clearly suggests that the French paraquat formulation offers a substantial margin of safety in dogs compared to an equivalent 100g/l formulation of Gramoxone. The formulation would probably achieve a 10 fold safety factor based on the AUC value obtained at 64mg/kg. I would suggest that a 200g/l version of this French paraquat formulation containing the same concentration of PP796 (1.5g/l) would be equally as safe in dogs and provide a safer alternative option to Gramoxone.

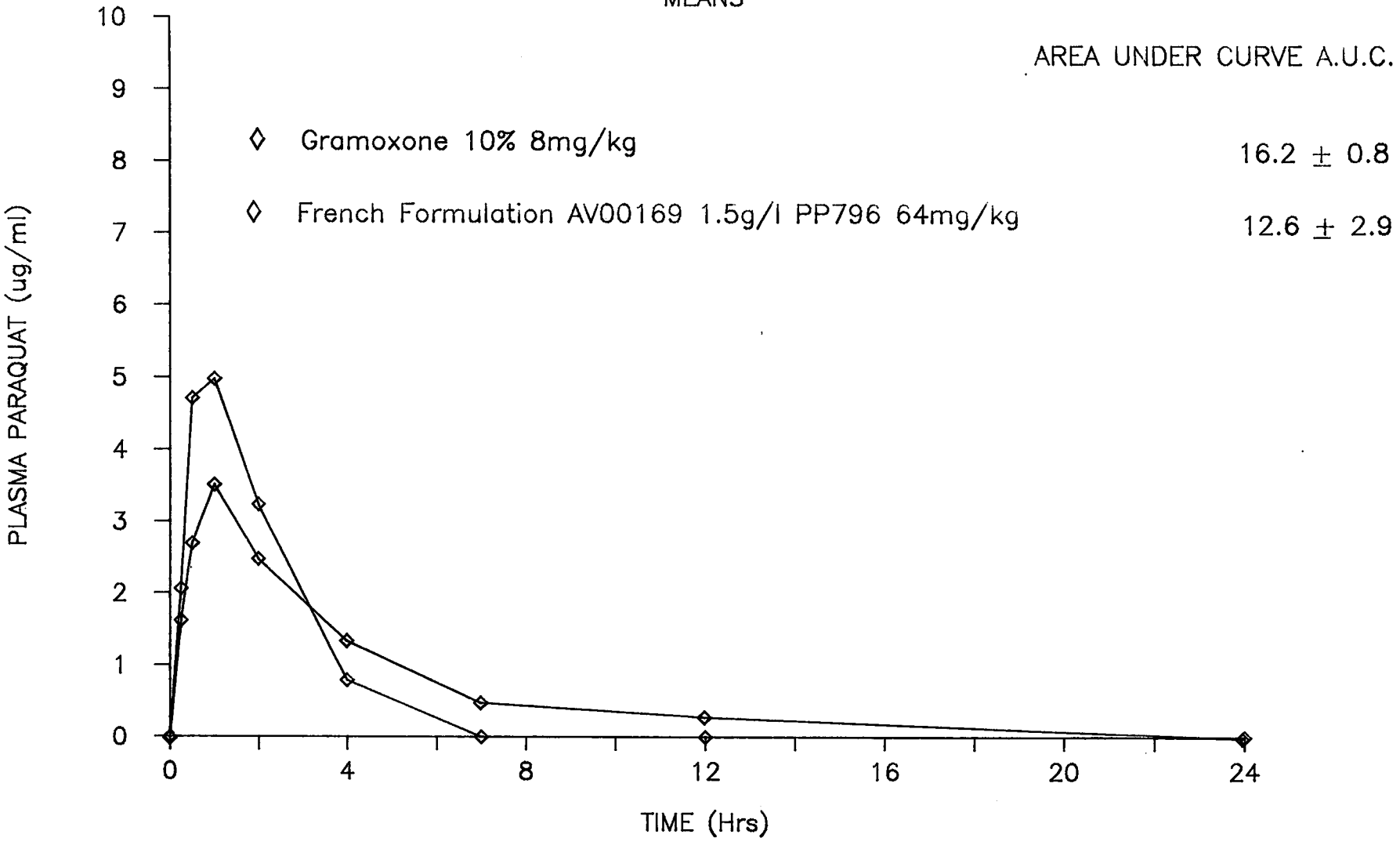
On reviewing the available data from the literature and the French Poison Service, there may be evidence to suggest that the incidence of reported paraquat poisonings and mortality have fallen since the introduction of the 1.5g/l level of emetic in France in the mid 1980s. A review of paraquat poisonings in France by Bismuth et al (J Toxicol. Clin Toxicol, 19 (5), pp461-474, 1982) clearly shows a high incidence of mortality (71%) following paraquat ingestion between 1972 and 1981.

I am unable to find evidence that paraquat poisoning in France since introduction of a paraquat formulation containing 1.5g/l emetic has had no effect on reported poisonings or reported deaths attributed to the herbicide. Indeed, the number of cases appears to be very low. If increasing the level of PP796 by 3 fold in France has reduced the number of fatal poisonings, this information would help in resolving some of the technical, regulatory and toxicological issues we would face in the development of a Gramoxone or Magnoxone formulation containing 1.5g/l PP796.

J R HEYLINGS
Biochemical Toxicology

FORMULATION STUDIES XD1328

MEANS



From
Dr J R Heylings
Biochemical Toxicology

ICI Central Toxicology Laboratory

Alderley Park
Macclesfield Cheshire SK10 4TJ

Tel: 0625 582711
Telex: 669095/669388
Fax: 0625 582897

To
G A Willis
N N Sabapathy

Copies to
PSAC Sub-committee members

R C Scott	G J A Oliver
T Green	J W Botham
E A Lock	S E Jaggers
R S Morrod	

Your ref	Our ref	Direct line	Tel ext	Date
	JRH111/JJB	Redacted - EU PII	Redacted - EU PII	12 June 1991

PARAQUAT HUMAN DATA

Over the past few months I have been collating data on paraquat human poisonings from various internal and external publications in order to gauge the potential impact of reducing the oral toxicity of the herbicide. This data represents a significant proportion of reliable information from 1979-1990 and represents territories where clinical data has been accurately documented (see Table). As a result the database does not include third world territories where paraquat poisoning has also been documented.

I have analysed the data for three primary parameters: (i) the mortality rate from confirmed paraquat poisonings, (ii) the average amount of paraquat consumed in grammes (based on product concentration) and (iii) the impact of increasing the lethal oral paraquat dose by 5 or 10x on these mortality statistics.

In terms of mortality, the combined data show a **76% mortality** rate in the territories which are represented. This figure is compatible with previous figures I have seen reported, both inside and outside the Company.

On consideration of the amount of paraquat ingested, it would appear that the average amount of paraquat ingested is about 10 grammes or about 3 lethal doses, based on data from five separate studies (see Table). I believe this to be at variance with what I understood to be the average lethal doses consumed, ie 10-20 lethal doses for paraquat suicides which represent 95% of all poisoning cases.

In order to comment on the impact of increasing the lethal dose of paraquat on mortality, I have chosen a single subset of data from Japan. This comprehensive set of data from ICI Japan was sent to us in 1988. One hundred paraquat cases were studied in detail. The cases involved different products, all of which contained the emetic PP796. At that time we analysed the data for time-to-emesis versus dose, plasma paraquat versus dose, and effect of product

continued....

dilution (eg Preeglox versus Gramoxone). We are now aware that time-to-emesis should be measured in minutes not hours, and that rapid, but brief, emesis induced by PP796 is totally different from the irritancy-induced prolonged emesis caused by the corrosive effect of paraquat. Furthermore, I believe that a sub-effective dose of emetic PP796 was present in all cases which consumed less than 5 lethal doses of paraquat.

On reviewing this same database, I have examined the distribution of a subset of 69 cases where a reasonably accurate measure of volume and concentration of product ingested was made in order to calculate the grammes of paraquat ingested. In consultation with our statistics department at CTL, I present the data from this study as shown on the attached figures.

Human LD_{50} for Paraquat (Figure 1)

Dividing the population into band widths of 1.5 grammes of paraquat enables an LD_{50} curve to be plotted for the 69 human paraquat poisoning cases. As expected, there is a steep dose response between 0.5 and 2 lethal doses which is a well known feature of paraquat lethality in man and experimental animals. The calculated human LD_{50} for paraquat was 3.1 grammes. Had all the cases involved Gramoxone, this would have been equivalent to about 15ml of product - a widely agreed and previously stated LD_{50} volume. There were seventeen people who ingested less than the LD_{50}. This fits very well with the 25% survival rate observed in the 69 cases.

→ MLD = 50 mg/kg

Volume of Paraquat Ingested (Figure 2)

A cumulative frequency plot was made for the 69 human paraquat poisoning cases. This data fits a polynomial distribution very well (r=0.99). From this curve, the average, or more correctly, the median volume of ingested product was 67ml. The majority of the cases involve a 200g/l product, but also include more dilute products such as Preeglox.

200g/l = 200 mg/ml
67ml = 13400 mg
= 206 mg/kg

Grammes of Paraquat Ingested (Figure 3)

The actual amount of paraquat ingested (based on product strength) was calculated for the same 69 human poisoning cases. A cumulative frequency plot was drawn in order to calculate the average or median amount of paraquat ingested. As shown on Figure 3, the average amount of paraquat ingested was 6.9 grammes. This corresponds to 2.2 x LD_{50}.

Improvement in Oral Toxicity of Paraquat and Estimated Survival (Figure 4)

Data presented in Figures 2 and 3 have used volume and weight of paraquat as the ordinate. By combining the LD_{50} value of 3.1 grammes with the plot of weight of paraquat, the same data can be presented using "number of lethal doses" as the ordinate. Figure 4 therefore shows the proportion of a population which includes any given number of lethal doses of paraquat. Thus, an improvement of 5-fold in oral toxicity would include 75% of cases. These figures may correspond to the respective survival rates for 5 and 10x safening. Likewise, an improvement of 10-fold in oral toxicity would include 90% of cases. This is, of course, assuming that this Japanese database is accurate and representative of the population as a whole. However, there are three features which suggest to me that this data is representative of typical paraquat poisonings. Firstly, the steep sigmoidal

dose curve has many of the characteristic features of paraquat lethal dose plots in animals. Secondly, the calculated human LD_{50} of 3 grammes paraquat is widely accepted. Thirdly, the percentage survival rate (~25%) fits many previously published studies which involve combinations of suicide and accidental poisonings.

Interestingly, if 'Magnoxone' were proven to be 15x safer in man and caused an increase in survival from 24% to 90%, this would not only include all accidental poisonings, but the few cases which do consume 15-20 lethal doses (½ pint) would probably find the new formulation (with its thickening properties) very difficult to drink.

A particularly important feature of the Japanese data occurs at the lower end of the lethal dose scale. The curve rises steeply between 0 and 5 lethal doses. It includes all accidental poisonings and probably most of the para-suicides. It includes 75% of all paraquat poisoning cases in this study. It therefore suggests that even a 5-fold safety factor such as may be caused by increasing the level of emetic by a factor of 5, would have a considerable and measurable impact on the accidental and suicide poisoning statistics for paraquat.

Dr J R Heylings
Biochemical Toxicology, CTL

HUMAN PARAQUAT POISONINGS

AUTHOR	YEAR	LOCATION	SPAN	CASES	% MORTALITY	AVERAGE LETHAL DOSES INGESTED
Howard (ICI)	1979	UK	1977	68	60%	2
Bismuth et al	1982	France	1972-81	28	61%	-
Zilker et al	1983	Germany	1974-81	21	76%	-
Hart and Whitehead	1984	UK	1977-83	210	78%	3
Proudfoot et al	1987	UK	1986	23	65%	-
Ohno (ICI Japan)	1987	Japan	1986-7	69	75%	2-3
Suzuki et al	1989	Japan	1988	51	84%	-
Yamaguchi et al	1990	Japan	1981-7	160	80%	5
Houzé et al	1990	France	1984-8	17	82%	1-2

Combined data : 490 mortalities from 647 cases (76%)

Fig 1.

JAPANESE HUMAN DATA (1986 – 7)

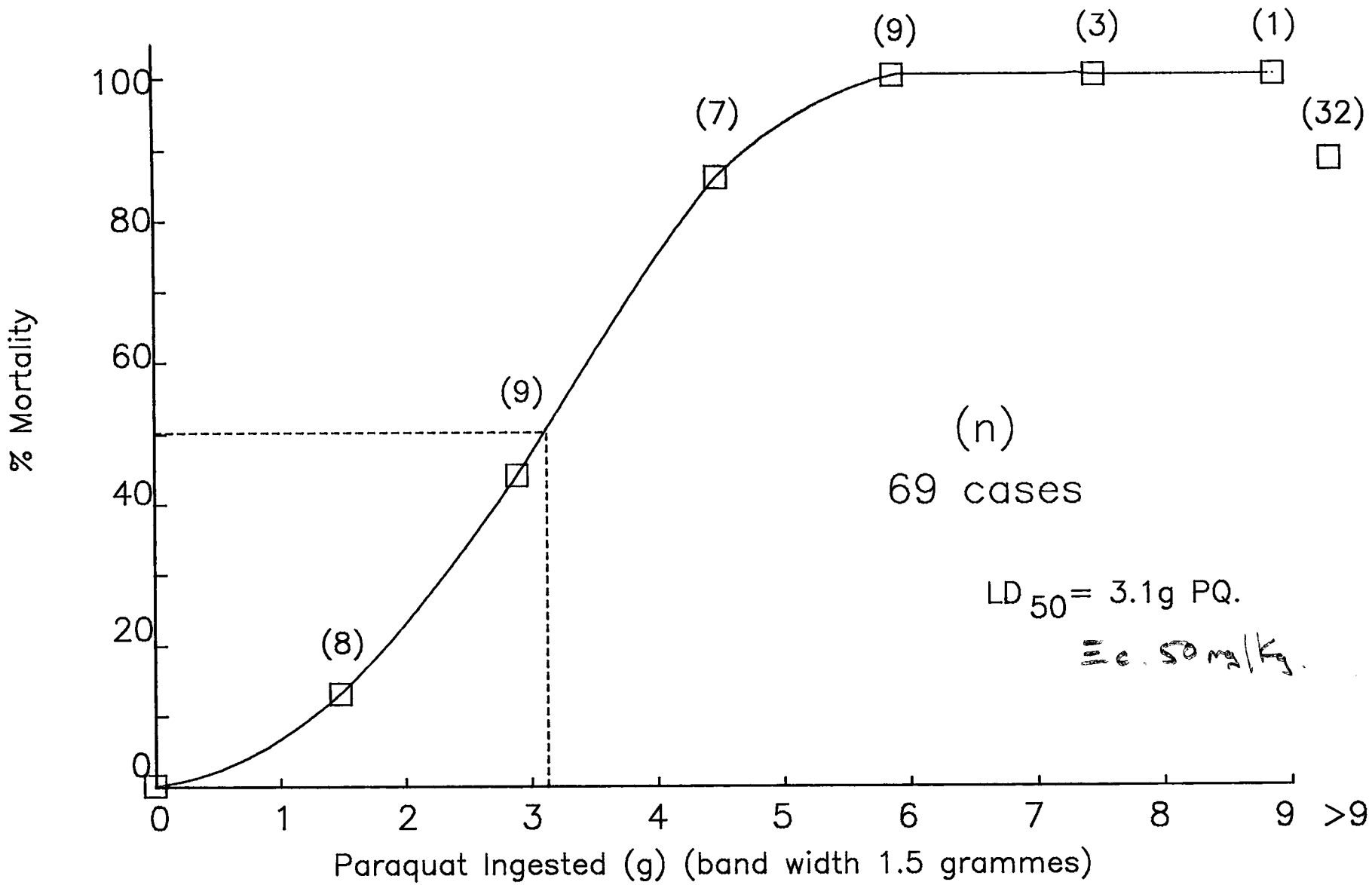


Fig 2.

JAPANESE HUMAN DATA (1986–7)

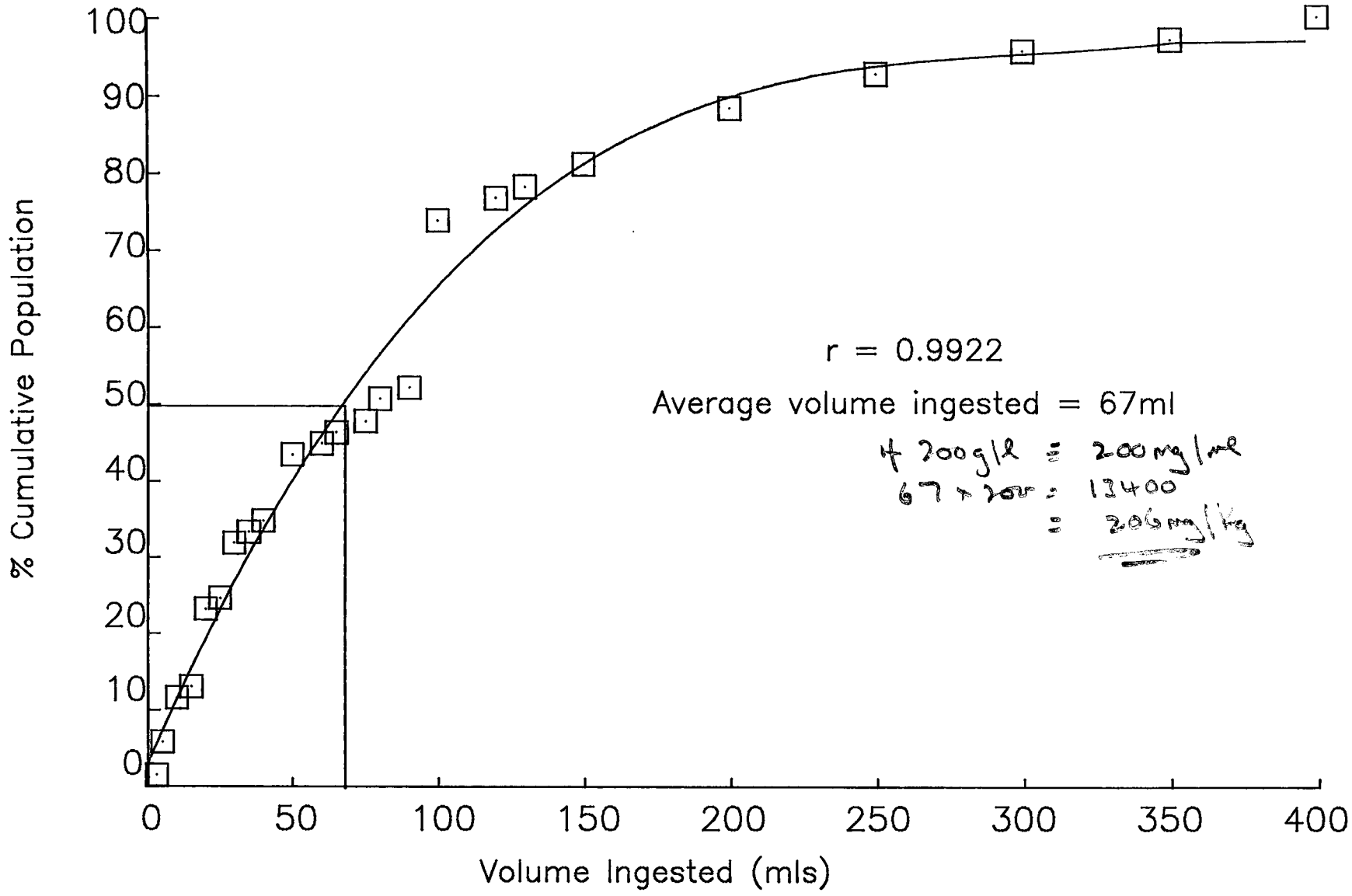


Fig 3.

