SAFER PARAQUAT FORMULATIONS

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ABSTRACT

This report summarises progress made by the Safer Paraquat Formulations project. A multiple emulsion formulation is identified which is recommended for further development. Based on the understanding gained of the factors which affect paraquat uptake in the gastrointestinal tract, a conventional formulation is proposed which may also satisfy the project criteria and be more financially attractive.

KEYWORDS

PARAQUAT
SAFER FORMULATIONS
EMETIC
PURGATIVE
MULTIPLE EMULSIONS
GELLING AGENTS

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RECOMMENDATIONS

1. Continue with the current levels of resources within Formulation R&D and Biochemical Toxicology to further define and optimise the lead multiple emulsion formulation E181 (JF 12255).

Action: Formulation R&D/Biochemical Toxicology (CTL)

2. Initiate formulation process scale-up, pack storage stability and product application testing studies using optimised JF 12255.

Action: Formulation R&D

3. Initiate formulation research and development of the magnesium sulphate/magnesium trisilicate/emetic option and confirm the toxicological profile and biological efficacy.

Action: Formulation R&D

4. Ensure protection of the synergistic effects of multiple emulsions and magnesium salt based formulations with the emetic through patents or publication as appropriate

Action: Patents Section

5. Carry out a detailed commercial review to cover the stratetic use of safer formulations of paraquat. Define the registration, toxicology and bioefficacy packages required

Action: Products/Development Departments

6. Consider the case for raising the level of emetic in current 'Gramoxone' formulations to improve safety margins

Action: Products Department

SUMMARY

Despite loss of market share due to glyphosate price reductions, sales of paraquat are still forecast to rise through the 1990s within an expanding non-selective contact herbicide market. However, 'Gramoxone' and other paraquat based products continue to face pressure from regulatory authorities due to the incidence of human paraquat poisonings, mainly suicides. Commercial assessment indicates that a toxicologically safer formulation is required to provide a strategic response to deregulation.

Collaborative research between Biochemical Toxicology, Formulation R & D and Biology has been directed toward devising safer formulations of paraquat to meet the following criteria:

(i) 5-fold reduction in toxicity relative to paraquat AC which will extend to a 10-fold reduction in toxicity for 100g ion/l products

(ii) at least 90% biological efficacy relative to paraquat AC

(iii) an incremental cost of formulation not exceeding £1000/tonne PQ ion (£ '87)

The majority of research effort has been focussed on multiple emulsion formulations. The acute toxicity of more than 300 multiple emulsions has been assessed in the rat. Promising formulations have been studied in detail in dogs, a species which closely resembles man in terms of paraquat absorption and toxicity.

Early work demonstrated the possibility of devising multiple emulsion formulations which satisfied the project criteria. However, these formulations dispersed poorly and left unacceptable agglomerated deposits in spray application trials. Recent work has resulted in an experimental formulation which eliminates the latter problem and satisfies the project criteria. The safety of this formulation is devised from the intrinsic properties of the multiple emulsion (2-3x) combined synergistically with the emetic PP796 which, at 0.12%, contributes a further 2-3x safening. The upper limit of safety of this formulation has not yet been established but it is estimated to be at least 5x safer than 'Gramoxone'. Furthermore, the time to vomit, a critical parameter in the prevention of paraquat poisoning following oral ingestion, was significantly reduced compared to that observed with 'Gramoxone'.

Preliminary dermal toxicity experiments have shown that normal spray dilutions of the multiple emulsions are less irritant than 'Gramoxone'. Most significantly, the multiple emulsion concentrates were not classified as corrosive and their irritant effect was reversible.

The herbicidal properties of the lead multiple emulsion are judged to be equivalent to 'Gramoxone' based on results obtained in UK field trials.

Much has been learned about the physico-chemical properties and process requirements of multiple emulsion formulations during the research phase. Despite the novelty of the technology, the probability of achieving a commercially acceptable product is assessed as good.

Throughout the research programme a fundamental understanding of the parameters which affect paraquat uptake in the gastrointestinal tract has been gained. An active calcium dependent uptake process thought to be involved in paraquat absorption has been demonstrated to be antagonised by magnesium ions. Hence the addition of magnesium salts to paraquat AC results in a lowering of toxicity. Use of magnesium sulphate as a source of magnesium ions resulted in a further reduction in toxicity, thought to be due to increased motility by purgation of the region of paraquat uptake. Furthermore, addition of magnesium trisilicate results in the formation of a highly viscous gel on contact with gastric juice. This has the effect of reducing gastric emptying. The combined effect of antagonism of calcium ions, purgation and gastric gelling have been demonstrated to safen 'Gramoxone' by at least 3-fold in the dog. Experiments are currently underway to assess the combined effect of this formulation with the emetic; an overall 5-fold safening factor is anticipated. Although the proposed concentrations of magnesium sulphate and magnesium trisilicate are at the limits of solubility in 'Gramoxone' it should be possible to develop a conventional product from this formulation. The cost of such a product would be significantly less than a multiple emulsion and will require less capital investment for manufacture.