JAPANESE HUMAN DATA (1986–7)

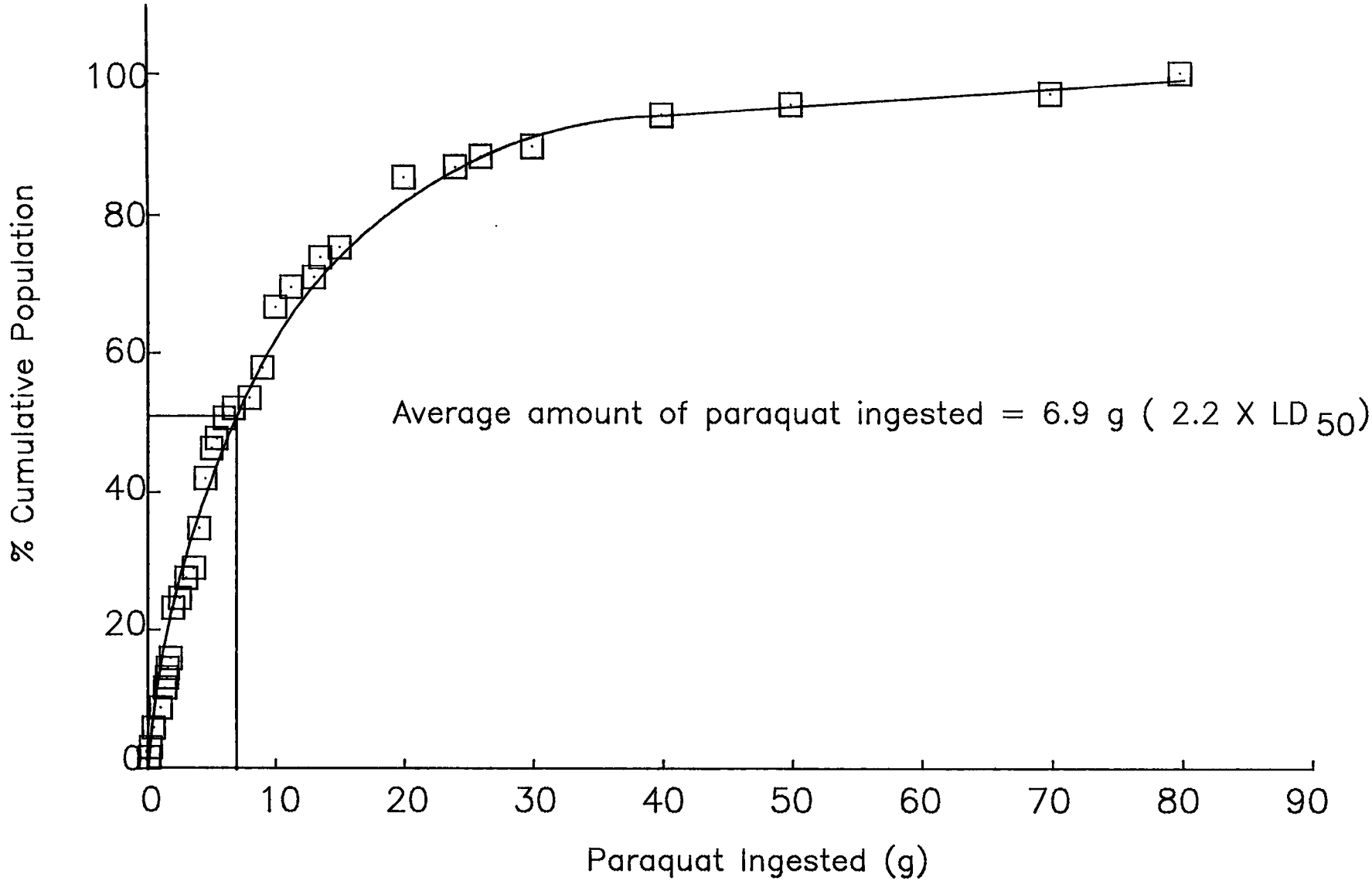
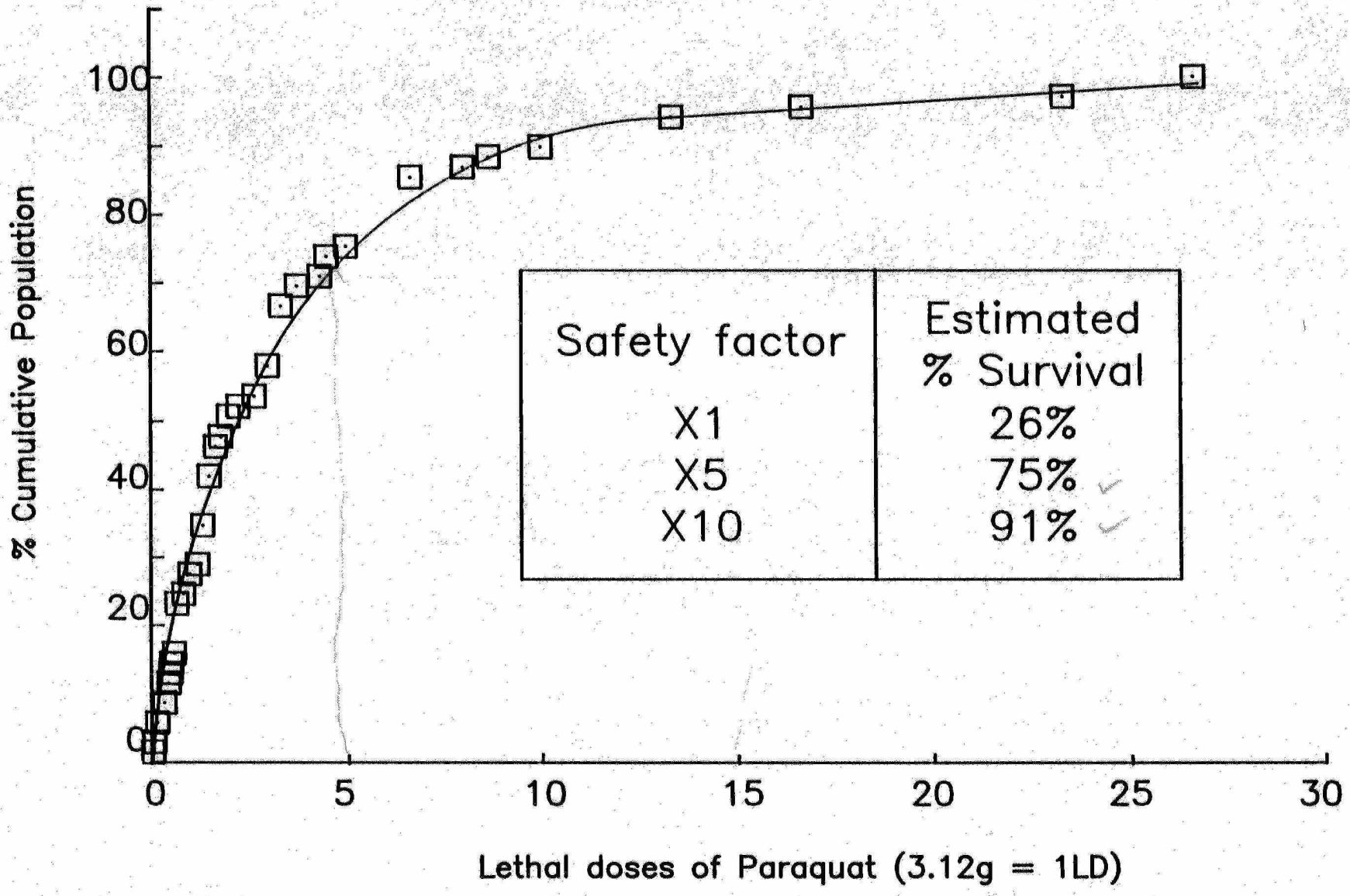


Fig. 4

JAPANESE HUMAN DATA (1986–7)



Safety factor	Estimated % Survival
X1	26%
X5	75%
X10	91%

SYNG-PQ-03709705_R

From
Dr J R Heylings
Biochemical Toxicology

ICI Central Toxicology Laboratory

Alderley Park
Macclesfield Cheshire SK10 4TJ

Tel: 0625 582711
Telex: 669095/669388
Fax: 0625 582897

To
G A Willis - PSS Fernhurst
N N Sabapathy - " "

Copies to
A Starling - Dev Dept, Fernhurst
R C Scott
S E Jaggers
R S Morrod

Your ref	Our ref	Direct line	Tel ext	Date
	JRH110/JJB	Redacted - EU PII	Redacted - EU PII	21 May 1991

ORAL TOXICITY OF PARAQUAT AND GLYPHOSATE

Stuart Jaggers pointed out a couple of interesting papers to me on acute glyphosate poisoning in man published recently in The Lancet and also in Human and Experimental Toxicology. The papers suggest that the actual 'Roundup' formulation is considerably more toxic than the active ingredient itself. I believe that it is quite widely accepted that the surfactants are the primary reason for the acute toxicity of Monsanto's product.

Having studied the clinical evidence I would suggest that the technology we are developing for 'Magnoxone' would also reduce the extensive gastrointestinal irritation caused by the surfactants in Roundup. Thus, removal of gastric acid by Magnesium Trisilicate and purgation with Magnesium Sulphate is highly likely to reduce the acute toxicity following oral dosing with Roundup.

Another interesting comparison between 'Magnoxone' and Roundup is the volume of product which is lethal to man. It is suggested that the average amount of Roundup ingested by non-survivors is 184ml (range 85-200ml). If man shows a similar shift in lethal dose with 'Magnoxone', as has been observed in the dog, I would predict that the average lethal volume for the new paraquat formulation would exceed 225ml or 45 grams of paraquat. This would make our potential product less toxic by volume orally than Roundup.

It is widely accepted that antacids such as Magnesium trisilicate reduce the incidence and severity of gastrointestinal erosions caused by a variety of irritating substances. These include ethanol, aspirin, mineral acids as well as surface active agents, such as bile salts. Prior to 'Magnoxone', incorporation of antacids in formulations of pesticides has not been considered before. The impact of protecting man from gastrointestinal irritation may have broader implications for other pesticide formulations outside the bipyridyl arena.

continued....

Magnesium trisilicate BP is the preparation most commonly used in the United Kingdom for the purpose of antacid prophylaxis in obstetric practice. (Crawford et al. Anaesthesia 39, 535-9, 1984). The objective here is to maintain the pH value of the mother's gastric contents above the level 3.0, so that if aspiration regurgitation does occur, she will not be at risk of developing the acid-aspiration syndrome. The amount of trisilicate present in 10ml of 'Magnoxone' (the potential minimal lethal dose of Gramoxone) is well within a therapeutic human antacid dose.

Dr J R Heylings
Biochemical Toxicology

HLA class II antigens of the patient's T-cell-rich fraction obtained on day 17 were examined and DRw13, DRw52, and Ia antigens were recognised on T cells and on B cells, pointing to T-cell activation.

Post-tranfusion GVHD usually develops in patients with an immature or an impaired immune system.[3] However, POE is seen in patients who are not obviously immunodeficient. Compared with the GVHD seen after bone marrow transplantation post-transfusion GVHD develops early and rapidly with severe pancytopenia and carries a poor prognosis. The symptoms in POE resemble those of post-transfusion GVHD. The patient described here was not immunodeficient and his HLA phenotype changed to that of a blood donor over 18 hours. The increase in cytotoxic T cells and the T cell activation are evidence of GVHD.

Engraftment of donor lymphocytes was established easily in this patient, perhaps because one of his HLA haplotypes is identical to the donor's homozygous haplotype so that he could not resist engraftment. The donor's haplotype (Aw33-CBL-B44-DRw13-DQ[illegible]) is found in 5·8% of Japanese so the risk of blood from a donor homozygous for this haplotype being given to a heterozygote is about 1 in every 3000 transfusions. When blood comes from different donors the risk will increase.

POE is fatal in about 65% of cases after gastroenterological surgery, and is almost always fatal after open heart surgery. In Japan fresh blood and platelet concentrates containing lymphocytes are often used during these operations and these preparations are not usually irradiated first. This would explain the high incidence of POE (1 in 300 to 400 in open heart surgery).[1] Since POE is untreatable it must be prevented either by using blood that has been stored for more than 10 days to allow lymphocytes to lose immunocompetence[4] or by irradiating (1500–3000 rad) fresh blood and platelet concentrates.

We thank Prof Takeo Juji for useful discussions.

KAZUHIKO ITO
HISAHIRO YOSHIDA
KEN YANAGIBASHI
YUTAKA SHIMADA
MASAYUKI IMAMURA
TAKAYOSHI TOBE
YUICHI AKIYAMA
HIROH SAJI
ETSUKO MARUYA
TAKEMITSU HOSOI

Department of Transfusion Medicine,
Kyoto University Hospital,
606 Kyoto, Japan;
First Department of Surgery
and Department of Paediatrics,
Faculty of Medicine,
Kyoto University;
and Kyoto Red Cross Blood Centre

1. Ino T, Matsuura A, Takanashi R, et al. GVHD like syndrome following transfusion at the time of surgery. *Geka* 1986; **48**: 706–12 (in Japanese).
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4. Harada M, Matsue K, Mori T, Ishino C, Kodo H, Hattori K. Viability of lymphocytes in stored blood: Their surface markers, mitogenic response and MLC reactivity. *Acta Haematol Jap* 1979; **42**: 30–34.

TOXICITY OF HERBICIDE CONTAINING GLYPHOSATE

SIR,—Dr Sawada and colleagues (Feb 6, p 299) present an important message about the relative safety of the active herbicidal ingredient glyphosate and the surfactant in the roundup formulation. It is often assumed, wrongly, that agrochemicals are orders of magnitude more toxic than more familiar chemicals in toiletries and household products. However, readers might conclude from the wording of Sawada and colleagues' letter that roundup is comparable in toxicity to paraquat when in fact it is comparable in safety to many toiletries and household products containing surface active ingredients.

Paraquat and roundup are safe if stored, handled, and used in accordance with the manufacturer's instructions. For a given quantity ingested, roundup is less toxic than paraquat and has a good margin of safety even when ingested with suicidal intent (less than 20% fatalities from Sawada's data). We have never claimed roundup to be non-toxic—all chemicals have a toxic dose. However, we have records of many cases of survival after ingestion of quantities of roundup estimated to be several hundred millilitres.

Sawada et al do not tell us about medicines and alcohol, which are frequent concomitants of suicidal self-poisoning; without these details the contribution of any of the ingredients in roundup to death cannot be assessed. In this company's wider experience, deaths are often associated with aspiration pneumonia after suppression of the protective reflexes by alcohol consumption.

Monsanto (which identified the cases in Sawada's report to him) is not aware of any deaths, including those in Sawada's series, following accidental ingestion. Such cases are often children, and the average dose in survivors needs to be corrected for that fact. All deaths from roundup poisoning have followed deliberate suicidal ingestion, often in conjunction with other substances. We are cooperating with the International Program of Chemical Safety (division of environmental health) and the International Development Research Centre to provide information packages for use in poisons centres which do not have access to extensive databases. These packages will include information of products formulated with glyphosate. It may be that the collation of our world-wide case experience for this exercise will lead to a review which will give the fullest possible clinical details on a wider spectrum of cases.

May I take the opportunity of reminding those who might have to treat cases of poisoning that, though an organic molecule containing phosphorus, glyphosate is not an inhibitor of cholinesterase. Atropine (other than in normal therapeutic doses of 0·6 mg, if indicated for some other reason) has no place in treatment and oximes should not be administered. In general, treatment should be supportive and symptomatic. There is some evidence that activated charcoal may adsorb surfactant, and this is likely to be beneficial in view of the probable contribution of this ingredient to the toxicity.

Monsanto Europe SA,
Ave de Tervuren 270–272,
1150 Brussels, Belgium

JOHN R. JACKSON

IMPORTING KHAT

SIR,—Dr Goudie's letter (Dec 5, p 1340) is based on pharmacological studies on animals and on a few case-reports of induced psychosis. The one publication in the list of references dealing with general medical aspects of *khat* (or *qat*, a more accurate transliteration) chewing is dated 1972. More recent studies, based on thorough observations in the field, have pointed out that most of the negative health aspects of *khat* chewing are anecdotal rather than factual.[1] One of us has discussed at length the far from exclusively negative social and economic implications of *khat*.[2] Before sounding the alarm on what is an important element of life for some sections of immigrant populations, it may be advisable to weigh the evidence dispassionately, as we would/should do for ouzo, cassava, or maple syrup.

Niels Andersensvej 9A,
2900 Hellerup, Denmark

SHELAGH WEIR
M. C. THURIAUX

1. Kennedy JG, et al. A medical evaluation of the use of qat in North Yemen. *Soc Sci Med* 1983; **17**: 783–94.
2. Weir S. Qat in Yemen: Consumption and social change. London: British Museum Publications, 1985.

ARSENIC AND CANCERS

SIR,—Arsenic is widely distributed in nature and mainly transported in the environment by water. High-arsenic artesian well water has been related to skin cancer and to blackfoot disease, a unique peripheral vascular disorder confined to the south-west coast of Taiwan Island.[1,2] The Taiwan study has been used to set safety levels for arsenic.[3,4] In 1985 we reported very high mortality rates from internal organ cancers among residents of the area,[5] and the increase was significantly associated with the use of high-arsenic artesian well water.[6] However, a dose-response relation between the level of arsenic ingested and cancer has not been reported.

We have analysed cancer mortality of residents in the blackfoot disease endemic area based on 899 811 person-years observed from 1973 to 1986. The studied population was grouped into three strata (ie, below 0·30, 0·30–0·59, and 0·60 or more parts per million [ppm]) according to the arsenic level of the drinking water, which

Human & Experimental Toxicology (1991), **10**, 1–8

Acute Poisoning with a Glyphosate-Surfactant Herbicide ('Round-up'): A Review of 93 Cases

Alan Ronald Talbot, Mon-Han Shiaw, Jinn-Sheng Huang, Shu-Fen Yang, Tein-Shong Goo, Shur-Hueih Wang, Chao-Liang Chen & Thomas Richard Sanford[1]

Departments of Critical Care Medicine and [1]Radiology, Changhua Christian Hospital, 176 Chunghua Road, Changhua, Taiwan 50022, Republic of China

Between 1 January 1980, and 30 September 1989, 93 cases of exposure to herbicides containing glyphosphate and surfactant ('Roundup') were treated at Changhua Christian Hospital. The average amount of the 41% solution of glyphosate herbicide ingested by non-survivors was 184 ± 70 ml (range 85–200 ml), but much larger amounts (500 ml) were reported to have been ingested by some patients and only resulted in mild to moderate symptomatology. Accidental exposure was asymptomatic after dermal contact with spray (six cases), while mild oral discomfort occurred after accidental ingestion (13 cases). Intentional ingestion (80 cases) resulted in erosion of the gastrointestinal tract (66%), seen as sore throat (43%), dysphagia (31%), and gastrointestinal haemorrhage (8%). Other organs were affected less often (non-specific leucocytosis 65%, lung 23%, liver 19%, cardiovascular 18%, kidney 14%, and CNS 12%). There were seven deaths, all of which occurred within hours of ingestion, two before the patient arrived at the hospital. Deaths following ingestion of 'Roundup' alone were due to a syndrome that involved hypotension, unresponsive to intravenous fluids or vasopressor drugs, and sometimes pulmonary oedema, in the presence of normal central venous pressure.

Introduction

Herbicides containing glyphosate (N-phosphonomethyl glycine) are an alternative to paraquat and have recently been used in attempted suicide. Glyphosate acts primarily as a competitive inhibitor of 5-enolpyruvoylshikimic acid-3-phosphatesynthase,[1,2] an enzyme essential to the synthesis of aromatic amino acids in plants. Since this enzyme is absent in animals, glyphosate is considered to have low mammalian toxicity. The acute oral LD_{50} of glyphosate is reported to be approximately 4–6 g kg^{-1} in rat and rabbit.[3,4]

Changhua Christian Hospital is the receiving hospital for a large agricultural area in central Taiwan. During the period 1975–1989, a total of 7035 cases of acute poisoning were seen, of which 80% were due to agricultural chemicals, and 17.6% of the entire series was due to herbicides.[5] This paper reports our experience with cases of poisoning with herbicides containing glyphosate.

Methods

A list of 12 herbicides registered as containing glyphosate was obtained from the Taiwan Agricultural Agents and Toxic Substances Research Institute. The official Monsanto 'Roundup' product was sold under the Chinese names *lan-da*, *hao-ni-chun*, and *nian-nian-chun*. Roundup is a commercial product containing 41% glyphosate (360 g l^{-1} of free glyphosate) as the isopropylamine salt, 15% surfactant (polyoxyethyleneamine, POEA) and water.[6]

Emergency room records were reviewed for the period from January 1, 1974, to September 30, 1989, for diagnoses related to drug poisoning, poisoning with agricultural agents, poisoning due to unknown agents, 'suicide' with cause not listed and cases of dead-on-arrival (DOA). The charts from this selection were then reviewed and only those recording exposure to glyphosate herbicides were included in the present series. Exposure to

Correspondence: A. R. Talbot, Department of Critical Care Medicine, Changhua Christian Hospital, 176 Chunghua Road, Changhua, Taiwan 50022, Republic of China.

'glyphosate herbicide' was diagnosed by history and verification of the agent by hospital staff (transcription of the label of the bottle into the chart records). Since other agricultural agents are often involved in poisoning in our area, paraquat was excluded in all cases by the dithionite test for paraquat in urine,[7] and organophosphate poisoning was similarly excluded by monitoring plasma pseudocholinesterase levels by colorimetry (Cholinesterase-NA test, Wako Junyaku Kogyo Coy, Osaka, Japan).

All data were retrospectively extracted from the medical records. Other agents (either agricultural chemicals or alcohol) ingested at the same time were noted, as was the trade-name of the glyphosate product.

The amount ingested was usually given in descriptive terms such as 'a mouthful' or 'a half a bottle'. For statistical purposes, arbitrary volumes were assigned to the phrases that occurred frequently. 'A little' or 'a spoon' was 5 ml, 'a mouthful' was 25 ml, 'a small cup' was 100 ml, and 'a bottle' was taken as 300 ml (bottles available commercially were 150 ml, 300 ml, 600 ml and 1000 ml, but the 300 ml bottle was most commonly used by our patients). Cases in which the amount was not recorded or was not known were excluded from the analyses based on amount ingested.

The degree to which a patient exhibited clinical signs and symptoms was classified into four groups (asymptomatic, mild, moderate, severe) as summarized in Table 1.[8] The severity of intoxication was also quantitated by the APACHE II system using the diagnostic category weight for aspiration/poisoning/toxic to calculate the risk of death.[9]

Organ involvement was defined as positive if laboratory tests of organ function abnormal for our hospital were recorded at least once in the chart. For renal function, this was serum levels greater than 106.8 μmol l^{-1} for plasma creatinine (Cr), 7.12 mmol l^{-1} for blood urea nitrogen (BUN); for liver enzymes, greater than 40 iu l^{-1} for both aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Chest radiographs (CXR) were classified as normal or abnormal, and the degree and type of pulmonary oedema was scored using a standard technique.[10] Bilateral alveolar exudates in the presence of normal central venous pressure were diagnosed as adult respiratory distress syndrome (ARDS). Gastrointestinal tract (GIT) involvement was judged by symptoms (sore throat, oesophagitis, abdominal pain, dysphagia), the presence of melaena or haematemesis, and endoscopy findings when performed. Central nervous system (CNS) involvement was documented if the patient was confused or comatose and other causes had been excluded, but was not differentiated from confusion secondary to hypercapnia or hypoxaemia. Haematological evaluation included a peripheral smear and differential count of leucocytes, with a leucocyte count above 9000 mm^3 defined as 'increased' (leucocytosis). Roger's test[11] was used to test for seasonal trends in incidence.

Table 1 Classification of severity in acute poisoning with Roundup herbicide.

Classification	*Description*
Asymptomatic	no complaints, and no abnormalities on physical or laboratory examination.
Mild	mainly GIT symptoms (nausea, vomiting, diarrhoea, abdominal pain, mouth and throat pain) that resolved within 24 h. Vital signs were stable, and there was no renal, pulmonary or cardiovascular involvement.
Moderate	GIT symptoms lasting longer than 24 h, GIT haemorrhage, endoscopically verified oesophagitis or gastritis, oral ulceration, hypotension responsive to intravenous fluids, pulmonary dysfunction not requiring intubation, acid–base disturbance, evidence of transient hepatic or renal damage, or temporary oliguria.
Severe	pulmonary dysfunction requiring intubation, renal failure requiring dialysis, hypotension requiring treatment with pressor amines, cardiac arrest, coma, repeated seizures, or death.

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Results

The final series in which the agent was satisfactorily identified by history and label consisted of 93 patients with seven deaths (Table 2). The first death was seen in 1984.

The different glyphosate products ingested included *nian-nian-chun* 88 cases (including the two with other pesticides); *hao-ni-chun* two cases; *ri-ri-hao* one case; and *jia-lin-sai* two cases. The majority (90 out of 93) were the official Monsanto product.

The number of cases was smallest in the winter months, with an average of two per month for November and December, and showed a peak in the summer months of June (average 10 per month) to September (averge 18 per month). Roger's coefficient of seasonal variation[11] was 8.97 and was significant at the $P < 0.025$ level. This reflected the trend to increased numbers during the summer seen here in poisoning with other agricultural agents.[5]

The age distribution of the patients is shown in Table 3. There is a peak for both sexes in the 20–30-year-old age group; males were more common in the age groups above 30 years. Male patients were twice as frequent as female patients. The average age for the entire series was 37 ± 17 years ($n = 93$). The average age of survivors was 36 ± 16 years ($n = 86$), and for non-survivors the average age was 58 ± 19 years ($n = 7$).

Aspiration pneumonitis occurred in one case, and the patient survived. In the five fatalities due to glyphosate herbicide alone, the patient was alert and cooperative on admission to hospital, and had not ingested alcohol. Of the 86 survivors, five had taken glyphosate herbicide while drinking alcohol and two had mixed it with fruit juice. All other intentional ingestions were of the undiluted 41% glyphosate herbicide.

The amounts ingested are compared to the clinical symptoms in Table 4. The numbers are suggestive of a dose-response relationship, with increasing amounts resulting in more severe symptoms; however, the patients' estimates of the amount ingested, and the conversion ratio used in this paper may be inaccurate. Two points that can be made are that reported large amounts did not necessarily cause serious symptoms ('moderate' range up to 500 ml), and severe symptoms (death) only resulted from oral ingestion of undiluted herbicide in amounts above 85 ml.

The risk of hospital death compared to clinical symptoms is shown in Table 5. All patients with a favourable outcome had a risk less than 0.2, whereas all eight of those with a risk score above 0.2 were in the severe group and seven of them died.

All cases of accidental exposure (dermal two cases; eating vegetables that had been sprayed four cases) were asymptomatic. Gastrointestinal ingestion by mistake (seven cases) was usually of a small amount that was immediately rejected, and resulted only in minor mouth discomfort.

Atropine and pralidoxime were given to three cases, one of whom was the non-survivor who ingested both glyphosate and parathion.

The times from ingestion to arrival at hospital are shown in Table 6. The average time was

Table 2 Yearly incidence of poisoning with glyphosate herbicides. Numbers in parentheses indicate deaths.

Year	*Male*	*Female*	*Total*
1980	1	0	1
1981	2	1	3
1982	0	2	2
1983	0	1	1
1984	4 (1)	4	8
1985	3	1	4
1986	3	3	6
1987	9	3 (1)	12
1988	14 (2)	12 (1)	26
1989*	23 (1)	7 (1)	30
Total	59	34	93

* The series ends on 30 September

Table 3 Age distribution of patients poisoned with glyphosate herbicides.

Age range	*Female*	*Male*	*Total*
0–10	0	1	1
11–20	4	7	11
21–30	15	10	25
31–40	7	14	21
41–50	3	4	7
51–60	2	11	13
61–70	3	7	10
71–80	1	3	4
81–90	0	1	1

Table 4 Comparison of severity of clinical symptoms with estimated amount of glyphosate herbicide ingested. Amount (mean ± s.d.) and range are in ml. Severity groups as described in Table 1.

Severity	*Number*	*Amount*	*Range*
Asymptomatic	13	17 ± 16	5– 50
Mild	38	58 ± 52	5–150
Moderate	22	128 ± 114	20–500
Severe	4	184 ± 70	85–200

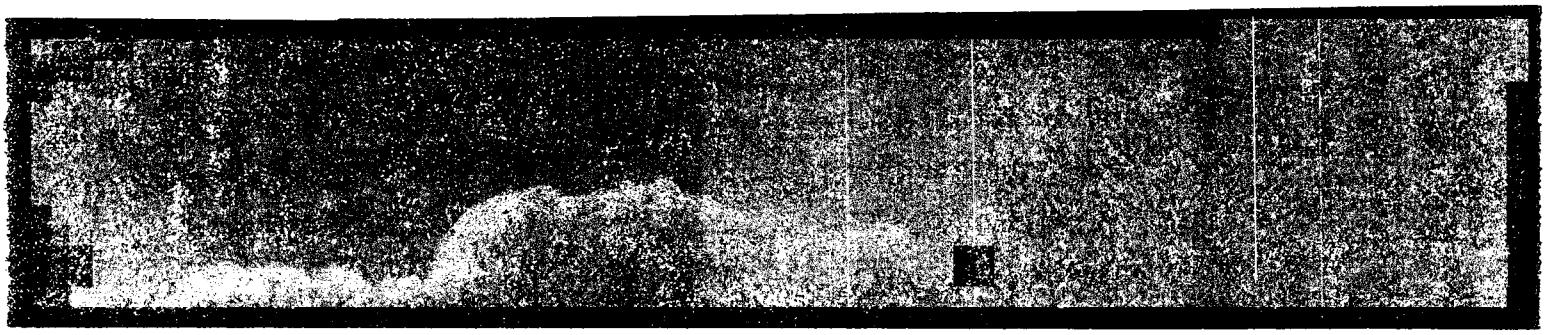


Table 5 Risk of hospital death compared to clinical group.

*Risk**	*Asymptomatic*	*Mild*	*Moderate*	*Severe*
0.0–0.1	18	39	19	"
0.1–0.2	"	6	4	"
0.2–0.3	"	"	"	1
0.5–0.6	"	"	"	1
0.6–0.7	"	"	"	2
0.8–0.9	"	"	"	2
0.9–1.0	"	"	"	1
Total (n = 93)	18	45	23	7

* Risk calculated from APACHE II score in first 24 h.[9] Risk levels (0.3–0.5) not represented in this series have been omitted from the table.

Table 6 Time (h) from ingestion of glyphosate herbicide to arrival at the emergency room.

Category	*Number*	*Mean ± s.d.*	*Min*	*Max*
Survived	80	12.2 ± 19.3	0.1	85.3
Dead	6	4.7 ± 3.1	2.5	11.5
Accident	8	26.6 ± 31.8	0.7	85.3
Suicide	79	10.1 ± 16.1	0.1	83.3
Overall	87	11.6 ± 18.7	0.1	85.3

around 12 h, with a range of 10 min to nearly 3 d. The average time in fatal cases was very short (4.7 h); however, death resulted despite medical treatment. Of the seven deaths in this series, two involved ingestion of multiple agricultural agents: one was *nian-nian-chun* with paraquat, and the other was *nian-nian-chun* with parathion. Neither of these patients could be resuscitated in the emergency room. The averge time from ingestion of glyphosate herbicide to death was 26.4 ± 26.4 h (range 3.7–38.5 h): five died within the first 12 h (two were dead on arrival), one died within 24 h, and one died at 38.5 h after ingestion. Death was due to pulmonary oedema or hypotension unresponsive to intravenous fluids or pressor agents. For cultural reasons, autopsies were not performed.

The incidence of signs and symptoms in the 74 symptomatic patients in the series is presented in Table 7. GIT symptoms 66% (49/74) were most common, with upper GIT symptoms similar to ingestion of a corrosive agent (sore throat, mouth ulcer, dysphagia, epigastric pain) being most frequent. Vomiting was poorly recorded because the patient had already been treated by gastric lavage and emetics, or gave a poor history. GIT haemorrhage occurred in 12% (6/49) of patients with GIT symptoms (8% of all patients with symptoms). Paralytic ileus was not seen, and pancreatitis was not evident clinically, although serum amylase was measured in only two cases.

Endoscopy was performed in 23 cases and was abnormal in 22. The oesophagus was involved in 39% (9/23), with ulcers (one case) and superficial (six cases) or corrosive (two cases) oesophagitis. The stomach was involved in 96% (22/23), as gastritis (17 cases) and ulceration (five cases). The duodenum was involved in 26% (6/23) as duodenitis (four cases) and duodenal ulcer (two cases). A lower GIT endoscopy in one case showed colitis.

Pulmonary dysfunction was documented in 23% (17/74) of cases. Asymptomatic mild hypoxaemia (arterial oxygen tension less than 11.3 kPa while breathing room air) was frequent, and chest radiographs were usually normal. There were eight cases of pulmonary oedema diagnosed as ARDS, of whom only one survived. Case 1 is an example.

The cardiovascular system was recorded as abnormal in 18% (17/74) of cases. The arrythmias and abnormal electrocardiograms (ECG) were usually sinus tachycardia or sinus bradycardia. In contrast to Japanese reports,[19] atrio-ventricular block was not seen. Hypotensive shock occurred in all seven of the non-survivors. This hypotension sometimes responded initially to hydration, but then recurred within hours. It was not due to hypovolaemia (as judged by central venous pressure, haemotocrit, and blood urea nitrogen) and did not respond to fluid resuscitation or vasopressor agents (Case 2 is an example).

Renal abnormalities occurred in 14% (10/74) of cases. They all had elevated serum creatinine

Table 7 Incidence of signs and symptoms (n = 74). All – number of patients with data recorded for this organ. No – number of patients with data recorded for this organ who had the sign/symptom.

Organ	*All*	*Sign/Symptom*	*No*
GIT*	49	sore throat	32
		mouth ulcer	32
		dysphagia	23
		epigastric pain	16
		melaena	6
		vomiting	1
Smear	48	leucocytosis	48
		shift-to-left	1
Lung	17	abnormal CXR	9
		aspiration	1
		intubation	7
		respirator	7
Heart	13	shock	7
		arrythmia	4
		abnormal ECG	7
Kidney	10	oliguria	3
		haematuria	3
		Cr increased	9
		BUN increased	0
Liver	14	GOT increased	14
		GPT increased	8
CNS	9	confused	3
		coma	6

* Endoscopy performed in 23 cases. Smear – peripheral smear for leucocytes and differential count. Other abbreviations (GIT, CXR, Cr, BUN, AST, ALT, CNS) as in the text.

(but only above 176.8 μM l^{-1} in three patients), and sometimes oliguria (3/10). Haematuria occurred in three cases. Blood urea nitrogen was normal in all cases.

Liver enzymes were mildly elevated (less than twice normal) in 19% (14/74) of cases. In all cases, AST was elevated, and in eight of them ALT was also elevated. The time course of these changes is unknown, as most patients had only one measurement recorded in the chart.

CNS symptoms occurred in 12% (9/74), two of whom followed cardiovascular resuscitation in the emergency room. Glasgow Coma Scale (GCS) scores ranged from 9 to 13, and improved rapidly without treatment.

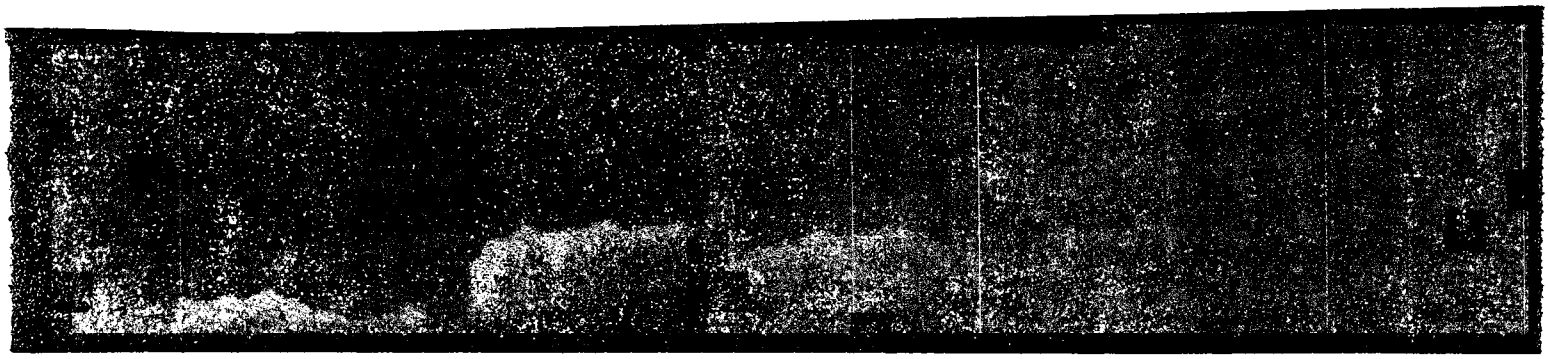
The peripheral smear showed leucocytosis in 52% (48/93) of cases. The incidence of leucocytosis appeared to be related to the clinical group (asymptomatic 30% (4/12), mild 53% (24/45), moderate 65% (15/23) and severe 71% (5/7)). A shift to the left occurred only in the patient with aspiration pneumonitis.

Case reports

Case 1. A 78-year-old male drank 'a bottle' of *nian-nian-chun* at 11:00 p.m. with suicidal intent. He was treated [Redacted - EU PII] 45 min later with gastric lavage, ultracarbon, oxygen, and intravenous fluids. Urine was negative for paraquat, and plasma cholinesterase was 8211 U l^{-1} (normal 2816–6971). On arrival, his blood pressure was 21/12 pKa, pulse 102 beats per minute, respiratory rate 28 per minute, and he was dyspnoeic. Lung examination revealed diffuse rhonchi and wheezing. The haemoglobin was 14.5 g 100 ml^{-1}, white cell count 8800 mm^3, platelets 197000 per mm^3, blood urea nitrogen 7.83 mM l^{-1}, plasma creatinine 132.6 μMol l^{-1}, blood glucose 8.03 mM l^{-1}. Electrolytes (sodium, potassium, chlorine, calcium) and enzymes (AST, ALT were normal. Arterial blood gases while breathing room air were pH 7.06, PaO_2 4.62 pKa, $PaCO_2$ 8.76 pKa.

Chest radiographs showed pulmonary oedema progressing to the characteristic picture of ARDS (Figure 1). He was intubated and ventilated with

Figure 1 Chest radiograph of patient with glyphosate poisoning (Case 1). The vascular pedicle width is normal as is the heart size, but perihilar irregularities are evident. Patchy involvement of the right lower lobe and peripheral infiltrates in the right upper lobe are prominent. Perviascular and peribronchial cuffing are present.



positive end expiratory pressure (PEEP), but hypoxaemia showed little improvement (PaO_2 5.32–6.65 pKa on 60% inspired oxygen and PEEP 5 cmH_2O), and he developed hypotension that did not respond to intravenous dopamine and fluids. Cardiac arrest occurred 13 h after ingestion.

Case 2. A 60-year-old male drank 'a bottle' of *nian-nian-chun* at 8:30 a.m. with suicidal intent. Vomiting occurred immediately. He was treated by a local doctor with an 'injection' (probably atropine), and was then transferred to the emergency room at 2:00 p.m. On arrival, he was confused (GCS of 11) but 30 min later was fully conscious (GCS 15) with normal pupillary reflexes. Blood pressure was 16/11 pKa, pulse 90 beats per minute, respiratory rate 24 per minute, and temperature 36.5 C. The haemoglobin was 13.9 g 100 ml^{-1}, with a white blood cell count of 17,400 (band 6%, segmented 87%, lymphocytes 10%). Electrolytes (sodium, potassium), plasma creatinine, blood urea nitrogen, and enzymes (AST, ALT) were within normal limits. Blood glucose was 3.74 mM l^{-1}. Urine paraquat was negative and plasma cholinesterase was normal.

He was treated with gastric lavage and ultracarbon. A toxicology screen for barbiturates, phenytoin, carbamazepine, and alcohol was negative. Arterial blood gases on 3 l min^{-1} oxygen by face mask were pH 7.24, PaO_2 15.4 pKa, $PaCO_2$ 4, 23 pKa. A chest radiograph was normal. The resting electrocardiogram showed no abnormalities.

He was sent to the ward, but 2 h later, blood pressure was 9/5 pKa and respiration was regular and shallow. The patient was transferred to the intensive care unit, but the hypotension failed to respond to intravenous fluids and vasopressor agents. Lung fields remained clear, and the bedside ECG showed sinus tachycardia (130–160 beats per minute). Cardiac arrest occurred 14 h after ingestion.

Discussion

There are few reports of the effects of glyphosate or Roundup on man.[12,13] The annual report of the American Association of Poison Control Centers does not specifically refer to it.[14] The US Environmental Protection Agency has an unedited series of 109 pesticide incidents involving glyphosate[15] reported during the period 1966–1980. Of them, 94 involved glyphosate alone and 15 cited glyphosate in combination with other ingredients. In the 91 incidents involving humans and glyphosate alone, four persons were hospitalized, 80 received medical attention, and nine were affected but did not receive medical attention. There were no deaths.

Reports discussing post-mortem measurement of glyphosate refer to three deaths but not in clinical detail.[16,17]

In Japan, glyphosate is sold under the trade-name 'Roundup' (Monsanto Co, St Louis, MO, USA). The Japanese experience was summarized by Sawada[18,19] in a series of 56 cases with nine deaths. The Monsanto Company commented that it was comparable in safety to many toiletries and household products containing surface-active ingredients.[20] The Sawada series is the one referred to in most reviews.[21–23] Details of one death[16] and three survivors[24,25] have been reported, but other case reports (Table 8) have appeared only as abstracts,[26–31] and it is not clear how many of them are duplicated in the Sawada series.

The first case in the series was seen in 1980, one year after the introduction of Roundup to Taiwan, and there has been a dramatic increase in the number of cases since 1987. The results for the amount ingested (Table 4) suggest a dose-response relationship, but this needs to be investigated with more accurate identification of the dose by, for example, measurement of the plasma level of herbicide. Glyphosate and its

Table 8 Summary of reports from Japan. Amount – amount of Roundup ingested (ml); nr – amount ingested not recorded; HP – charcoal haemoperfusion; HD – haemodialysis; Ref – reference number. Where reports refer to the same patient, multiple references are shown.

Age	*Sex*	*Amount*	*Outcome*	*Ref.*	*Comment*
60	F	200	Dead	26, 27	HP, shock, coma, acidosis
73	M	100	Survived	23, 25, 28	endotracheal intubation
27	M	10	Survived	23, 25, 28	endotracheal intubation
36	M	100	Dead	29	hypotenstion, pulmonary oedema
52	F	280	Survived	30	HP pancreatitis, pulmonary oedema
64	F	nr	Survived	31	HD acidosis, hypotension
48	F	nr	Dead	31	acidosis, hypotension
31	M	50	Survived	31	sore throat, headache
72	F	30	Survived	31	diarrhoea, renal dysfunction
37	F	50	Survived	24	acidosis, creatinine increased

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2,13 The annual report of
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tically refer to it.[14] The US
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cidents involving glyphos-
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e accurate identification of
nple, measurement of the
icide. Glyphosate and its

p ingested (ml); nr –
haemodialysis; Ref –
efere s are shown.

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ubation
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pulmonary oedema
potension
nsion
dache
dysfunction
ine increased

major plant metabolite, aminomethyl phosphonic acid (AMPA), have been measured by gas chromatography,[32] high-performance liquid chromatography,[33,34] gas chromatography/mass spectrometry,[16] and nuclear magnetic resonance spectroscopy,[17] but none of those techniques are likely to be available in referring hospitals where most of the cases occur.

There was only one case of aspiration pneumonitis, and the outcome was favourable. Oral ingestion was not associated with 'aspiration pneumonia after suppression of the protective reflexes by alcohol consumption',[20] and the reference to unpublished records of 'many cases of survival after ingestion of quantities of roundup estimated to be several hundred millilitres'[20] is not supported by our findings.

The pharmacokinetics of glyphosate in man are unknown. Plasma glyphosate about 7 h after ingestion was 69.2 ppm in one case, but was undetectable (less than 10 ppm) by the second day and over the next 4 days.[24] In another case, a spot urine on arrival at the hospital 30 min after ingestion contained a high concentration of glyphosate (15,100 μg ml^{-1}), which had become undetectable (less than 2.5 μg ml^{-1}) by day 3.[25] In rats, a single oral dose was 15% (males) to 35–40% (females) absorbed, with a small amount of biliary excretion and enterohepatic circulation.[4]

Symptomatology in the present series agreed with the series from Japan,[18,19] although the incidence of individual effects varied. The lower incidence of GIT symptoms in our series may be due to under-reporting in the chart, as, until recently, the cases were considered very safe and received little attention after admission to hospital. Vomiting and diarrhoea were obscured by medical treatment, which included gastric lavage, emetics, and bowel washout in some cases. We have seen no cases of paralytic ileus. The mucosal ulceration documented by endoscopy, resembles that of other corrosive agents. Studies using the gastric mucosa of dogs showed that pure glyphosate, the surfactant contained in Roundup, and the Roundup formulation, all caused injuries similar to those made by 0.25 N HCl.[35–37]

The toxicity of the commercially available herbicide may be due to constituents other than glyphosate.[38,39] Sawada has proposed that the syndrome in fatal cases resembles that of toxicity due to the surface-active agent,[18,19,40] although other commercially available products containing surfactants are usually benign.[14] In beagle dogs using concentrations equivalent to those in Roundup, pure glyphosate caused increases in heart rate, cardiac output, and blood pressure.[41] The surfactant from Roundup, and the commercial Roundup preparation, both caused decreases in these parameters. All three agents caused increases in mean pulmonary artery pressure and both systemic and pulmonary vascular resistance. Sawada[19] suggested that the cardiovascular shock was due to hypovolaemia. In our series, haematocrit, blood urea nitrogen, and central venous pressure did not support hypovolaemia as the cause of the shock that developed after rehydration.

Uncoupling of mitochondrial oxidative phosphorylation has been suggested as one lesion in glyphosate poisoning,[42,43] but technical difficulties make the results difficult to interpret. There is variability in the mitochondrial State 4 respiration rates displayed, and they were then used to calculate respiratory control ratios. Moreover, the mitochondria recover after a single addition of ADP, in contrast to the result with dinitrophenol, which causes true uncoupling. In rats given glyphosate intragastrically for 2 weeks, glyphosate decreased the hepatic level of cytochrome P450 and monooxygenase activities, and the intestinal activity of aryl hydrocarbon hydroxylase.[44] The clinical significance of these biochemical abnormalities is unclear.

In conclusion, the toxic syndrome resulting from the massive ingestion of Roundup herbicide consists of mucosal and gastrointestinal irritation, hypotension, and pulmonary insufficiency. Effects on organs other than the gastrointestional tract tend to occur with increasing amounts ingested. The data suggest that those over 40 years of age, who ingest more than 100 ml, are at the highest risk of a fatal outcome.

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(Received 22 May 1990; accepted 31 July 1990)

From: J R Heylings
Biochemical Toxicology

To: L L Smith

Date: 29 March 88

Ref: JRH/SAB/21

Ext: [redacted]

Copies to: R C Scott

PREEGLOX L STUDY IN RATS: EFFECT OF SURFACTANT

We have just completed a toxicity study in the rat where two formulations of PREEGLOX L were compared. One PREEGLOX formulation (Y00061/134/001) was prepared as close as possible to the current manufactured product and contained both emetic and surfactants. The other PREEGLOX FORMULATION (Y00061/135/001) was formulated as close as possible to the above but was devoid of surfactants.

Following an overnight fast, four dose levels (20-100mg/kg) of each PREEGLOX formulation was administered orally by gavage to groups of 5 male rats. In all cases neat formulation was used and dose levels (calculated as mg/kg paraquat ion) varied by altering the volume of concentrate given. Animals were monitored for 10 days for signs of toxicity.

Results and Conclusions

The results for each PREEGLOX formulation are shown below. Interestingly, all the mortalities occurred within the first three days following dosing. Beyond this time all the surviving animals were normal. It is quite clear from this study that the PREEGLOX formulation containing surfactants is considerably more toxic to rats. Mortality data was transformed by logit fit and approximate LD_{50} values computed. The estimated LD_{50} values were 40 and 85mg PQ ion/kg for PREEGLOX with and without surfactant respectively.

MORTALITY FIGURES

Dose mg/kg PQ ion	PREEEGLOX L with surfactants	PREEGLOX L without surfactants
20	1/5	0/5
50	2/5	1/5
75	5/5	2/5
100	5/5	3/5
LD_{50}	40mg/kg	85mg/kg

Cont....