During the course of this work important conclusions have been reached regarding the role of the emetic (PP796). It has been found that increasing the concentration of emetic in 'Gramoxone' by a factor of 5 resulted in a minimum of a 2-3 fold safety factor over standard 'Gramoxone'.

TOXICOLOGY OF NEW FORMULATIONS OF PARAQUAT

Jon R Heylings and Lewis Smith

SUMMARY

During the last 3 years the majority of the CTL effort on the safer paraquat formulation programme has centred on the Multiple Emulsions. In addition, we have applied some of the fundamental research knowledge on paraquat absorption to develop an additional approach to reduce the oral toxicity of the herbicide. More recently, the role of the emetic (PP796) has been more fully investigated with regard to its potential in aqueous paraquat concentrates such as GRAMOXONE, and as a synergistic or additive safety factor in novel formulations. There are therefore 3 discrete areas of investigation within the safer paraquat formulation programme. Progress to date in each area is summarized as follows:

1. High Concentrations of Emetic in GRAMOXONE

Increasing the concentration of emetic in an aqueous concentrate of GRAMOXONE by a factor of 5 resulted in a minimum of a 2-3 fold safety factor over standard GRAMOXONE.

2. Multiple Emulsion Formulations

An intrinsic safety factor of 4-5X over GRAMOXONE can be achieved with E90 and E140. An Emulsion which has acceptable spray and field trial characteristics such as E181 is approximately 5X safer than GRAMOXONE. In the case of E181, the safety factor is a combination of a 2-3 fold intrinsic safening caused by emulsification, plus an additional 2-3 fold safening by increasing the emetic to 0.12%.

3. Magnesium Sulphate and Trisilicate Formulations

Addition of the purgative, magnesium sulphate, and the gel forming magnesium trisilicate to GRAMOXONE resulted in a minimum of a 3 fold safety factor over GRAMOXONE alone. It is expected that inclusion of 0.12% emetic will further safen this formulation to an accceptable level.

Introduction and Objectives of the Safer Paraquat Formulation Programme

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. As a consequence of these research studies on paraquat absorption an additional strategy in the development of a novel safer paraquat formulation was investigated. This involved the use of additives to GRAMOXONE, in particular the sulphate and trisilicate salts of magnesium, in order to manipulate gastrointestinal functions and thereby reduce paraquat absorption. During the course of these studies and from data generated during the Emulsion programme, the role of the emetic PP796 in paraquat formulations was also examined. This report therefore centres on three areas of paraquat absorption: (i) the effect of high concentrations of emetic in GRAMOXONE, (ii) the development of a safe and sprayable Multiple Emulsion, and (iii) the effect of agents which affect gastrointestinal function as additives to GRAMOXONE.

1. HIGH CONCENTRATIONS OF EMETIC IN GRAMOXONE

In 1977, a pyrimidine compound triazolopyrimidine (PP796) was added to paraquat formulations because it had emetic properties in all vomiting species including dog and primates (Rose, 1976) and man (Bayliss, 1973). This compound had reached the clinical stages of development at ICI Pharmaceuticals in 1973 but was withdrawn due to its lack of efficacy in various disease states and because of its high incidence of nausea and vomiting during human volunteer and clinical trial studies (Bayliss, 1973). It was decided to utilize the emetic effects of this compound in paraquat formulations and a dose level of PP796 which was thought at the time would induce vomiting following a lethal dose of the herbicide was included in GRAMOXONE (Rose, 1977). A dose level of 5mg in an adult receiving a minimum lethal dose of paraquat (eg 2g paraquat or 10ml GRAMOXONE) was therefore added to aqueous paraquat concentrates as a safener.

Over the following 5 years paraquat poisoning cases were monitored to determine whether inclusion of emetic had significantly reduced the number of mortalities attributed to the herbicide. A total of 640 cases of paraquat poisoning were reviewed by Hart and Whitehead in 1984 (unpublished data). There was no definitive evidence from this large

database that inclusion of emetic had resulted in a reduction in oral toxicity of paraquat. On reviewing more recent data with the emetic conducted by Brammer and Robinson in 1985 and 1986, it becomes clearer that the original decision to add 0.05% emetic to GRAMOXONE was probably an underestimate of the effective emetic dose in man. The time-to-vomit parameter is extremely critical to remove non-absorbed paraquat. Recent studies suggest that animals must remove the herbicide within 20 minutes of ingestion in order to survive a lethal dose of paraquat. In order to achieve this, available data suggests that the minimum concentration of emetic in GRAMOXONE should be some 5 times higher than currently used. Studies were therefore conducted to examine the safening potential of increased emetic in GRAMOXONE.