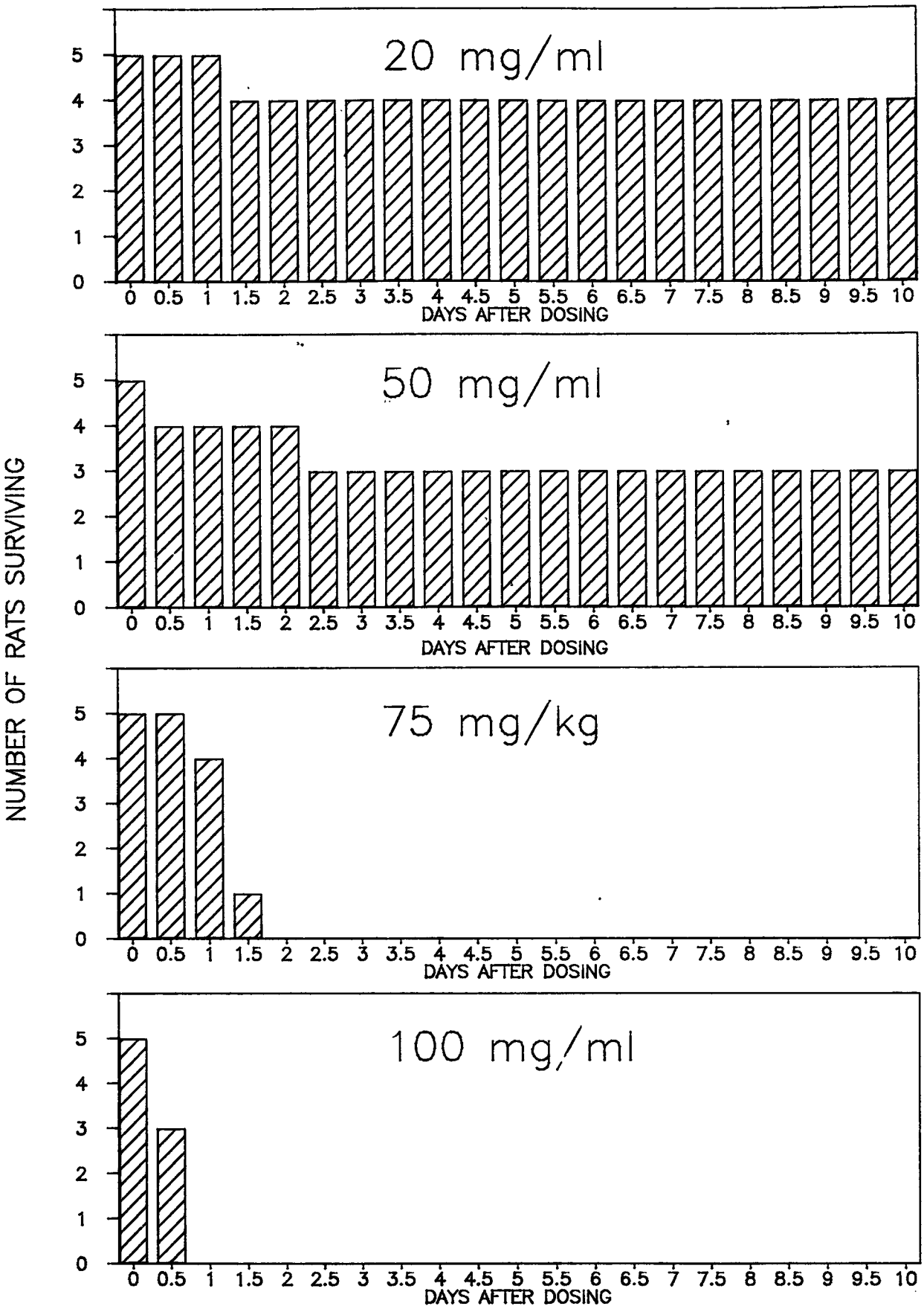
L L Smith 29 March 88

The results of this study are in agreement with a previous study carried out by Metabolism and Pharmacokinetics Section in 1986 (Report No CTL/L/1428) which demonstrated in rats that inclusion of surfactants in PREEGLOX L enhanced paraquat absorption, the amount of paraquat found in urine, and the overall toxicity to this species.

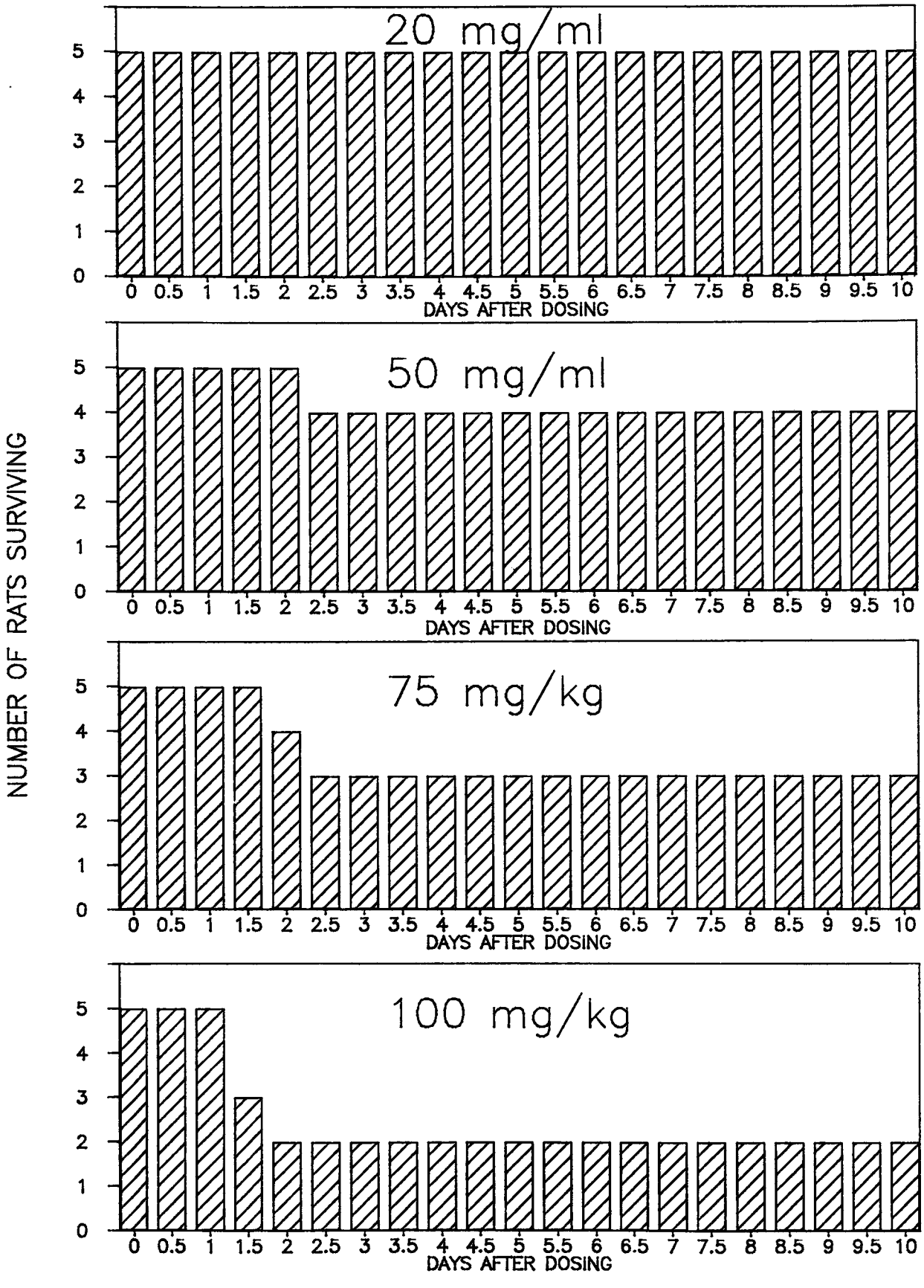
Although this phenomenum has not been observed in the dog, and therefore is unlikely to occur in man, I suggest a further study in rats to delineate the possible contribution of diquat to the enhanced toxicity observed with PREEGLOX L containing surfactants. I suggest a three way comparison between GRAMOXONE UK, GRAMOXONE S and GRAMOXONE W (all 20% PQ ion).

JON R HEYLINGS

PREEGLOX L WITH SURFACTANTS



PREEGLOX L WITHOUT SURFACTANTS



From: J R Heylings
Biochemical Toxicology

To: M A Collins
Toxicity

Date: 11 May 1987

Ref: JRH/LCM/07

Ext: Redacted - EU PII

Copies to: L L Smith
G H Pigott
R C Scott
I Wyatt
L Pinto

INTERIM REPORT MAY 11 1987

PARAQUAT - EFFECT OF SDS FORMULATION ON ABSORPTION IN DOGS.

CTL STUDY NUMBER: XD1252

PACT: 422

Nine dogs have been dosed on two separate occasions with either TAL GRAMOXONE (8mg/kg) or SDS PARAQUAT (50mg/kg).

In the first part of the study the effect of two dilutions of SDS were studied. In the second part fresh vs old SDS was compared along with dosing volumes. In addition, the amount of formulation lost by emesis was quantified.

SDS PART I (March 26, 1987)

The effect of two different strengths of SDS PARAQUAT were compared with TAL GRAMOXONE control group. Three dogs received a 1:10 dilution of SDS and three a 1:7 dilution of the SDS formulation both containing 50mg/kg paraquat ion. A further three animals received a standard TAL GRAMOXONE formulation (8mg/kg PQ ion).

RESULTS

(i) Clinical observations.

In the TAL GRAMOXONE group, two of the three animals vomited within the first 2 hours of dosing. These dogs were active and alert thereafter. All six animals receiving SDS PARAQUAT vomited within 80 minutes following gavage dosing. Some of these animals vomited later in the day and others had diarrhoea. On the following day all nine dogs were symptom-free and feeding normally.

(ii) Plasma Paraquat.

Plasma paraquat profiles are shown in Figure 1. The peak values as well as the AUC values were similar between groups. Thus, despite a six fold difference in dose level of paraquat ion there was no proportional increase in the amount of paraquat absorbed into the bloodstream. Furthermore, there were no differences between the two strengths of SDS PARAQUAT.

Cont....

M A Collins 11 May 87

SDS PART II (April 28, 1987)

In the second part of this study the doses were kept the same but this time the dose volumes were adjusted to 2.5ml/kg for all three groups. One of the SDS groups received freshly prepared formulation and the other a sample which had been prepared 2 weeks earlier. In addition, the volume and concentration of paraquat in vomit samples was recorded.

RESULTS

(i) Clinical Observations

In the TAL GRAMOXONE group (8mg/kg) only one animal vomited. All three dogs were active and alert thereafter. The six animals receiving SDS PARAQUAT (50mg/kg) all vomited and diarrhoea was apparent in some cases. Dog 604 remained subdued and died on day 6. The remaining eight animals showed no clinical signs of toxicity following the study.

(ii) Plasma Paraquat

Plasma paraquat profiles are shown in Figure 2. Peak values and AUC for the TAL GRAMOXONE group were very similar to those observed in Part I of the study despite the formulation being dosed at 28 times the total volume. Five of the six SDS-treated dogs showed similar results to Part I of the study. Dog 604 (which died on Day 6) was an exception. This animal absorbed more than ten times the amount of paraquat compared to the others, hence the higher mean values in group two. Essentially, there was no difference between fresh and old samples of SDS PARAQUAT when data from dog 604 is excluded.

(iii) Paraquat lost by Emesis

An important feature throughout this study with SDS PARAQUAT is that all dogs receiving the formulation have vomited. In Part II the volume of material lost by emesis was around 100ml (range 60-165ml). Following assay of paraquat in these samples it was found that on average about one half of the dose of SDS PARAQUAT was lost by emesis. Interestingly dog 604 only lost 25% of the dose given by emesis which is probably the major contributing factor to the sequelae observed with this animal.

Next Phase of the Study

Dog 604 has been replaced from our stock animals of equivalent age and body weight. Due to the importance of ascertaining the mechanism of reduced absorption of SDS PARAQUAT, it is proposed to investigate a 50mg/kg dose level of SDS PARAQUAT which contains no emetic.

DR J R HEYLINGS
Biochemical Toxicology

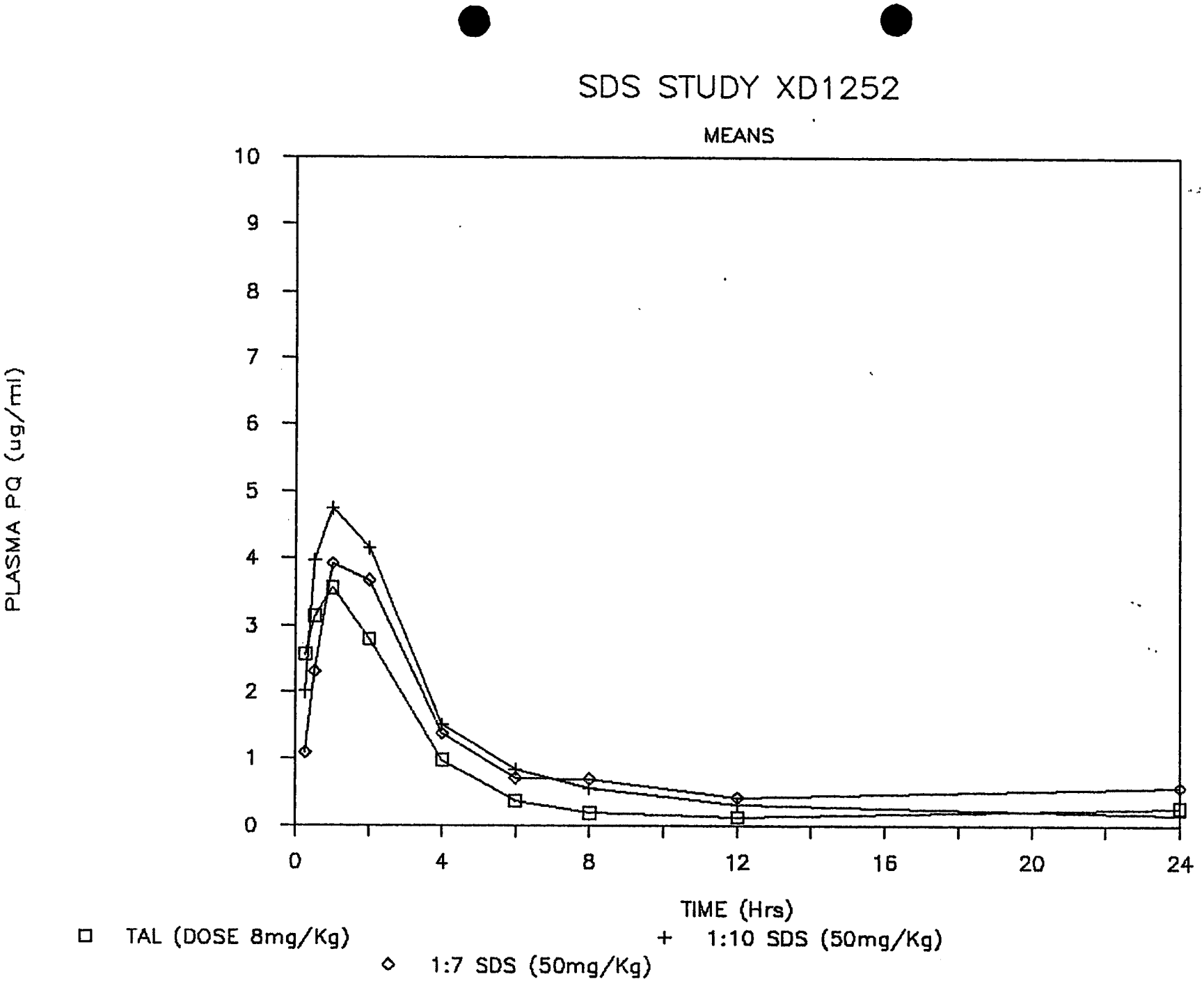

SDS STUDY XD1252
MEANS
PLASMA PQ (ug/ml)
10
9
8
7
6
5
4
3
2
1
0
0
4
8
12
16
20
24
TIME (Hrs)
TAL (DOSE 8mg/Kg)
1:10 SDS (50mg/Kg)
1:7 SDS (50mg/Kg)

FIGURE 1

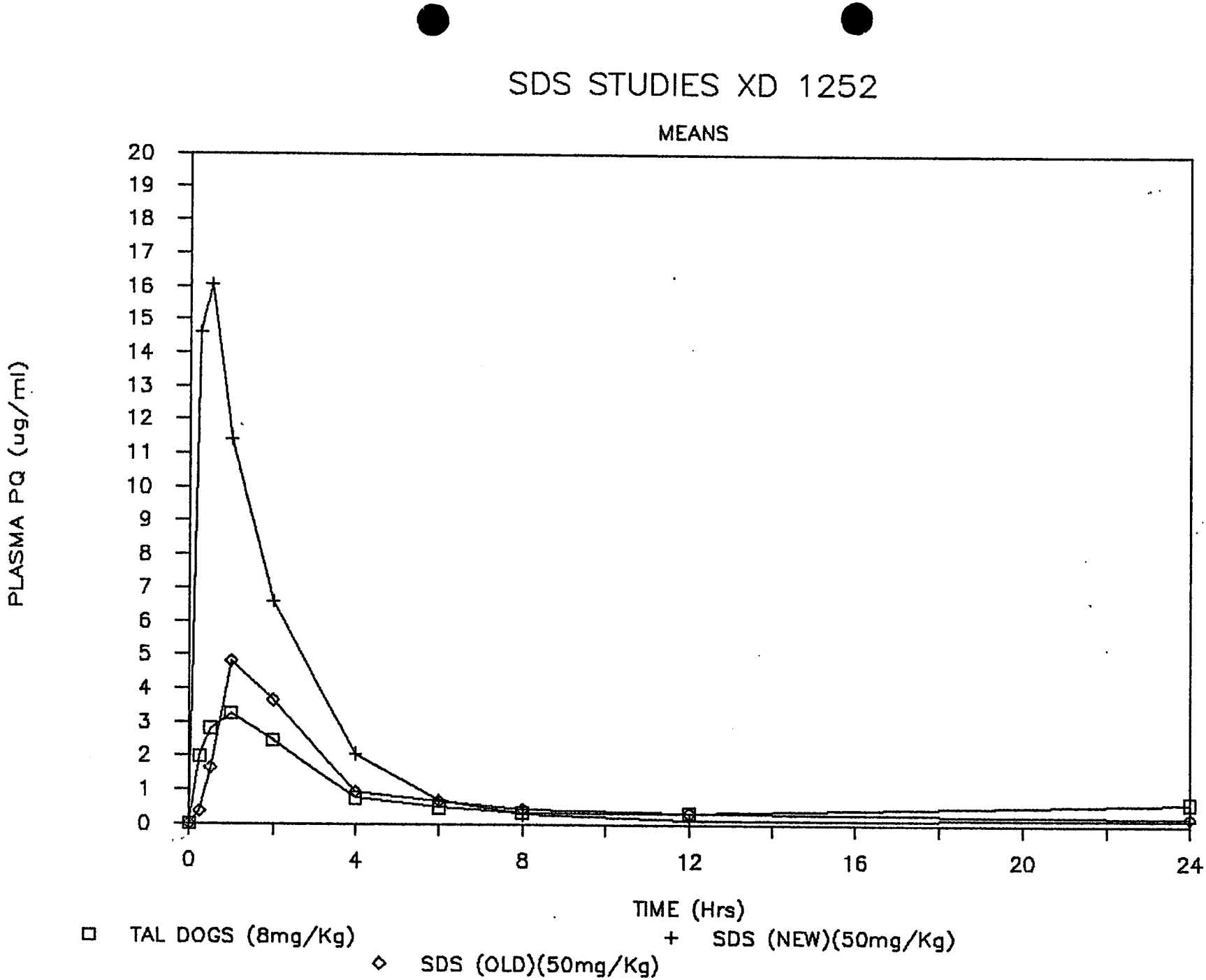

SDS STUDIES XD 1252
MEANS
PLASMA PQ (ug/ml)
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
4
8
12
16
20
24
TIME (Hrs)
TAL DOGS (8mg/Kg)
SDS (NEW)(50mg/Kg)
SDS (OLD)(50mg/Kg)

FIGURE 2

From: J R Heylings
Biochemical Toxicology

To: L L Smith

Date: 8 April 87

Ref: JRH/SAB/04

Ext: Redacted - EU PII

Copies to: I Wyatt
R C Scott

Dextran Sulphate Formulations

Objective

Dextran sulphate (DS) has been demonstrated to protect animals from paraquat toxicity when dosed subsequently to paraquat. We have investigated combinations of paraquat and dextran sulphate which may be useful as a safer paraquat formulation. Acute toxic effects in rats have been studied in addition to in vitro binding studies using a 5000 MW DS sample from Sigma.

Binding Studies

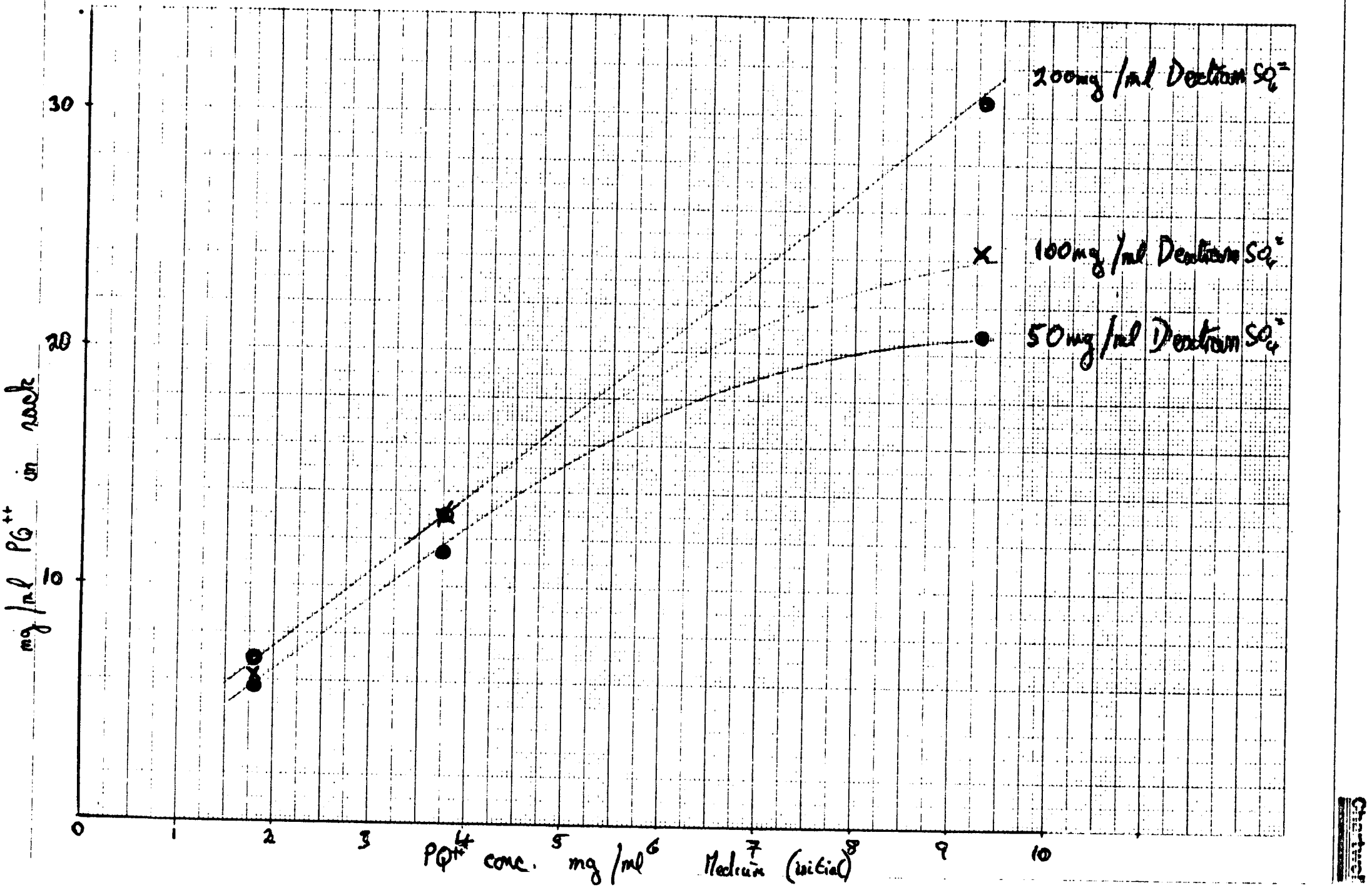
Dialysis experiments were carried out using membrane which excludes 2000 MW. DS in buffer (1ml) was contained inside the membrane and placed in 20ml buffer containing ^{14}C-paraquat. The system was left at 4°C overnight. The DS concentration was 50, 100 and 200mg/ml and the external paraquat concentration 2-10mg/ml PQ ion (10-50mM). DS bound a consistent amount of paraquat and this effect was linear until a ratio 10:1 to 20:1 DS to paraquat (w/w) was obtained. The ability to bind was saturable at this level (See Fig 1).

Rat Studies

Rats (n=5 per group) were dosed by gavage with combinations of DS and PQ. A dose 200mg/kg PQ ion was used in all cases. This is about twice the LD50 in this species. The ratios of DS:PQ used were 2:1, 4:1 and 8:1 (w/w). Animals were monitored for 10 days prior to termination. At a dose ratio of 2:1 only two of five animals survived. At 4:1 all but one animal survived the full time course and at 8:1 all the animals survived ten days. It therefore appears that dose ratios in excess of 4:1 (DS:PQ) are necessary to afford protection against paraquat toxicity in the rat.

DR J R HEYLINGS
Biochemical Toxicology

Figure 1. Binding of Dextran Sulphate and Paraquat in vitro



From: J R Heylings
Biochemical Toxicology

To: M A Collins
Toxicity

Date: 1 April 87

Ref: JRH/SAB/04

Ext: Redacted - EU PII

Copies to: L L Smith
G H Pigott
R C Scott
I Wyatt
L Pinto

INTERIM REPORT MARCH 30, 1987

PARAQUAT - EFFECT OF FORMULATION ON ABSORPTION IN DOGS.

CTL STUDY NUMBER: XD1236

PACT: 404

Nine dogs were dosed with paraquat formulations containing 8mg/kg paraquat ion. One group of 3 animals received TAL GRAMOXONE. The other two groups received the formulation B246 and E471. The emulsion formulations each contained 5% of primary emulsifier and were formulated with soya bean oil (B246) and isopar oil (E471) (See Appendix 1). Experimental numbers and groups are shown in Appendix 2.

RESULTS

(i) Clinical Observations
Following oral dosing all nine dogs remained active and alert. One of the TAL GRAMOXONE dogs vomited at 15 and 26 minutes and had green mucoid faeces at 70 minutes. One of the B246 dogs was slightly subdued at 1-2 hours but otherwise normal. One of the E471 dogs vomited at 90 minutes and had diarrhoea at 12 hours. On the following day all nine dogs were feeding normally and were free of symptoms.

(ii) Plasma Paraquat Profiles
Plasma paraquat concentrations were determined by Radioimmunoassay and by HPLC. Both methods gave essentially similar results (Fig 1 and 2). In both cases paraquat was absorbed to a greater extent following TAL GRAMOXONE compared with the multi-emulsions B246 and E471. Peak plasma levels and area under the curve (AUC) were calculated for each dog (see Table). Using peak height as an indicator of absorbed paraquat, the two emulsions appear similar and about one half of the value of TAL GRAMOXONE. On analysis of AUC, formulation B246 offers the greater safening compared to TAL GRAMOXONE. In all cases the peak plasma level occurred around 1-2 hours after dosing. Interestingly, the dog which vomited in the TAL GRAMOXONE group had the highest plasma paraquat levels and the plasma concentration at 15 min after dosing was the peak value for that animal.

Cont...

M A Collins

30 March 1987

Next Phase of the Study

The two emulsions B246 and E471 offered around a two fold sefening in likely paraquat absorption over TAL GRAMOXONE. It is therefore proposed to increase the dose of paraquat with these formulations to levels equivalent to that observed with 8mg/kg TAL GRAMOXONE. The dose of TAL GRAMOXONE used in the next phase will remain at 8mg/kg to investigate intra-animal variation.

DR J R HEYLINGS
Biochemical Toxicology

PARAQUAT: EFFECT OF FORMULATION ON ABSOPRTION IN DOGS.

CTL STUDY NUMBER: XD1236

APPENDIX 1

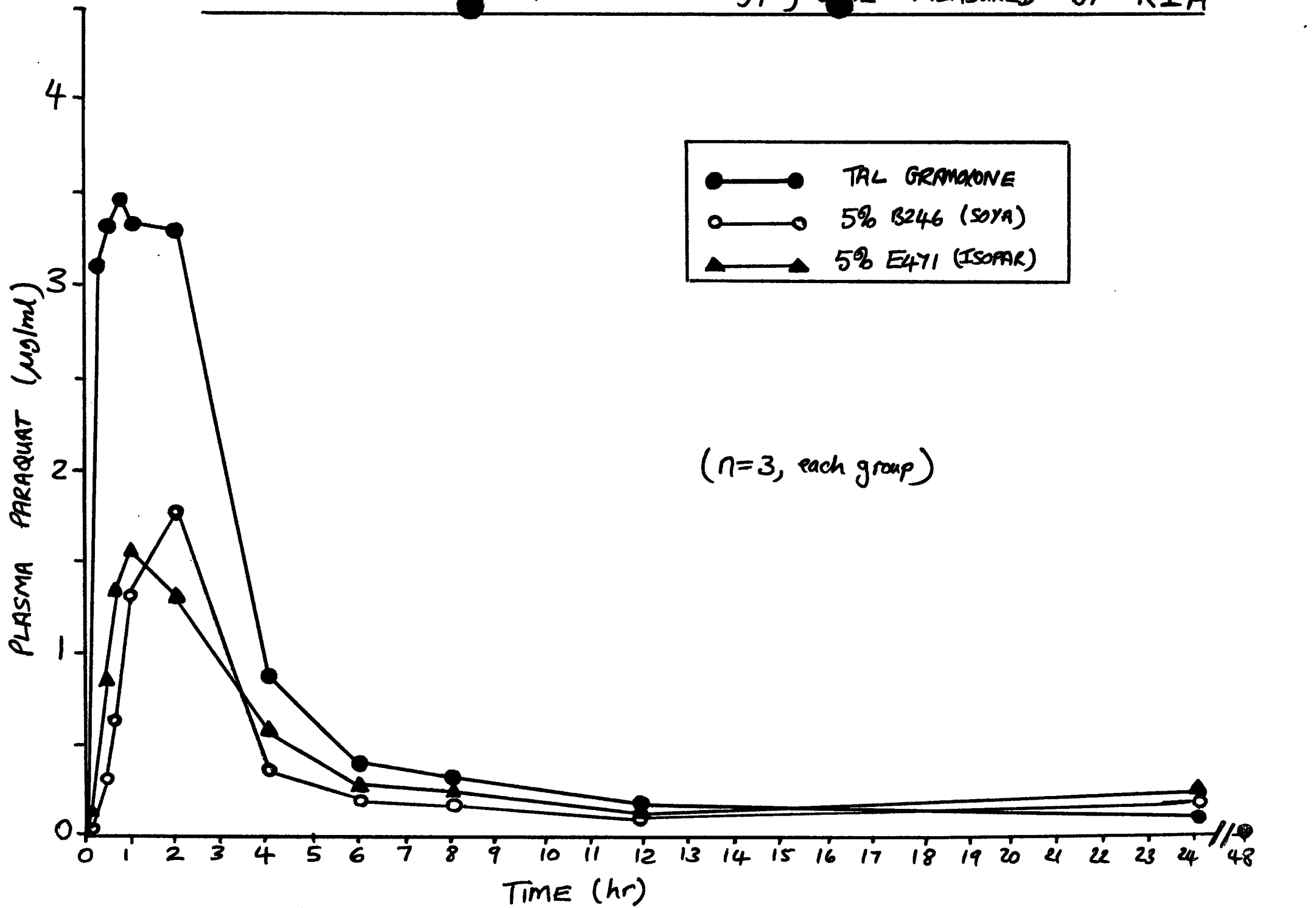
Identification of Formulations Used.

Group	Formulation	Dose mg/kg PQ ion	Dose Volume ml/kg
1	TAL GRAMOXONE	8	0.088
2	5% B246 (SOYA)	8	0.085
3	5% E471 (ISOPAR)	8	0.085

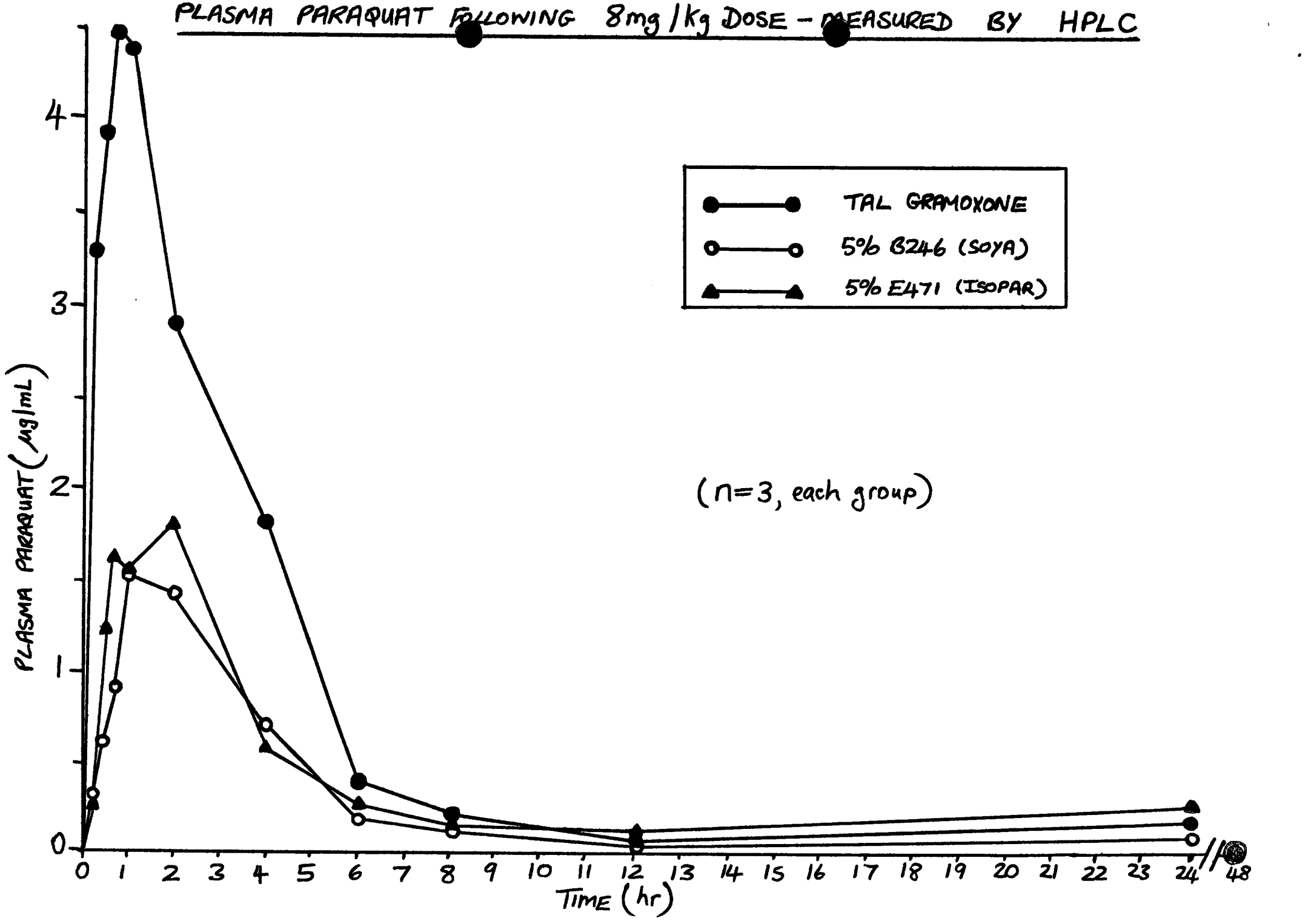
APPENDIX 2

Animal Details, Unique and Experimental Numbers and Housing Layout.

Rep	Unique No	Exp No	Group	Cage No	Pen
1	1339/86	504	2	1	114
	1338/86	501	1	2	113
	1340/86	507	3	3	112
2	1277/86	505	2	4	111
	1279/86	502	1	5	110
	1280/86	508	3	6	109
3	1306/86	509	3	7	108
	1309/86	503	1	8	107
	1308/86	506	2	9	106



SYNG-PQ-03709730_R

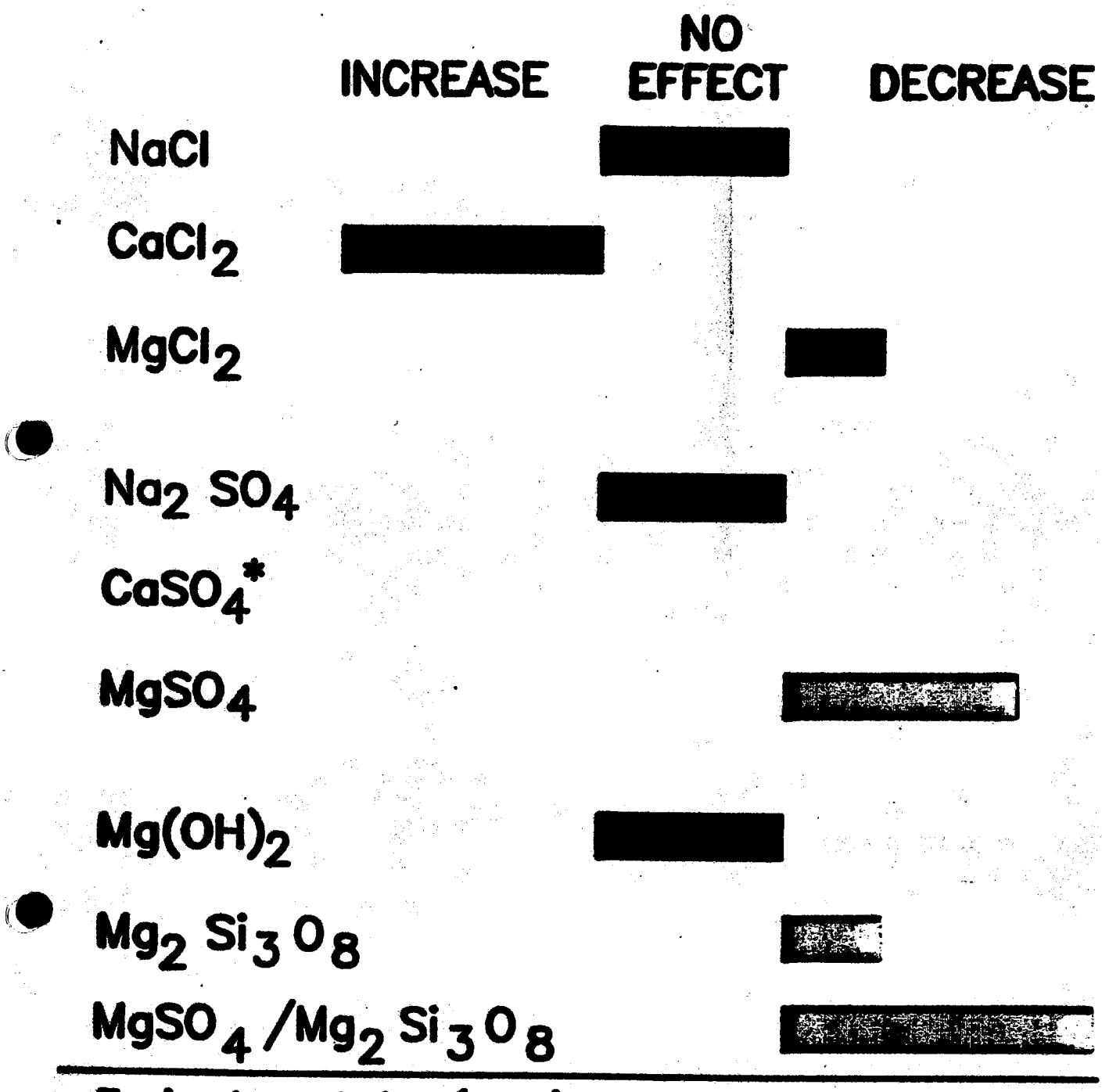

PLASMA PARAQUAT FOLLOWING 8mg/kg DOSE - MEASURED BY HPLC
TAL GRAMOXONE
5% B246 (SOYA)
5% E471 (ISOPAR)
(n=3, each group)
PLASMA PARAQUAT (µg/mL)
TIME (hr)
0
1
2
3
4
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 48

PLASMA PARAQUAT CONCENTRATION AS MEASURED BY RADIOIMMUNOASSAY

	DOG	PEAK PLASMA PARAQUAT (µG/ML)	AUC (µG/ML/HR)
	501	3.45 (2HR)	13.11
TAL	502	3.25 (2HR)	13.60
	503	7.50 (15MIN)	22.02
	MEAN	4.73	16.24 (15.33)*
	504	0.99 (1HR)	4.27
B246	505	3.25 (2HR)	11.53
	506	1.48 (2HR)	12.64
	MEAN	1.91	9.48 (9.26)*
	507	1.68 (1HR)	18.19
E471	508	1.90 (1HR)	10.30
	509	1.29 (2HR)	8.23
	MEAN	1.62	12.24 (12.83)*

(* CALCULATED AUC FROM HPLC ANALYSIS)

EFFECT OF VARIOUS ELECTROLYTES ON THE ORAL TOXICITY OF PARAQUAT IN RATS

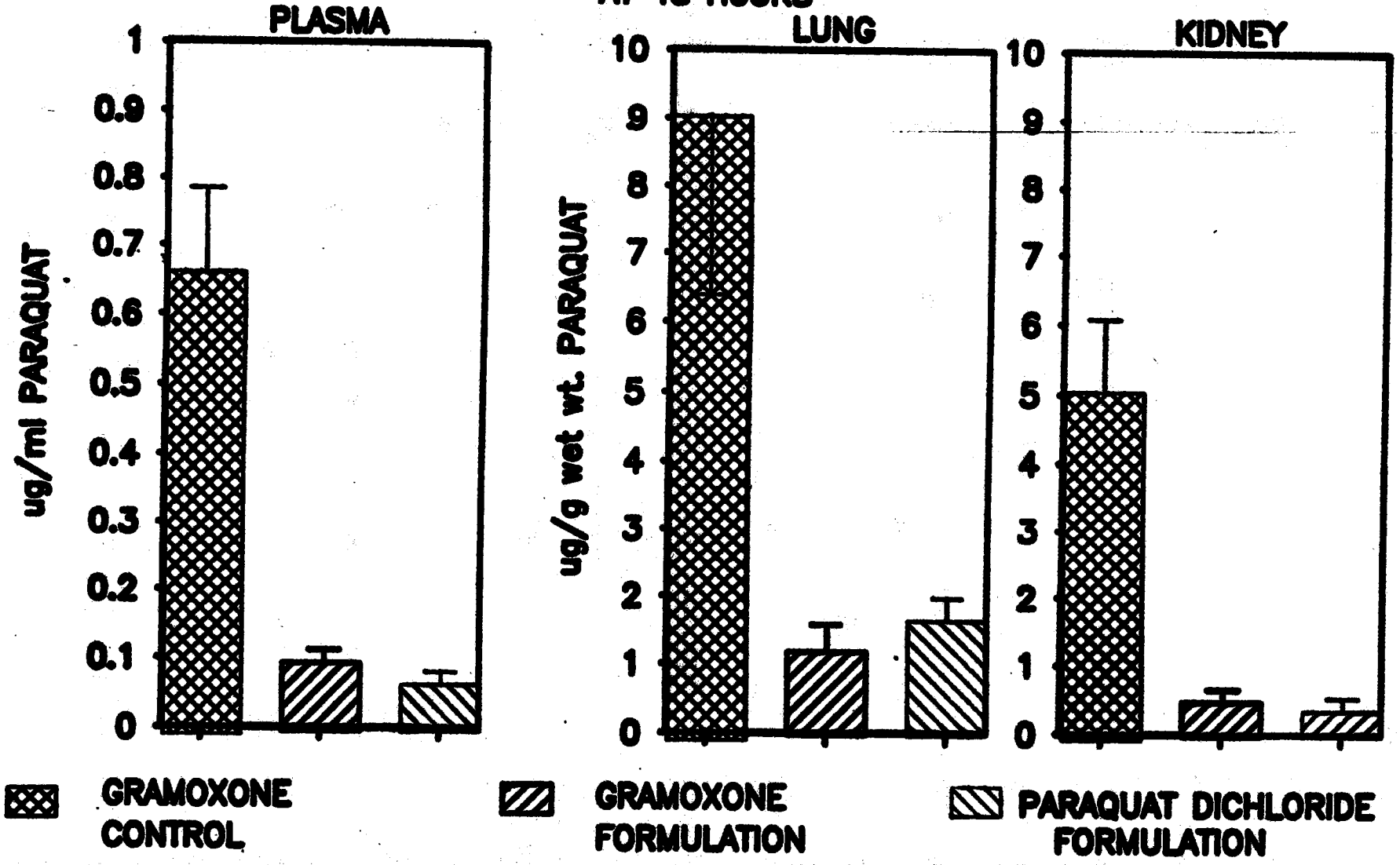


Equimolar solutions(1.5M) as additives to GRAMOXONE Final concentration 100g/l PQ^{2+}; dosed at 1ml/kg. at 100, 150 and 200mg PQ^{2+}/kg

* Insoluble

ABSORPTION OF PARAQUAT IN THE RAT

A COMPARISON OF GRAMOXONE WITH PARAQUAT DICHLORIDE FORMULATED WITH 1.5M $MgSO_4$ AND 1.5M MAGNESIUM TRISILICATE AT 200 mg/kg AT 48 HOURS



DEVELOPMENT OF A SAFER FORMULATION OF PARAQUAT

Toxicological objective : in 1987

To develop a new formulation of paraquat which is less well absorbed from the Gastrointestinal tract. Target is a 10 X improvement in oral toxicity in man compared to GRAMOXONE.

1. **Slow release formulation by EMULSIFICATION** — **CTL/Jealott's Hill**

2. **More rapid removal by EMESIS** — **CTL**

3. **More rapid removal by PURGATION** — **CTL**

TKC March 1989

Copy to C.L.S.