Studies in the Dog with High Emetic Concentrations

Development of a safer formulation has encompassed both an intrinsic safety factor and a dilution factor for the final product. Conventional GRAMOXONE contains 20% paraquat and 0.05% emetic. This is equivalent to a 400:1 ratio of bipyridyl:emetic. This ratio is critical in our calculation of increased emetic. Based on a low strength GRAMOXONE containing 10% paraquat, increasing the emetic by 2.5X results in a 5X change in bipyridyl:emetic ratio. Thus, a 10% GRAMOXONE containing 0.12% emetic was prepared by dissolving extra emetic (as solid) in the GRAMOXONE solution. This formulation was dosed orally by capsule to 3 dogs at 16mg/kg, a lethal dose of paraquat. The dogs had been starved overnight and food withheld for 12 hours after dosing. This was the first ever study where a lethal dose of GRAMOXONE has been dosed as a neat concentrate with high levels of emetic. Previous studies which showed reduction in plasma paraquat with high emetic doses used dosing solutions containing 0.3% paraquat with food (Brammer et al, 1986). As shown in Figure 1, the plasma profile following dosing was very similar to a control group of 3 dogs which received a 4mg/kg dose of a 10% GRAMOXONE containing 0.025% emetic. Thus, despite the 4 fold difference in paraquat dose level, the plasma area-under-curve (AUC) values were almost identical. None of the 4mg/kg (low emetic) dogs vomited and all were normal clinically. All the 16mg/kg dogs vomited with a mean time to first vomit of 19 ± 4 minutes after dosing. These dogs vomited several times upto 2 hours after dosing

but no further emesis occurred thereafter. These dogs were feeding and behaving normally within hours of the lethal paraquat dose. Thus, alteration of the bipyridyl:emetic ratio by 5X results in a minimum of a 2 fold safety factor over conventional GRAMOXONE. Further studies at higher dose levels of GRAMOXONE are planned to determine the overall safety factor of high emetic formulations.

(1) Bayliss, P.F.C. (1973). A summary of clinical results of the phosphodiesterase inhibitor ICI 63197 in a variety of disease states. Report No: PH 20992B.

(2) Brammer, A. and Robinson, M. (1985). PP796: Emetic dose response study in dogs. CTL Report No: CTL/T/2459.

(3) Brammer, A. and Robinson, M. (1986). Emetic study in paraquat treated dogs. CTL Report No: CTL/T/2471.

(4) Rose, M.S. (1976). The effect of administration of an emetic (PP796) on paraquat toxicity in dog and monkey. Report No: CTL/R/391.

(5) Rose, M.S. (1977). The concentration of PP796 required to produce emesis in experimental animals and an estimation of the emetic dose in man. CTL Report No: CTL/R/390(R).