TESTING STRATEGY OF MULTIPLE EMULSION FORMULATIONS OF PARAQUAT

FORMULATION RESEARCH (PPD)

Formulation stability / leakage

Microscopy
Centrifugation
Dialysis

BIOCHEMICAL TOXICOLOGY (CTL)

Rat study

Ten day study following toxicity of LD50 x 2 oral dose in 5 animals

Dog study

Plasma profile, AUC, clinical signs of toxicity in 3 animals at sub LD50 oral doses
c.f. Gramoxone

Repeat Dog study

Increase dose in proportion to shift in AUC: 8, 16, 32, 48, 64, 80mg/kg
Full pharmacokinetic profile

WEED SCIENCE (PPD)

Biossay

Dispersibility
Sprayability
Phytotoxicity in 4 species

Herbicidal activity

Further sprayability tests
Full scale herbicidal trials

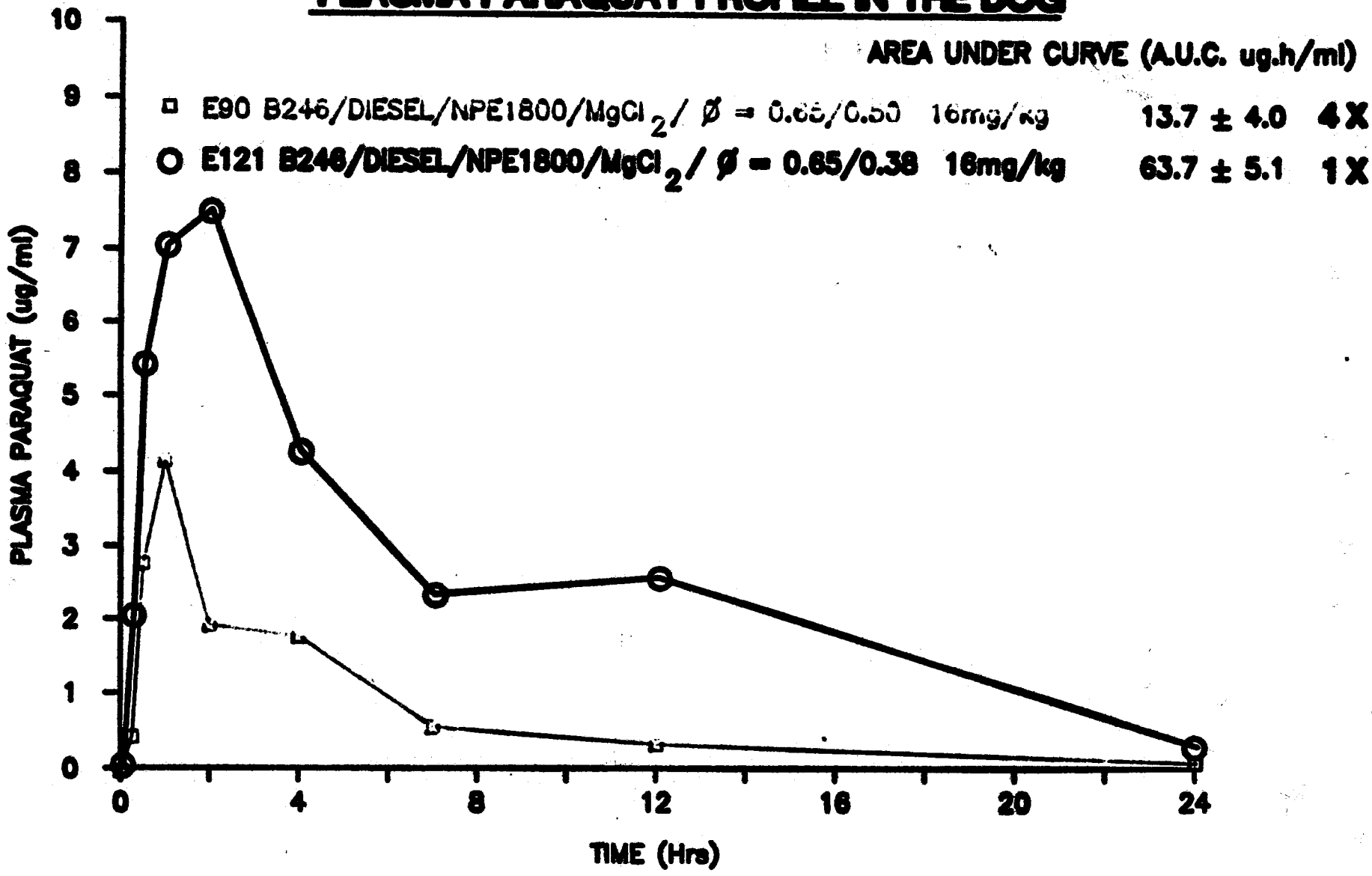
Field Trials

UK and other territories
Stability tests
Larger scale production

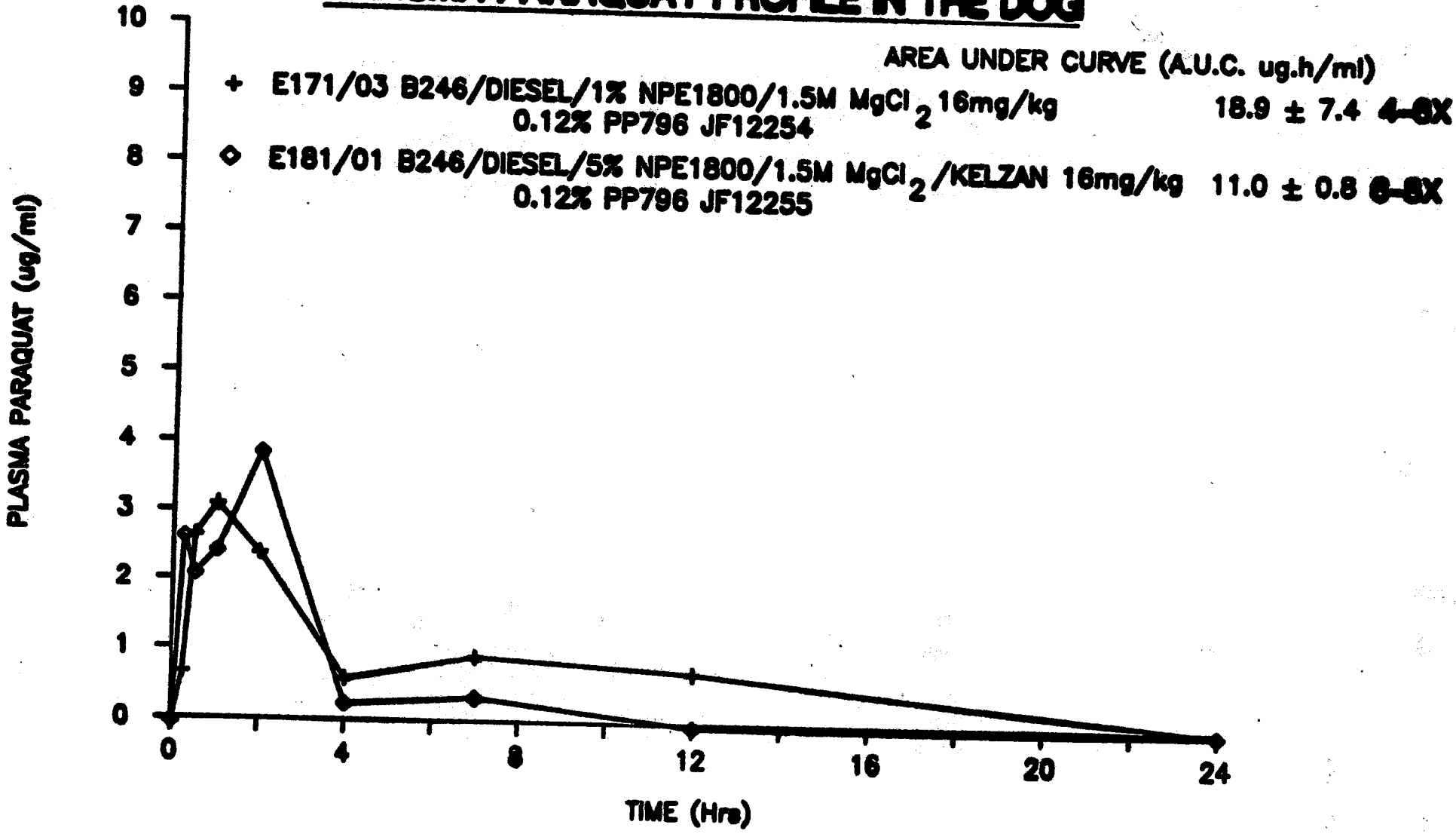
(FORMULATION SELECTION)

(FORMULATION SELECTION)

PLASMA PARAQUAT PROFILE IN THE DOG

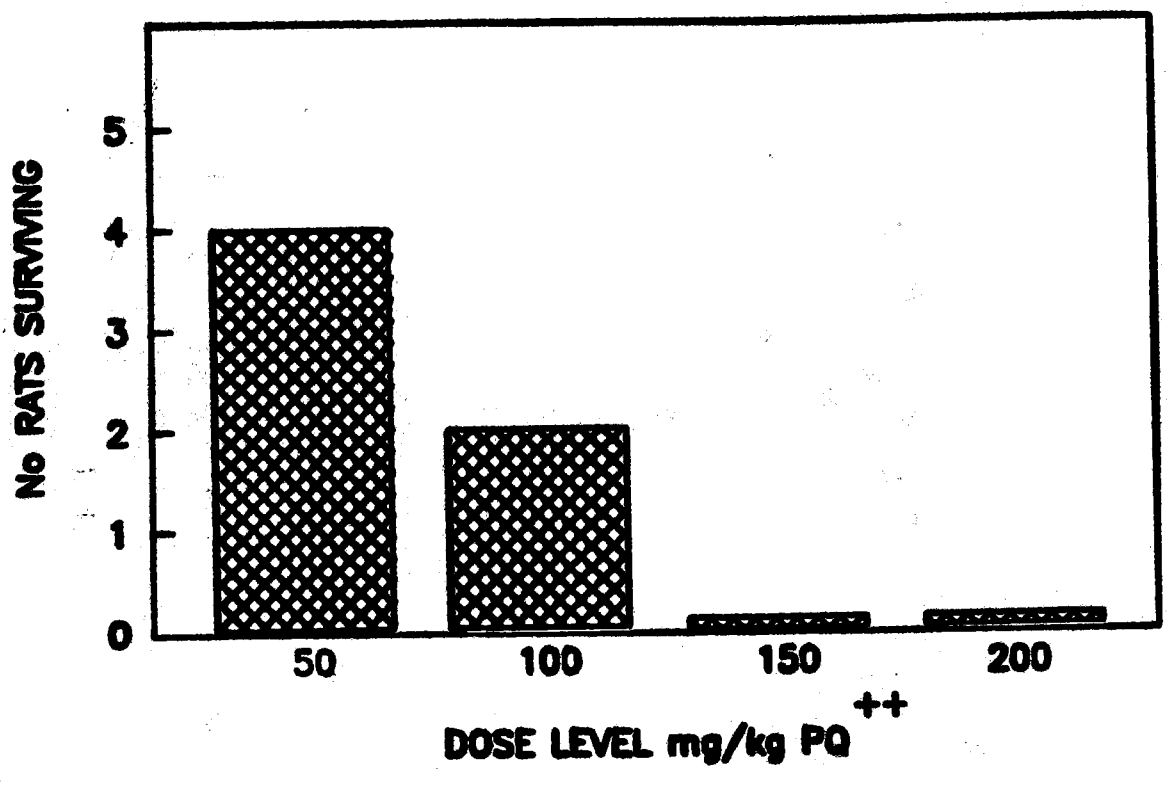


PLASMA PARAQUAT PROFILE IN THE DOG

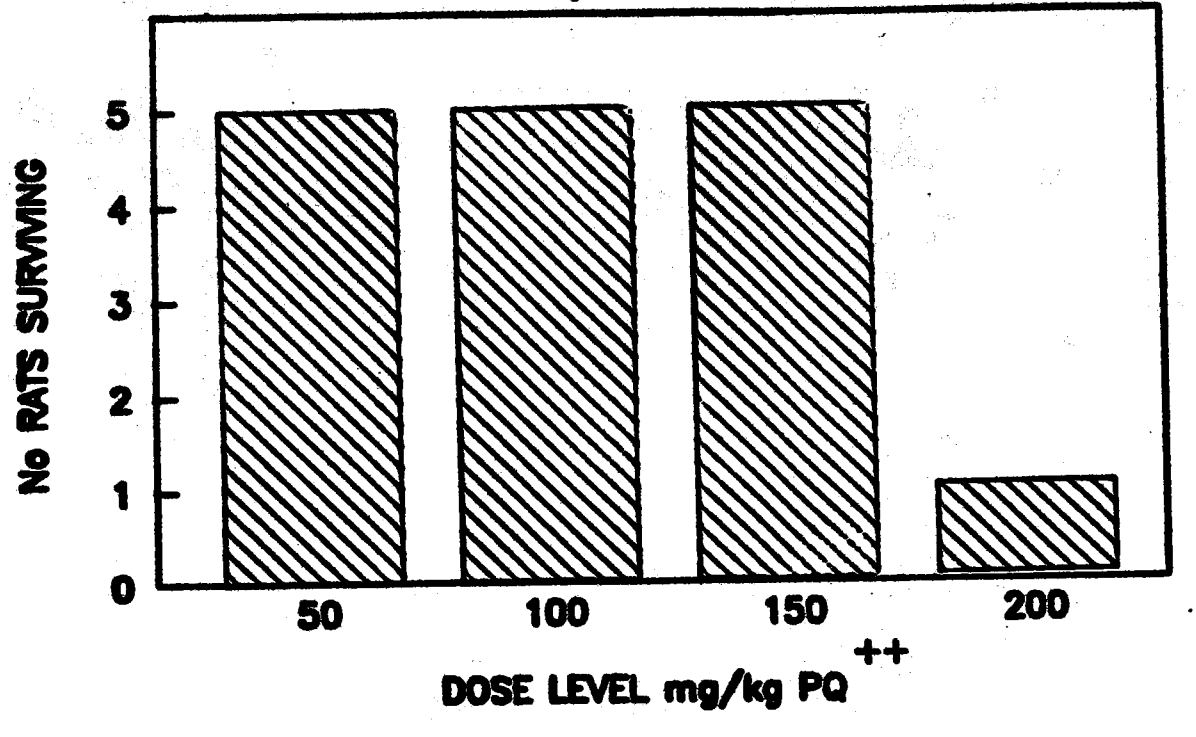


RAT SURVIVAL FOLLOWING AN ORAL DOSE OF PARAQUAT

GRAMOXONE + 0.12% PP796



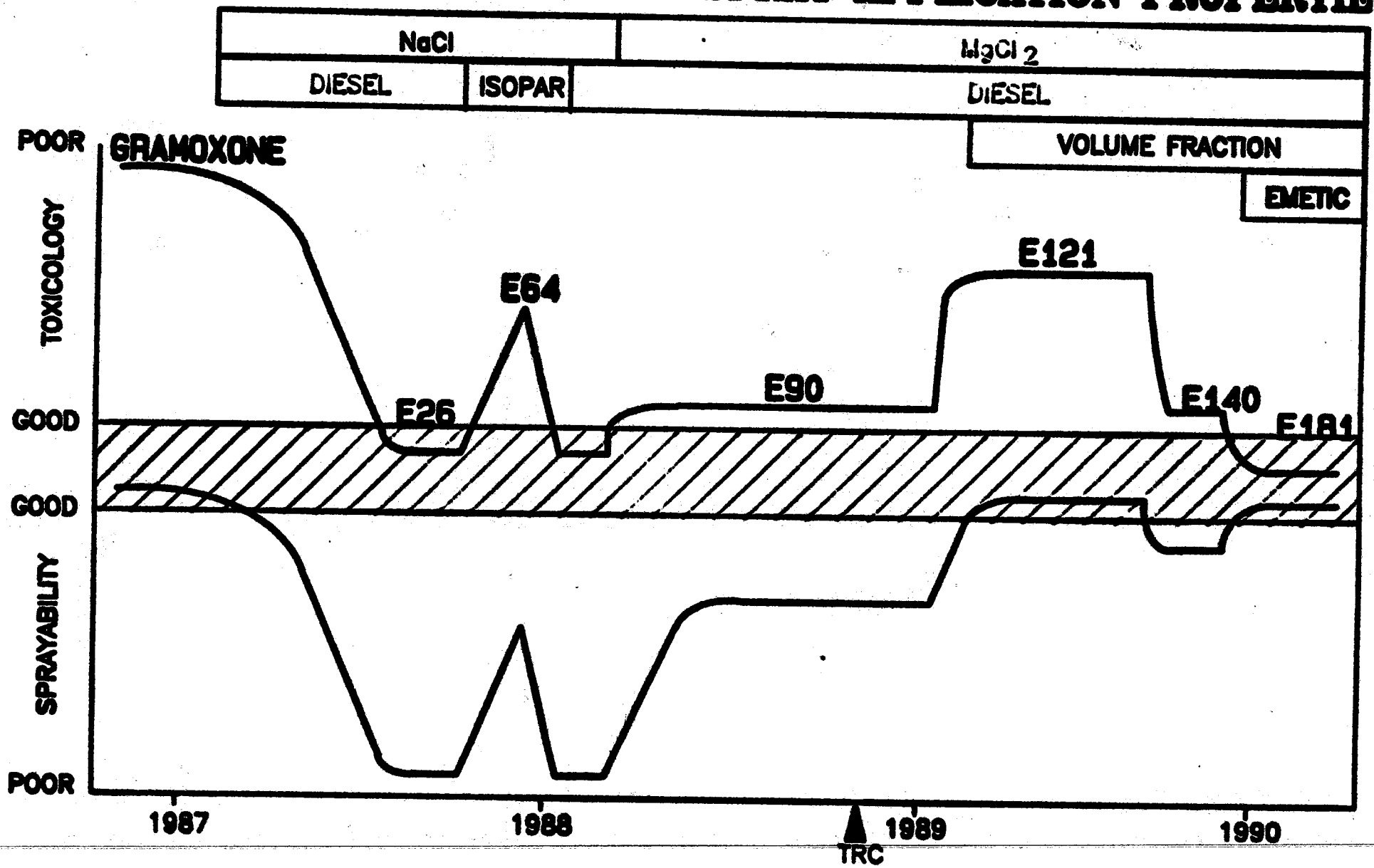
EMULSION 181/01 + 0.12% PP796 (JF12255)



MULTIPLE EMULSION PROGRAMME 1987 - 1990

EMULSION FORMULATION	AUC at 16mg/kg mean ± SEM, n=3 ug.h/ml	Safety Factor	Sprayability
GRAMOXONE	60 – 80	1X	V. Good
E26 1987	14.2 ± 3.0	6X	Poor
E64 1987	31.7 ± 1.0	2X	Fair
E82 1988	24.4 ± 0.2	3X	Fair
E90 1988	13.7 ± 4.0	5X	Fair
E121 1989	63.7 ± 7.1	1X	V. Good
E140 1989	28.2 ± 3.3	4X	Good
E171 1990 (JF12254)	18.9 ± 7.4	(3X)	V. Good
E181 1990 (JF12255)	11.0 ± 0.8	(5X)	V. Good

MULTIPLE EMULSION PROGRAMME (1987 –1990)
OPTIMIZATION OF SAFENING AND SPRAY APPLICATION PROPERTIES



Harry Swaine

TOXICOLOGY OF MULTIPLE EMULSION FORMULATIONS OF PARAQUAT

Introduction and Objectives

Paraquat is a potent contact herbicide that is potentially lethal to man if ingested. Once a critical plasma concentration is exceeded, active accumulation of paraquat in the lung occurs and death caused by pulmonary failure may result. There is no effective antidote for paraquat poisoning and measures designed to enhance the elimination of paraquat from the body have not proven satisfactory. Over the last three years we have directed paraquat research towards reducing the absorption of the bipyridyl herbicide from the gastrointestinal tract. A workgroup was established in 1986 between ICI Agrochemicals and CTL to investigate safer formulations of paraquat. The majority of this research has centred on the toxicology of Multiple Emulsion formulations which contain 100g/l paraquat ion. Emulsified paraquat reduces the bioavailability of the herbicide following an oral dose.

Over the last three years at CTL we have assessed the acute toxicity of more than 300 Emulsion formulations of paraquat in the rat. This includes around 200 different compositions plus various batches of formulations prepared by different processes. Certain Emulsions eg E26, E90, E121 and E140 have been studied in detail in dogs, a species which closely resembles man in terms of paraquat absorption and toxicity. Our effort during the last 12 months has been centred on the major formulation and process variables which affect both the toxicology and the sprayability of the Multiple Emulsion formulation. Our goal still remains to provide a formulation which clearly demonstrates a minimum of an intrinsic 5 fold reduction in oral toxicity compared to an equivalent aqueous GRAMOXONE concentrate. Since GRAMOXONE contains 200g/l paraquat, development of a 100g/l Emulsion formulation will hopefully result in an overall 10 fold reduction in oral toxicity.

In addition to the Emulsion research, a basic research programme on paraquat absorption is also being conducted at CTL. One objective of this research is to study the mechanism by which paraquat enters the bloodstream from the gastrointestinal tract. Furthermore, by gaining detailed knowledge on the site and kinetics of paraquat absorption in different species, current therapeutic approaches to paraquat poisoning may be improved.

Evaluation of New Paraquat Emulsion Formulations

Rodent Studies

All new Emulsion formulations are tested in rats before any dog studies are undertaken. In rats, the median lethal dose (MLD) for GRAMOXONE is about 90mg/kg paraquat ion. A minimum of a 2-fold safety factor with a new formulation is our minimum criteria to further investigate a new formulation in dogs. From experience, we have set our dose levels in rats within the 150-250mg/kg range where dose level represents the mg of paraquat ion in the 10% (or 100g/l) Emulsion formulation per kg bodyweight. Neat concentrate is dosed orally by gavage to five male rats per dose level. Clinical observations are carefully monitored for 10 days. Formulations which are non-toxic to rats at twice the lethal dose level of GRAMOXONE are deemed to be acceptable for further study. During the programme approximately 10% of Emulsion formulations have proceeded to dog studies for further evaluation.

Dog Studies

Paraquat toxicity in man closely resembles that in dogs. The principal reasons for this are the similarities in absorption, distribution and excretion of the bipyridyl following oral administration. Careful selection of Emulsions for dog studies is required in order to assess the toxicity of systems which not only have good intrinsic safening in rats but also have a high likelihood of being dispersible and sprayable in herbicidal trials. Our overall strategy is to develop not only a safer formulation of paraquat but also to ensure that there is a good likelihood of such a formulation becoming a successful product in terms of its spray characteristics and herbicidal efficacy. Following regular discussions between CTL and the Formulation Section, Jealott's Hill, about 30 different Emulsions have progressed to the dog during the course of the Emulsion programme. Our strategy in the dog studies is to intially test at a calculated sub-lethal dose of paraquat Emulsion. This is given orally by capsule as a neat concentrate to 3 dogs. A full plasma paraquat profile over 24 hours is then obtained and clinical signs monitored throughout. Total area-under-curve (AUC) is calculated and a mean value from three

dogs obtained. Dose levels are increased from 8, 16, 24, 32, 48 and 64mg/kg sequentially in separate studies until the AUC for a particular Emulsion formulation equates with a standard sub-lethal GRAMOXONE AUC profile for the same dogs. Thus, an estimate can be made as to the safety factor for any given Emulsion formulation. Our target is a minimum of an intrinsic 5X safety factor over GRAMOXONE in dogs.