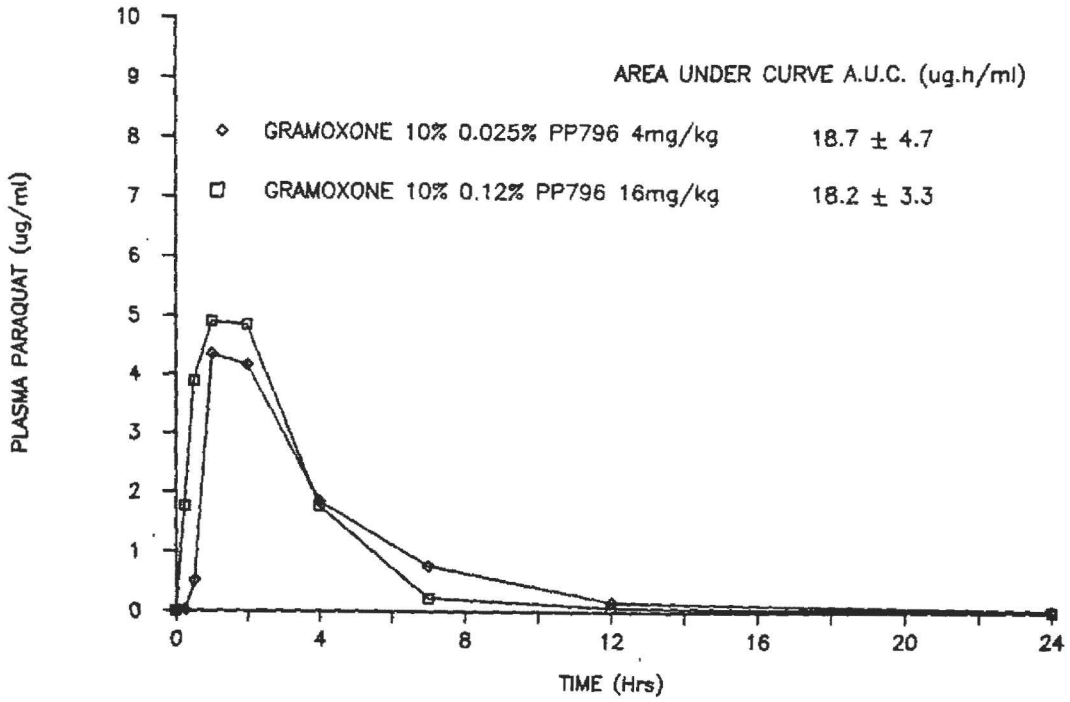

Fig 1. Effect of two formulations of GRAMOXONE in the conscious dog. Both formulations contained 10% paraquat dosed by capsule. Increasing the emetic (PP796) by 5 fold resulted in a very low plasma paraquat profile which was equivalent to a 4mg/kg dose of standard GRAMOXONE. Mean values are shown for 3 dogs per group.

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2. MULTIPLE EMULSION FORMULATIONS OF PARAQUAT

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 from over 300 different Emulsions approximately 10% of these have proceeded to dog studies for further evaluation.

Dog Studies

The dog is the best available animal model for man. 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 2). 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 3). 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 4, 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 5, using equal doses of paraquat (16mg/kg), 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 vomited 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 relatively low peak plasma value, but 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 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 this Emulsion 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) Emulsions 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.

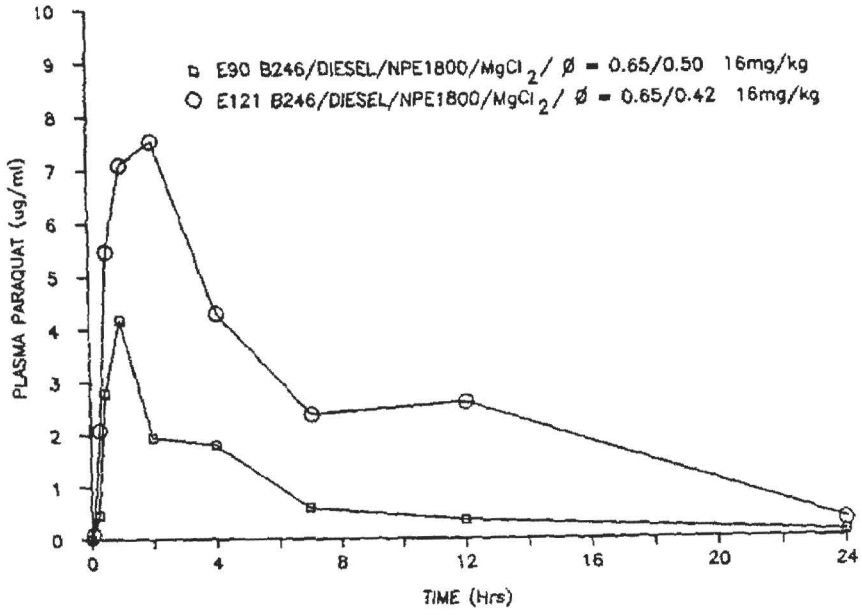

Fig 2. Effect of a single oral dose (16mg/kg) of two Multiple Emulsion formulations of paraquat in the conscious dog. Plasma paraquat levels are very different when the secondary volume fraction is altered. Emulsion 90 contains more oil and gave a much lower plasma AUC (13.7 ± 4.0µg.h/ml) compared to Emulsion 121 (63.7 ± 7.1µg.h/ml). Mean values for 3 animals per group are shown.