Progress from 1987-1990

By the end of 1987 we had identified a Multiple Emulsion formulation which had an intrinsic safety factor in the dog of 6X. This formulation; E26 (B246/Diesel/NPE 1800/NaCl) would not disperse well in water on dilution and this resulted in spray problems. Extensive studies with different oils, eg Isopar M, demonstrated improved dispersibility but reduction of safening in both rat and dog was invariably the result when diesel oil was replaced for the paraffinic Isopar M.

A breakthrough occurred during 1988 when we compared the properties of Emulsions containing different cations in the external phase. Substitution of NaCl for the divalent $CaCl_2$ or $MgCl_2$ not only improved dispersibility of the Emulsion, but also gave important information on the mechanism of gastrointestinal absorption of paraquat. The presence of calcium salts in Emulsions or GRAMOXONE enhanced the toxicity of paraquat. Conversely, magnesium salts, which competitively inhibit certain calcium-dependent processes in cells, caused a reduction in absorption and toxicity of the herbicide. Such a formulation as E90 (B246/Diesel/NPE 1800/$MgCl_2$) gave a clear 5X safety factor over conventional GRAMOXONE in dogs and also had improved dispersibility properties over the NaCl-containing E26. Field trial data and toxicology of E90 was presented at the TRC meeting in October 1988. This Emulsion had acceptable herbicidal properties but caused some flocculation problems and was not seen as an ideal candidate for further development.

The majority of our effort at CTL during 1989 focussed on the identification of an Emulsion which has even better spray properties than E90. A critical factor was found to be the volume fraction of the system. Reduction of the diesel oil in E90 gave rise to E121 which had improved

spray characteristics and lower flocculation. Unfortunately E121 gave an insufficient margin of safety. Despite extensive examination of potential process variables, E121 could not surpass the 2X safety factor in dogs (Figure 1). These studies reinforced the requirement for a minimum amount of diesel oil in the system to ensure a better toxicological profile.

Other methods for reducing flocculation were investigated during the latter half of 1989. In particular, E140 which maintains the 'safe' factors of system E90 in terms of volume fraction and magnesium content, but also contains polyvinyl alcohol (PVA) which reduced post-dilution flocculation. Our first example of system E140 gave a 4X safety factor in dogs (Figure 2). Subsequent batches of this Emulsion have given different degrees of safening and sprayability when prepared by different processes. Fortunately, safening and sprayability were not paradoxically related with this formulation. Emulsion E140 has an MLD in rats of 250mg/kg. In the dog only mild clinical observations were observed at 32mg/kg. Plasma paraquat profiles for E140 in dogs dosed at 8, 16 and 32mg/kg did not exceed a standard AUC for GRAMOXONE at 8mg/kg. The predicted MLD in dogs is 48mg/kg based on extrapolation of the AUC curve. This represents a 4X safety factor over GRAMOXONE. Thus, batches of this Emulsion which have both adequate safening and Field trial acceptability have been produced.

Toxicology of Multiple Emulsions E171 and E181

By the end of 1989 we had identified the major formulation factors in Multiple Emulsions which both reduce the intrinsic toxicity of paraquat and also those factors which caused flocculation and poor sprayability. We decided therefore to choose two of our Emulsion formulations which were felt to have a good probability of success as herbicide products, and to fully evaluate the toxicology of these Emulsions in rats and dogs. Emulsions 171 and 181 both contain the polymers B246 and NPE 1800, Diesel oil and $MgCl_2$ in the external water phase. The difference between them is that E181 contains 10% NPE 1800 and 0.1% Kelzan gel. E171 contains 1% NPE 1800 and no Kelzan. We also included emetic in these two formulations. During 1989, we examined whether inclusion of the emetic

(PP796) would interfere with the Emulsion process in any way as we move closer towards a commercially viable product which would contain safeners. We found that the emetic (0.12% w/v) in a 100g/l Multiple Emulsion formulation of paraquat had no effect on the emulsification process or the toxicity of paraquat Emulsion formulations in rats. Indeed, since the emetic partitions into oil well, it is possible that it will be delivered to the absorptive sites of the intestine at a faster rate than the paraquat which is retained inside the Emulsion droplets. Emulsions 171 and 181 were compared directly with a 100g/l GRAMOXONE formulation containing an identical concentration of emetic (0.12%). Thus, the intrinsic safening of Emulsion could be compared directly with GRAMOXONE under conditions of equal volumes of dosing solution and equal concentrations of both paraquat and emetic.

Rodent Studies

As shown in Figure 3, the rat survival profile following a single oral dose of paraquat as GRAMOXONE compared to paraquat as Emulsion were quite different. The median lethal dose (MLD) for GRAMOXONE was between 50 and 100mg/kg, which is in agreement with previous data. In contrast, the MLD for both Emulsion 171 and 181 was >150mg/kg. All animals received identical doses of paraquat ion and emetic. Rats have no vomit centre in the brain and as a consequence cannot remove the herbicide via emesis. This study clearly demonstrates that both Emulsion 171 and 181 have an intrinsic safening over GRAMOXONE which exceeds 2-fold in the rat. Further work is in progress at higher dose levels in order to determine the actual MLD of these Emulsion formulations in the rat.

Dog Studies

During the course of the Emulsion programme the vast majority of successes and failures of novel Emulsion formulations of paraquat have been determined at a dose level of 16mg/kg in dogs. This dose of paraquat is lethal to dogs with commercial aqueous concentrates of paraquat such as GRAMOXONE, GRAMOXONE L and PREEGLOX. Comparison of the plasma paraquat profiles at this dose level usually gives quite accurate predictions whether or not a new formulation will achieve the necessary safety margin

of 5X over GRAMOXONE. Since a minimum of a 2X safety margin had already been achieved in rats with Emulsions E171 and E181, we decided to omit the 8mg/kg dose in dogs and to proceed directly with an oral dose of 16mg/kg with these two Emulsions.

As shown in Figure 4, the GRAMOXONE treated group absorbed a significantly greater amount of paraquat from the gastrointestinal tract compared to Emulsion 181. The mean AUC for GRAMOXONE was 18.7 ± 4.7µg.h/ml, n=3. All peak paraquat plasma levels were higher in the GRAMOXONE group. All 9 dogs did vomit following dosing but the time to vomit was significantly delayed and more variable with GRAMOXONE compared to Emulsion 181. The mean time to first vomiting was 19 ± 4 minutes for GRAMOXONE. Dogs treated with Emulsion 171 had a very similar plasma paraquat AUC (mean 18.9 ± 7.4µg.h/ml, n=3) compared to GRAMOXONE. All animals had vomited within 20 minutes (mean time = 15 ± 3 min). Dogs dosed with E171 displayed few clinical signs and were normal by 24 hours. Emulsion 181 gave a very promising result. The plasma paraquat AUC for Emulsion 181 was very low (11.0 ± 0.8µg.h/ml, n=3). This represents a significant reduction in paraquat absorption compared to the GRAMOXONE group. Peak plasma paraquat values were also very low for this dose level and paraquat levels had returned to baseline within a 4 hours of dosing. All dogs dosed with E181 vomited within 10 minutes of dosing (mean time = 9 ± 0.6 min) and showed no further symptoms thereafter. Indeed, all nine dogs in the study not only survived a lethal dose of paraquat but were feeding normally within a few hours of dosing. This study suggests that a level of 0.12% emetic in GRAMOXONE probably results in at least a 2 fold safety factor compared to GRAMOXONE EXPORT. Emulsion 181 has a further intrinsic safety factor of at least 2 fold on top of this. The AUC value obtained with E181 is the lowest ever value observed during the course of the Emulsion programme at this dose level in dogs.

Based on a very large database of Emulsion formulations studied at CTL over the last 3 years we would suggest that Emulsion 181 would achieve our safety margin of 5X. Obviously, until higher dose levels are tested we cannot extrapolate with exact certainty how safe these Emulsions will be. However, the AUC value obtained at 16mg/kg (11.0 ± 0.8µg/ml) is significantly lower than a 4mg/kg dose of GRAMOXONE EXPORT (18.2µg.h/ml, n=3) which is a 4 fold difference in paraquat dose. Therefore, Emulsion 181 is likely to be at least four times safer on a volume basis than an equivalent concentration of paraquat as GRAMOXONE.

A summary of the toxicological properties of certain Multiple Emulsion formulations of paraquat is shown below. The safety factor of Emulsions 26-140 inclusive is based on extensive dog studies over the dose range 8-48mg/kg paraquat ion. Plasma paraquat area-under-curve (AUC) is shown for the 16mg/kg dose level which is a lethal paraquat dose for GRAMOXONE in this species.

FORMULATION	AUC at 16mg/kg mean ± SEM, n=3 µg.h/ml	Safety Factor	Sprayability
GRAMOXONE	60 - 80	1X	V. GOOD
E26 1987	14.2 ± 3.0	6X	POOR
E64 1987	31.7 ± 1.0	2X	FAIR
E82 1988	24.4 ± 0.2	3X	FAIR
E90 1988	13.7 ± 4.0	5X	FAIR
E121 1989	63.7 ± 7.1	1X	V. GOOD
E140 1989	28.2 ± 3.3	4X	GOOD
E171 1990	18.9 ± 7.4	(3X)	V. GOOD
E181 1990	11.0 ± 0.8	(5X)	V. GOOD

Skin studies with Multiple Emulsion Formulations of Paraquat

(i) Emulsions diluted to spray strength

The skin irritation potential of spray strengths of three Multiple Emulsion formulations of paraquat (E26, E82 and E90) have been compared to GRAMOXONE. The Emulsions all contain B246, Diesel oil and NPE 1800. The external water phase of Emulsions 26, 82 and 90 contains NaCl, $CaCl_2$ and $MgCl_2$ respectively. All formulations contained a nominal 0.4% w/v paraquat ion concentration. Skin irritation in four New Zealand White albino rabbits was observed following single four-hour applications of spray strength formulations. An aqueous spray strength dilution of GRAMOXONE (0.4% w/v) produced signs of slight to mild irritation following a single application to rabbit skin. Signs of slight irritation were observed following a single application of an aqueous dilution of Emulsion 26 (0.4% w/v). Aqueous dilutions of Emulsion 82 and Emulsion 90 (also containing a nominal 0.4% w/v paraquat ion) produced practically no irritation to signs of mild irritation. Thus, these preliminary data indicate that application of spray strength dilutions of Multiple Emulsion formulations of paraquat containing B246, NPE 1800 and Diesel oil are less irritant than GRAMOXONE when applied to rabbit skin.

(ii) Emulsion as neat concentrates

The above studies were repeated using GRAMOXONE diluted to 100g/l paraquat ion and Emulsion concentrates (100g/l paraquat) of E26, E82 and E90. GRAMOXONE caused irreversible damage to the stratum corneum and underlying dermis which was still present at Day 25. Such observations are consistent with skin corrosion. Emulsion 26 was a slight irritant in two animals and a mild irritant in two animals. Emulsion 82 was a moderate irritant in three and severe in one. Emulsion 90 was a severe irritant in three and moderate in one. Unlike GRAMOXONE, none of the Emulsions were classed as corrosive and the effects observed with Emulsions were reversible with all animals recovered by Day 14. On the basis of these preliminary studies these three Emulsions would be classified on a more favourable basis compared to GRAMOXONE.

Future Emulsion Studies

We are continuing to optimize the safening and sprayability properties of specific Multiple Emulsion types. Our current promising Emulsions which include E181 are being scaled up by various mixing processes. The toxicology of these systems will be systematically assessed in rat and dog studies at each stage of scale-up, simultaneously with spray and herbicidal trials. Repeat studies will also have to be carried out at CTL on Emulsions which have been stored up to several months at various temperatures. We envisage that there will be a reduction in the screening of new Emulsions in 1990, with the majority of our effort centred on a small number of formulations as potential development candidates.

Emulsion E140 which contains 100g/l paraquat and comprises the polymers B246 and NPE 1800, diesel oil, $MgCl_2$ and polyvinyl alcohol. Our current promising Emulsions such as E171 and E181 are

GASTROINTESTINAL MOTILITY AND SAFER PARAQUAT FORMULATIONS

Paraquat is absorbed rapidly but incompletely from the gastrointestinal tract following oral ingestion in man. One of the most important treatments following paraquat poisoning is early gastric lavage to remove as much of the non-absorbed herbicide as possible. GRAMOXONE contains an emetic (PP796) which, if a sufficient dose is given, will induce vomiting. Since the emetic itself has to be absorbed there is a latency between oral ingesting and emesis. Furthermore, since GRAMOXONE is a free-flowing liquid, it empties from the stomach into the small intestine (the site of paraquat absorption) within a few minutes which makes it more difficult to remove by emesis. Semi-solid formulations of high osmolarity empty from the stomach slowly and stimulate emesis directly on contact with the duodenal osmoreceptors. Furthermore, the presence of high tonicity in the small intestine causes a reflex clearance of this organ by purgation. Part of our research effort at CTL during 1989 has been to attempt to identify a formulation of paraquat which will have reduced absorption by means these enhanced effects on gastrointestinal motility.

Aqueous Paraquat Concentrates Containing Magnesium Sulphate

The acute toxicity of a single oral dose of GRAMOXONE containing various salts in the rat is summarised in Figure 5. Generally, Mg-based systems were least toxic with the sulphate producing the best safening in rats. In 1988, we demonstrated that GRAMOXONE containing calcium salts increased toxicity of paraquat. Most Ca uptake processes are antagonised by Mg. Furthermore, Mg salts were less irritant to the mucosa compared to other salts of equal tonicity. Acute toxicity studies in rats were used to characterise the GRAMOXONE-$MgSO_4$ formulation. A dose related reduction in toxicity occurred between 0.5-1.5M $MgSO_4$, where the formulation remained as an aqueous solution. Concentrations above 1.5M (40%) $MgSO_4$ began to salt out of solution. GRAMOXONE containing 1.5M $MgSO_4$ gave an MLD of 190mg/kg in the rat. This compares with 90mg/kg for GRAMOXONE alone.

In the rat, plasma paraquat analysis following GRAMOXONE-$MgSO_4$ gave a significant reduction in plasma paraquat levels from 4-48 hours after dosing. In dogs, the same GRAMOXONE $MgSO_4$ formulation was dosed orally to 3 animals at 8, 16 and 24mg/kg on three separate occasions one month apart. Although the lethal dose of GRAMOXONE alone in dogs is about 12mg/kg there were no clinical signs of paraquat intoxication at any dose. A common feature throughout was emesis within 30 minutes of dosing and a watery diarrhoea by 2-3 hours in all cases. Since a lethal plasma AUC for paraquat in the dog is around 50µg/ml.hr., we would predict that addition of $MgSO_4$ results in a formulation which is at least 2-3 times safer than GRAMOXONE. The plasma profile for paraquat following oral dosing with GRAMOXONE-$MgSO_4$ in dogs is shown in Figure 6.

We have also studied the small bowel transit of $MgSO_4$ in rodents. The transit time of a charcoal meal in mice, in the absence of paraquat, was used an an index of motility. An oral dose of 1.5M $MgSO_4$ caused the marker charcoal to move from pylorus to caecum (the length of the small intestine) in about half the time compared to control. Other salts and other purgative drugs are being compared in this model in order to identify the most effective stimulants of gastrointestinal motility.

Aqueous Paraquat Concentrate Containing Magnesium Sulphate and Trisilicate

It is our opinion that the combination of rapid effective emesis together with rapid small bowel clearance will further reduce paraquat absorption. Our current approach is to produce a gel on contact with gastric juice which will reduce gastric emptying. Magnesium trisilicate ($MgSi_3O_8$) has such properties and a combination of the purgative $MgSO_4$ and $Mg_2Si_3O_8$ in GRAMOXONE has increased the MLD above 250mg/kg in rats. The magnesium trisilicate reacts with gastric acid to produce silicon dioxide gel in the stomach. Slower delivery of paraquat into the the small intestine with the gel allows the latency of purgation to be overcome. Furthermore, the gel reduces the dissolution of paraquat in the gastrointestinal tract and actually binds the bipyridyl molecule at high concentrations. Dilutions of this concentrate by 3-fold releases bound bipyridyl and would therefore re-activate the herbicide. In vomiting species such as dog and man, a slowing of gastric emptying will allow the latency of both

purgation and emesis to be overcome. As a result more paraquat (as gel) would probably be removed by emesis and any formulation which enters the small intestine (the site of paraquat absorption) would be rapidly cleared by purgation. Studies in the dog at 24mg/kg paraquat ion have confirmed that a formulation of GRAMOXONE containing a combination of Magnesium Sulphate and Trisilicate is safer than $MgSO_4$ alone (Figure 6). A dose of 24mg/kg of GRAMOXONE containing both $MgSO_4$ and Trisilicate also gave a low plasma AUC (Figure 6). This formulation probably has a minimum of a 3-fold safety factor over GRAMOXONE. Higher dose levels are planned to determine if such a formulation will achieve our intrinsic 5-fold safety factor objective.

Paraquat products containing $MgSO_4$ are currently marketed as the solid formulations WEEDOL and PATHCLEAR. Furthermore, silicate systems have been used as thickening agents with the herbicide. Both salts are inexpensive, exempt from environmental and Regulatory problems. Studies with existing paraquat formulations suggest that these additives will not interfere with the herbicidal properties of paraquat. More research is required to optimize the formulation but it is possible that such as system would be a satisfactory addition to our paraquat product portfolio.

J R HEYLINGS
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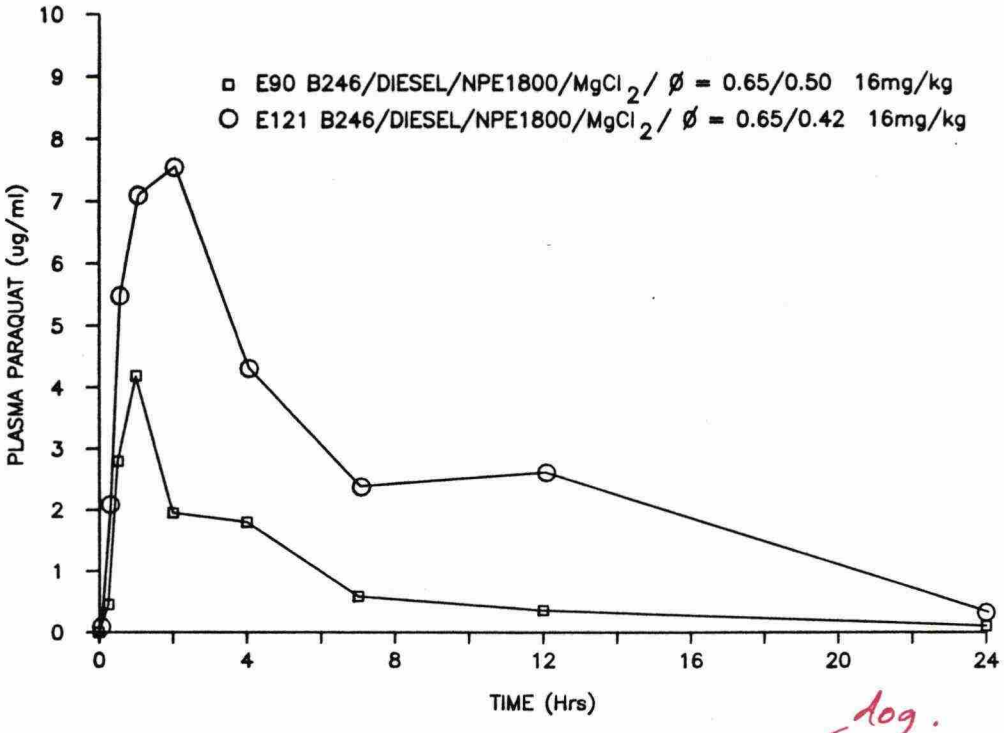

Fig 1. Effect of a single oral dose (16mg/kg) of two Multiple Emulsion formulations of paraquat in the conscious dog. Plasma paraquat levels are very different when the secondary volume fraction is altered. Emulsion 90 contains more oil and gave a much lower plasma AUC (13.7 ± 4.0µg.h/ml) compared to Emulsion 121 (63.7 ± 7.1µg.h/ml). Mean values for 3 animals per group are shown.

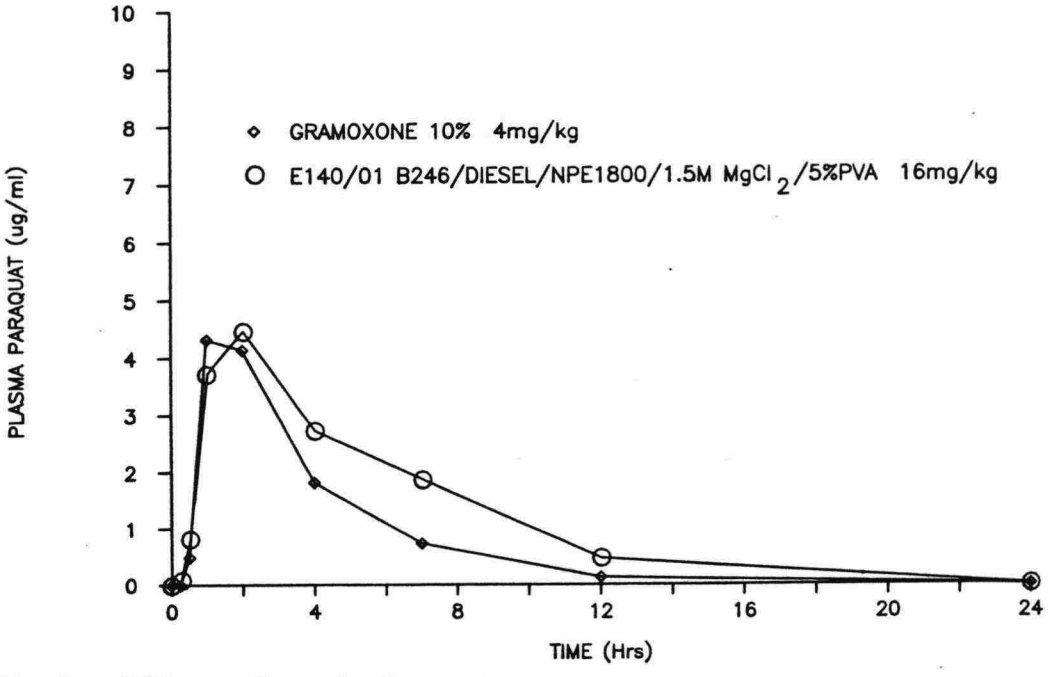

Fig 2. Effect of a single oral dose of the Multiple Emulsions formulation E140 at 16mg/kg in the conscious dog. For comparison a contemporary GRAMOXONE control at 4mg/kg gave a similar plasma profile despite the four-fold difference in paraquat dose.

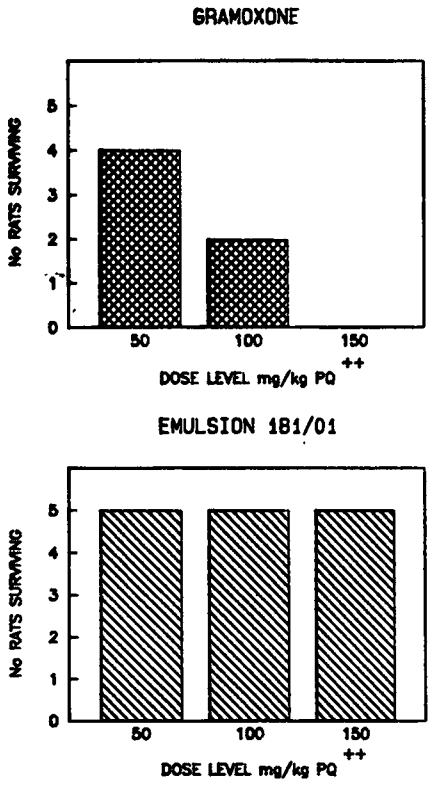

Fig 3. Effect of a single oral dose of paraquat (50-150mg/kg) as GRAMOXONE and Emulsion 181 in the rat. Survival rates are shown for groups of 5 animals per dose level over a 10 day period. Both formulations contained 10% paraquat and 0.12% PP796. The median lethal dose (MLD) for GRAMOXONE was 50-100mg/kg. Emulsion 181 has an MLD in excess of 150mg/kg in this species.

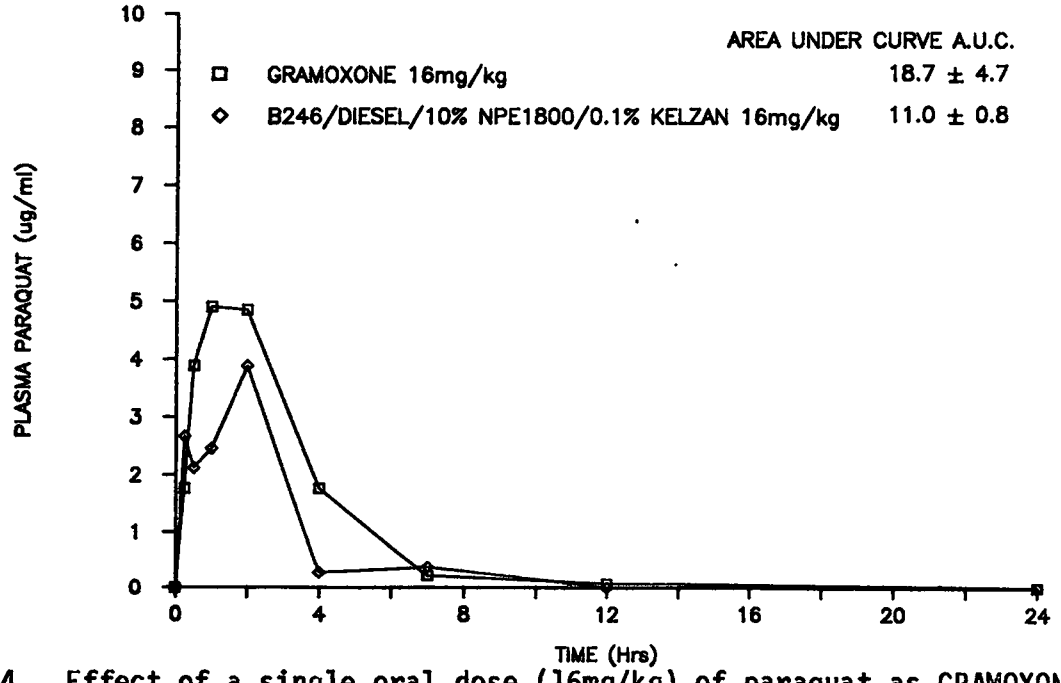

Fig 4. Effect of a single oral dose (16mg/kg) of paraquat as GRAMOXONE and Emulsion 181 in the conscious dog. The mean AUC value for Emulsion 181 was significantly lower than the GRAMOXONE control. Both formulations contained identical concentrations of paraquat (10%) and PP796 (0.12%). Mean values for 3 animals per group are shown.

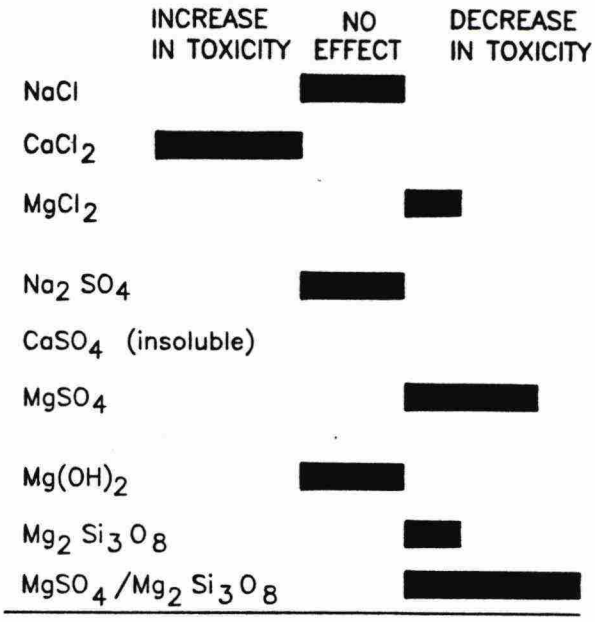

Fig 5. Effect of a various electrolytes on the oral toxicity of GRAMOXONE in the rat. Equimolar solutions (1.5^M) of each salt were added directly to 10% GRAMOXONE and dosed over the range 100-300mg/kg paraquat. Magnesium based salts reduced the oral toxicity of GRAMOXONE.

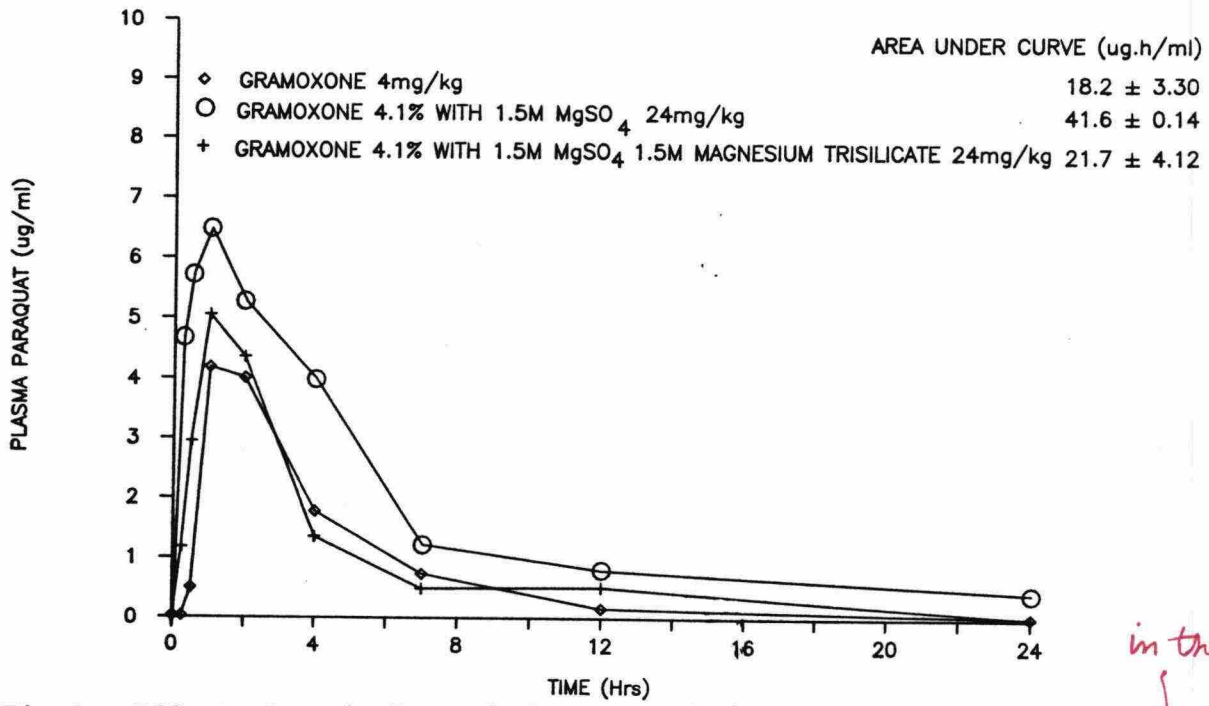

Fig 6. Effect of a single oral dose (24mg/kg) of GRAMOXONE containing $MgSO_4$ alone or in combination with Magnesium Trisilicate in the conscious dog. The gelling, emetic and purgative properties of the combination of both salts resulted in a reduction in plasma paraquat AUC to values which are equivalent to a 4mg/kg dose of GRAMOXONE. Mean values for 3 animals per group are shown.

APPENDIX

PARAQUAT : STUDIES ON THE MECHANISM OF GASTROINTESTINAL ABSORPTION

During the course of our research studies at CTL, we identified the jejunum as the principal site for paraquat absorption in rats. Studies both *in vitro* and *in vivo* confirmed that the absorption rate was more than ten fold greater across jejunum compared to the stomach. Once the importance of the small intestine had been established, the kinetics of paraquat uptake was more fully characterised using isolated mucosa from this region of the gastrointestinal tract.

Rat Isolated Mucosa

In vitro preparations of isolated mucosae can be kept viable for several hours when bathed by rapidly oxygenated solutions. Tissues are dissected free of outer muscle layers and a $1.8cm^2$ disc or tube of mucosa was mounted as a membrane between two separate Kreb's solutions. These solutions were gassed with 95% O_2 + 5% CO_2, pH 7.2 and maintained at 37°C. Viability of each mucosa was assessed by measuring the transmucosal potential difference (PD). A viable tissue which is undamaged will generate a stable PD of around 5-10mV under normal conditions. Damage to the tissue abolishes the PD as the permeability of the mucosa increases. Permeability damage to the tissue was determined by the kinetics of the non-absorbable marker mannitol. Paraquat absorption and tissue uptake was measured over 4 hours following exposure of the luminal side with a fixed concentration of the bipyridyl (containing ^{14}C-paraquat).

Under normal conditions of tissue oxygenation at 37°C, absorption of paraquat by rat isolated small intestine obeyed saturation kinetics. This suggests that a barrier to paraquat diffusion exists in the mucosa as shown in Figure 1. Inhibition of metabolism at 4°C resulted in paraquat absorption becoming an exclusively diffusional process across the same range of luminal paraquat concentrations. This suggests that the barrier to paraquat diffusion depends on tissue metabolism. Removal of this

barrier results in much greater rates of paraquat absorption at the same concentrations which demonstrated saturability. Evidence that mucus could act as a barrier to paraquat absorption in rats was achieved with the thiol reagent N-acetyl cysteine (NAC). This drug breaks the disulphide bonds of mucins and solubilizes the glycoprotein. Exposure of the luminal solution of rat small intestine to paraquat following NAC treatment resulted in a significant increase in paraquat absorption.

Dog Isolated Mucosa

There are differences in the paraquat plasma profile between rat and dog following a single oral dose. This may reflect different gut transit times between the species or may be due to differences in the mechanism by which paraquat is transported across the gastrointestinal mucosa. We adapted our current methodology to study paraquat absorption in isolated mucosa from dogs. Control adult male animals from various CTL studies were used. A 100cm section of small intestine was removed immediately after sacrifice and lumen rinsed thoroughly with warm Kreb's solution. Outer muscle layers were carefully dissected away from the underlying mucosa. This was divided into five segments each 5cm in length. These tubes of tissue were attached to the open ends of two glass tubes connected to a 25ml reservoir. All chambers were rinsed repeatedly with oxygenated Kreb's solution at 37°C and placed in an outer vessel containing 250ml of serosal side solution. Potential difference and permeability was used to determine viability of each mucosa.

Absorption was measured across a wide range of paraquat concentrations (2-100mg/ml) in each dog. Data was plotted as mucosal uptake in µmol paraquat/g wet wt/hr versus luminal concentration. As shown in Figure 2, mucosal uptake in the small intestine of dogs was linear between 2-100mg/ml. Unlike the rat, paraquat absorption in dogs is diffusional under normal conditions of tissue viability. The rate of absorption in the dog is very similar to the rate of passive diffusion in the rat at 4°C (Figure 2).

Mucus as a Barrier to Paraquat Diffusion

The most striking difference between the paraquat absorption kinetics in rat and dog was the fact that uptake of paraquat obeyed saturation kinetics in rat but was a diffusion process in dog. Since there is always a very large chemical gradient for paraquat to diffuse from the lumen into the mucosa in our studies, the saturability phase probably reflects a functional barrier to the bipyridyl which we have shown is dependent on tissue metabolism. Furthermore, since our tissue analysis also includes epithelium plus adherent mucus, we therefore investigated the capacity for intestinal mucins to bind the paraquat ion.

Mucus was collected from the small intestine of fasted rats and dogs post mortem by blunt scraping of the mucosa. A 50% suspension by weight in Kreb's solution was incubated with paraquat at 37° or 4°C for 15 minutes and then 1ml placed inside a dialysis bag to separate mwt <1200 from >2000. Paraquat was dialysed into a surrounding 50ml Kreb's solution for 6 hours at 37° or 4°C. As shown in Figure 3, the rate of paraquat dialysis is much slower in the presence of rat mucins compared to control aqueous conditions. The same quantity of dog mucin under the same experimental conditions had no effect on the rate of dialysis of paraquat. Table 1 shows the comparison between dialysis rates between the two species. At 4°C the rate of paraquat diffusion from mucus was slower but only rat mucins had the capacity to bind paraquat. Since the barrier to paraquat diffusion is lost in the rat isolated mucosa at 4°C, yet rat mucins in situ still bind the paraquat ion at this temperature, then this suggests that the rate of mucus secretion (and therefore the thickness of the barrier) is markedly reduced at 4°C. With this mucus barrier removed, paraquat will then diffuse readily into the mucosa and higher tissue levels will result.

The differences in mucus binding capacity for the paraquat cation between species probably represents a difference in the quality of the mucins. For instance, the extent to which paraquat will bind electrostatically to the anionic ester sulphate residues to form non-absorbable complexes will depend on the degree of sulphation of the mucin. Mucins from different

species vary in their degree of sulphation. Future studies will examine the paraquat binding characteristics of human mucins to determine if mucus is a permeability barrier to paraquat absorption in man.

Future Paraquat Research

We aim to continue studies on the paraquat absorption process in vitro using both rat and dog isolated mucosa. Collaboration with the University of Newcastle has enabled us to study both paraquat and polyamine uptake in isolated brush border membrane vesicles and human cultured enterocytes. In addition, we plan to study the absorption of paraquat in the presence of drugs which affect mucus secretion and fluid transport in the gastrointestinal tract. We have also set up a collaborative project with the Gastroenterological Unit at the University of Manchester to study small bowel transit time by ultrasonography. Finally, by recruiting a postdoctoral fellow from September 1989, we hope to characterise the mechanism of paraquat absorption in vivo, and to maintain a strong basic research programme to assist the development of safer paraquat formulations.

J R HEYLINGS

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EMULFORM (19.2.90)

FIGURE 1

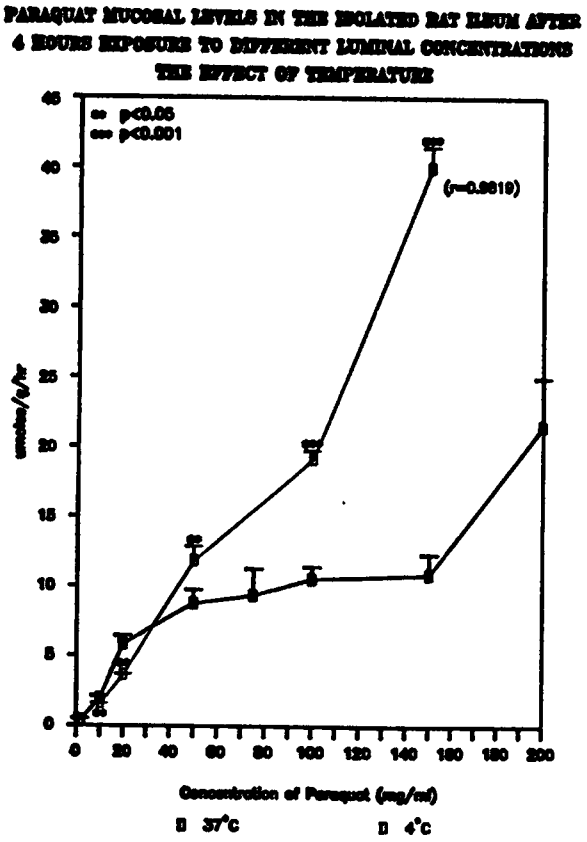


FIGURE 2

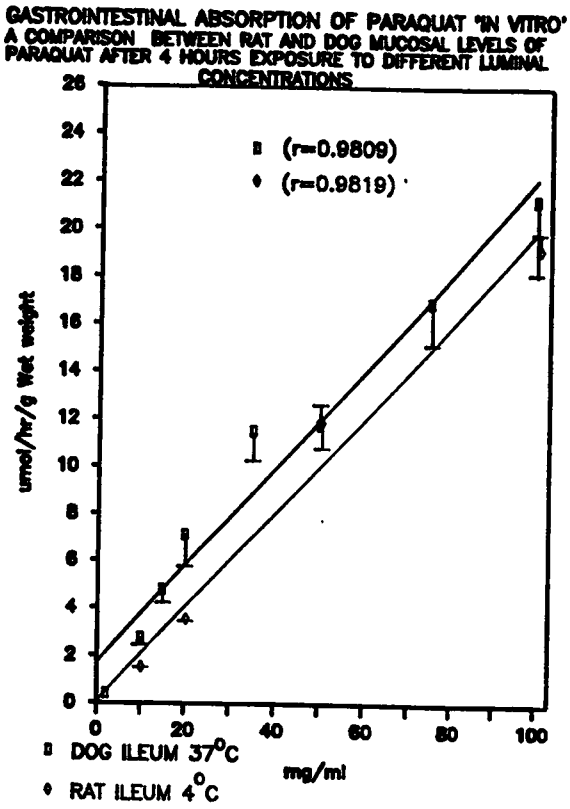


FIGURE 3

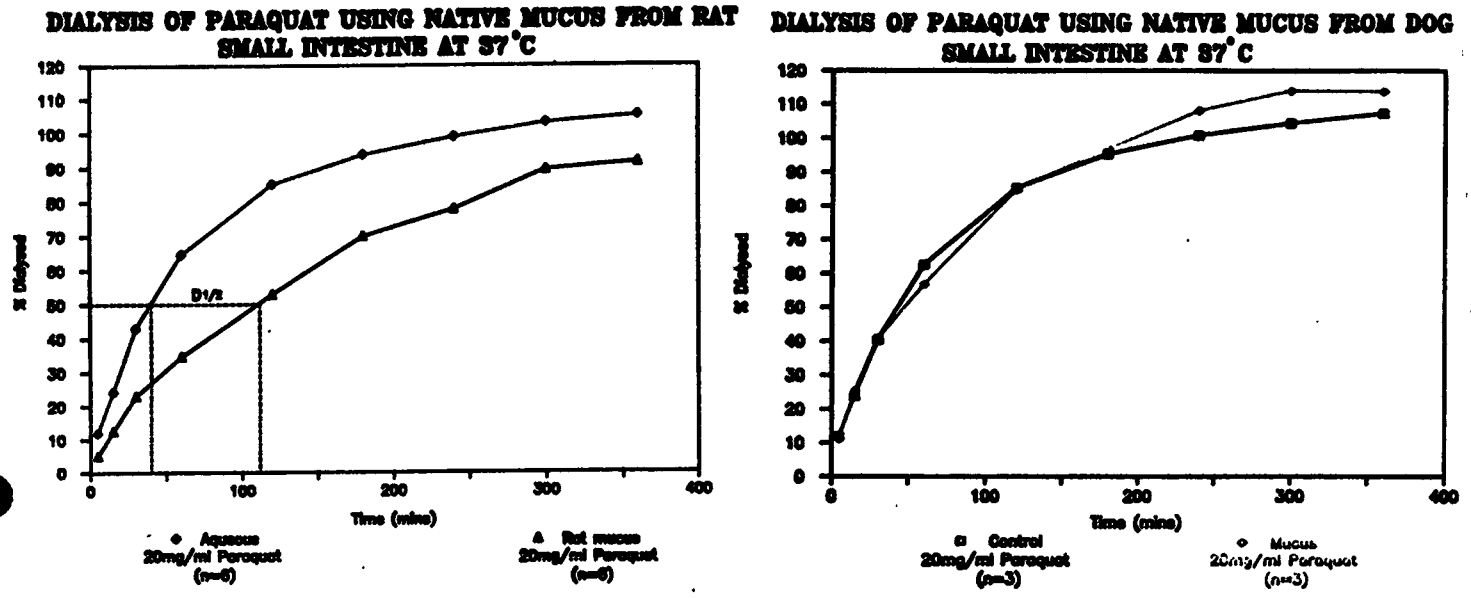
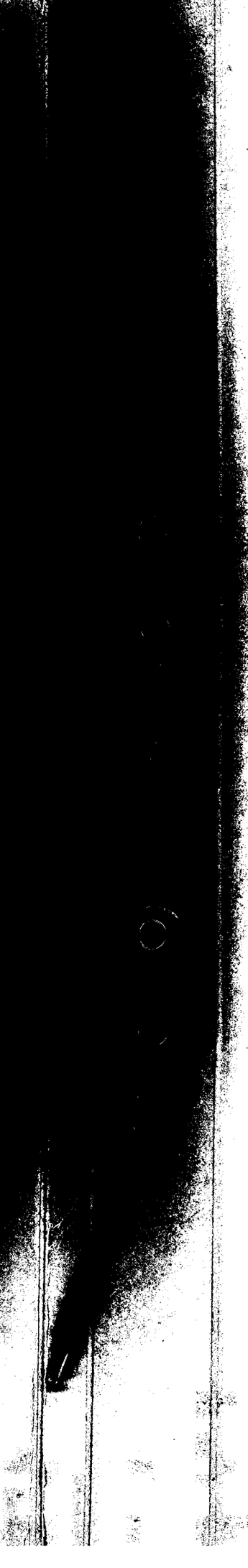


TABLE 1

Rate of Dialysis of Paraquat (20mg/ml) using Native Mucus from Rat and Dog Small Intestine

	D1/2 (mins)	
	37°C	4°C
Paraquat aqueous (n=6)	38.5± 3.1	125.5 ±7.0
Paraquat rat mucus (n=6)	93.1 ± 9.5*	252.8 ±15.5*
Paraquat dog mucus (n=3)	44.5± 0.3	114.0 ±8.8

*$p<0.001$



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SOUTHPORT U.K. Tel.0704-28866 Fax.0704-28814
LONDON OFFICE Tel.071-377-1777 Fax.071-247-6181