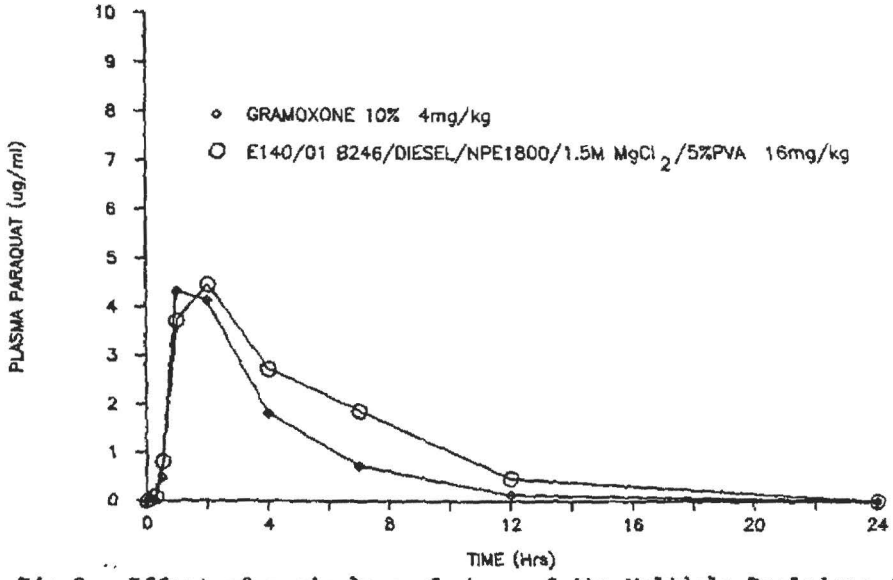

Fig 3. Effect of a single oral dose of the Multiple Emulsions formulation E140 at 16mg/kg in the conscious dog. For comparison a contemporary GRAMOXONE control at 4mg/kg gave a similar plasma profile despite the four-fold difference in paraquat dose.

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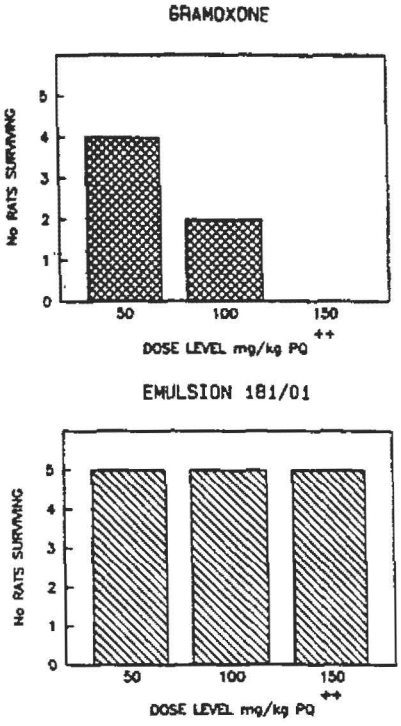

Fig 4. Effect of a single oral dose of paraquat (50-150mg/kg) as GRAMOXONE and Emulsion 181 in the rat. Survival rates are shown for groups of 5 animals per dose level over a 10 day period. Both formulations contained 10% paraquat and 0.12% PP796. The median lethal dose (MLD) for GRAMOXONE was 50-100mg/kg. Emulsion 181 has an MLD in excess of 150mg/kg in this species.

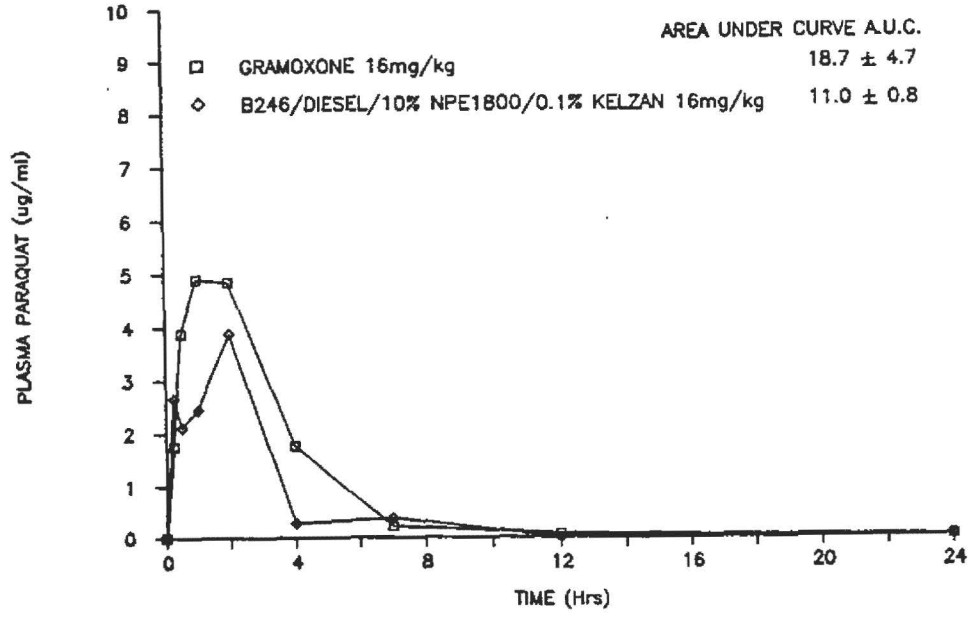

Fig 5. Effect of a single oral dose (16mg/kg) of paraquat as GRAMOXONE and Emulsion 181 in the conscious dog. The mean AUC value for Emulsion 181 was significantly lower than the GRAMOXONE control. Both formulations contained identical concentrations of paraquat (10%) and PP796 (0.12%). Mean values for 3 animals per group are shown.

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3. MAGNESIUM SULPHATE AND TRISILICATE FORMULATIONS OF PARAQUAT

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 ingestion 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 6. 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 7.

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 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 GRAMOXONE plus $MgSO_4$ alone (Figure 7). 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, and 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 a system would be a satisfactory addition to our paraquat product portfolio.

Future Studies

Our objective during 1990 is to establish as accurately as possible the safety factor of our new safer formulations of paraquat. A minimal amount of effort is required to establish the safety factor of GRAMOXONE containing a higher level of emetic. Such a system is almost certainly without storage stability, spray or herbicidal problems.

The Emulsion programme has discovered a formulation in E181 which has achieved our goal of safening and sprayability/herbicidal efficacy. Such a formulation will have to be scaled up and tested at CTL at various stages of the process development. Repeat testing will also have to be carried out on stored batches of such a new formulation.

Finally, the approach of producing a gel in the stomach *in situ* with maynesium trisilicate and removing non-absorbed paraquat from the gastrointestinal tract by purgation with magnesium sulphate will be continued. The intrinsic safety factor of this system for a 10% GRAMOXONE formulation will be assessed. Synergism with extra emetic in this formulation will also be addressed.

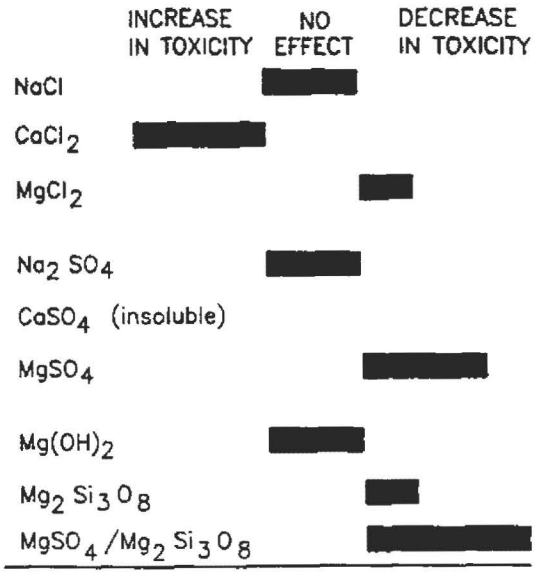

Fig 6. Effect of a various electrolytes on the oral toxicity of GRAMOXONE in the rat. Equimolar solutions (1.5M) of each salt were added directly to 10% GRAMOXONE and dosed over the range 100-300mg/kg paraquat. Magnesium based salts reduced the oral toxicity of GRAMOXONE.

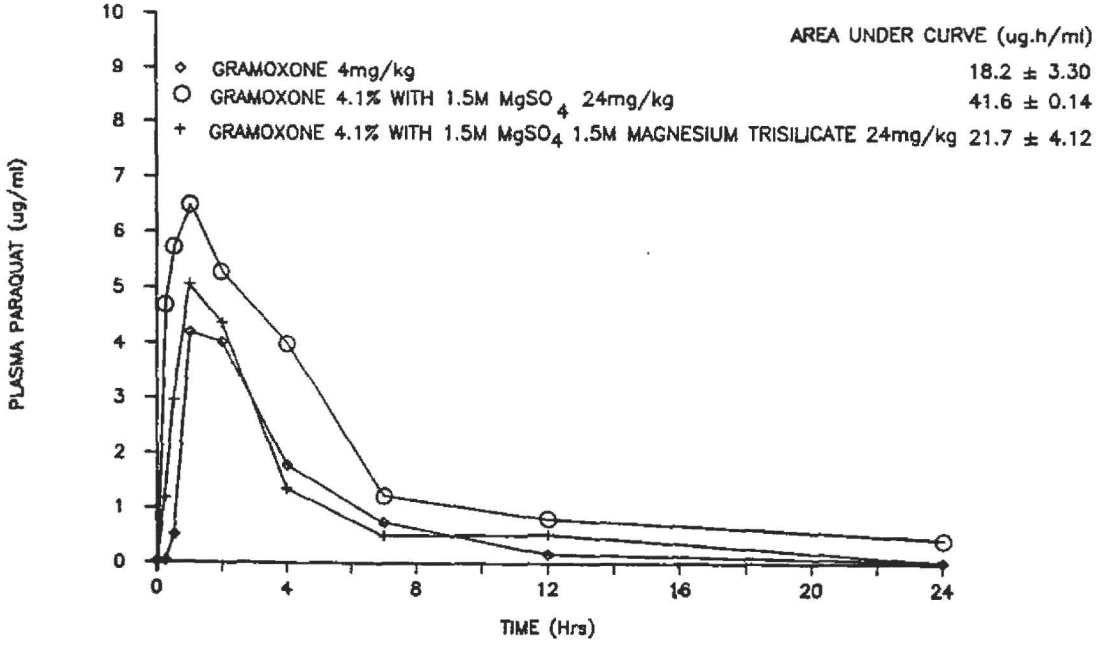

Fig 7. Effect of a single oral dose (24mg/kg paraquat) of GRAMOXONE containing $MgSO_4$ alone or in combination with Magnesium Trisilicate in the conscious dog. The gelling, emetic and purgative properties of the combination of both salts resulted in a reduction in plasma paraquat AUC to values which are equivalent to a 4mg/kg dose of GRAMOXONE. Mean values for 3 animals per group are shown.

2. FORMULATION RESEARCH (Carola G Sales and Tharwat F Tadros)

2.1 Introduction

In the last TRC Report we showed that acceptable toxicity reduction of paraquat dichloride could be achieved by formulating as a multiple emulsion. The principle of a multiple emulsion was discussed, highlighting the importance of producing an oil film coating around droplets of a paraquat dichloride concentrate. This oil film effectively encapsulates the paraquat ions, thus minimising the transport of ions to the external water medium.

Thereby, a degree of safening is obtained which is dependent on the properties of the oil film. These include the nature of the oil, the thickness of the film (the amount of oil used), the effectiveness of the emulsifiers used at the water-oil and oil-water interfaces, and any additives such as viscosity modifiers.

It was shown that a multiple emulsion could be prepared using B246, Diesel oil, Synperonic NPE1800 and 2 molar NaCl to give more than five fold reduction in toxicity, (based on measurement of absorption in the blood of dogs). Thus, it was demonstrated that a stable multiple emulsion with reduced toxicity could be prepared. However, this formulation did not disperse and gelled on storage, so was not a practical solution.

The main objectives in formulating an acceptable multiple emulsion were therefore, (1) to remove gelation of the multiple emulsion concentrate to give good initial dispersibility and dilution into water, and (2) to remove ensuing problems of poor dispersibility.

A major improvement in dilution properties resulted from the replacement of NaCl with $CaCl_2$ or $Mg\ Cl_2$ although the main advantage of this was to prevent gelation of the formulation. The initial dilution of these formulations is very good, with good strike and bloom. However, the ensuing aggregation of multiple emulsion drops to form insoluble-oil coagulates was unacceptable (flocculation). However, although these leave deposits on the filters of spray nozzles and inside the spray tanks, the paraquat has diffused out due to osmotic shock, and so herbicidal activity is maintained.

Subsequent work has concentrated on reducing this flocculation on dilution to an acceptable level. Dilution tests and knapsack sprayability assessments have shown that a creamed height of 5% on dilution of 4 mls of concentrate into 100 mls of water would be acceptable for field trials. The options available were those of reducing the amount of the oil present, adjustment of secondary emmulsification process and secondary emulsion interface variation using added polymers and alternative secondary emulsifiers.

2.2 Formulation Research Progress

2.2.1 Reduction of the amount of oil

A formulation containing the minimum amount of oil possible, whilst maintaining 100 g/l paraquat ion was developed to the point of field testing early in 1989. (This contained 13% oil). Extensive work was carried out to adjust the process of secondary emulsification for scale-up. Diesel fuel oil (E121) and Exxsol D80/Escaid 100 mixtures (E134) were used.

Good storage, dialysis, dilution, (4% flocculation) and sprayability were given, and although rat toxicity was good, the toxicity to dogs was found to be unacceptable.

2.3.2 Adjustment of secondary emulsification process

The formulation containing 25% oil phase was therefore re-evaluated (E90). This previously gave a four fold safety factor in dogs. After process adjustment, the creamed height was reduced to 7%. Initial dog tests at low dose levels showed reasonable toxicity reduction, but the flocculation was still not thought acceptable.

2.3.3 Secondary emulsion interface variation

Extensive work has also been carried out to adjust the secondary emulsion interface by either replacing NPE1800 or adding another surfactant (or polymer) to it. The work was carried out on the formulation containing 25% oil, as this was thought to be safer and more robust.

2.3.4 Addition of Polyvinylalcohol (PVA)

The addition of 5% PVA to the 1% NPE1800 reduced flocculation to 6% and knapsack dilution tests showed that sprayability was acceptable (E140). This formulation gave a four fold safety factor in dogs with the first batch, but subsequent batches have not proved as stable in rats. Also, instability of the formulation on storage has been observed.

2.3.5 Use of a static mixer

Work was directed towards improving the secondary emulsification process by means of a static mixer (a tube containing individual elements which cause the liquid flowing through to be mixed with a uniform shear pattern). This has proved very promising. Initial rat testing gave favourable results and flocculation was reduced to 6%. However, the procedure still needs refining due to the high viscosity differences of the two phases.

2.4 Alternative Secondary Emulsifiers

A wide range of alternative surfactants were investigated. The Pluronics and Tetronics proved to be most effective (ABA block copolymers of (poly)ethylene and propylene oxides; block copolymers of propylene oxide and ethylene oxide on ethylenediamine). In particular, P123 and T908 gave 5% creamed heights on dilution, but increased leakage of paraquat (especially for P123) and so were not screened for toxicity.

2.4.1 Increased NPE1800 concentration

It was found that increasing the NPE1800 concentration to 8% on the dispersed phase, with 0.1% Kelzan (Xanthan gum) presents helped reduce flocculation (E173). The creamed height appeared visually to be 8%; however, this cream was of a more loosely flocculated structure and therefore was expected to redisperse in the spray tank. A spray test was reasonably good overall, despite some deposits on filters.

Such a formulation was screened for toxicity, showing a much higher degree of safening in rats (all rats survived at 250 mg/kg). Unfortunately, this result was not substantiated by toxicity testing in dogs; which showed high plasma levels at a low doseage. Further increasing the NPE1800 concentration (up to 20%) as well as reducing the oil volume (to 15%) have eliminated flocculation whilst maintaining low dialysis. A spray test using the coke-can was very favourable. However, due to the poor dog toxicity result this line of approach was temporarily abandoned.

2.5 Incorporation of the emetic

One of the most promising toxicity results obtained so far has been due to the addition of emetic to the formulation containing 25% oil, 8% NPE1800 and 0.1% Kelzan (E173). When 1.2 g/l emetic (PP796) was added prior to doseing at 16 mg/kg in dogs, the level of paraquat in the plasma was reduced six fold.

The level of PP796 in Gramoxone is generally 0.5 g/l although the specification is 0.5 - 2 g/l. Therefore, initially, it was attempted to incorporate 2 g/l on the total formulation. As this was insoluble, 2 g/l in the external phase was used (1.2 g/l on the total formulation when using 13% oil).

Work was initiated on incorporating emetic into the less flocculating formulations containing 15% oil. Two formulations were prepared, one using the standard 1% NPE1800 (E171), and the other using 10% NPE1800 with 0.1% Kelzan (E181). Both gave good dialysis and dilution (4%, 2% respectively); and spray ability was thought excellent with virtually no filter blockage. It is hoped that a safer formulation can be made in this way, which can be developed for field trials and other large scale testing.

In fact, initial toxicity results from dog trials showed a distinct safening using 10% NPE1800 and 0.1% Kelzan. The similar formulation containing 1% NPE1800, did not so far satisfy the safety criteria. It may be that addition of a high concentration of surfactant and 0.1% Kelzan provides an extra safening factor due to the higher viscosity of the resulting formulation.

2.6 Twin Pack Concept

This concept was optimised (by P K Thomas) based on the primary emulsion containing B246 and diesel oil, which gave an eight fold safety factor in dogs. The surfactant solution consisted of a combination of Synperonic NPE1800, alkylglucoside and 1.5 NaCl. The mixture gave a three fold safety factor in dogs.

The main problem with this concept was that the paraquat concentration in the primary emulsion could not be increased above 100 g/l, and that the mixture only gave good dilution characteristics if used immediately.

2.7 Further Safening Aids

Further work is still needed to adjust the properties of the external water phase to cause gelation of the multiple emulsion in the gut environment. It is envisaged that this will be added to the final multiple emulsion to afford an extra degree of safening, in conjunction with that already gained due to the oil film, the emetic, and the magnesium ions.

Background research (by D J Brown) is also continuing on the encapsulation of the multiple emulsion drops by in situ polymerisation. This is looking very promising at the moment, although the overall level of paraquat needs to be increased.

2.8 SUMMARY

Improvements on last years safe formulation had to be made to give it dispersion on dilution and minimum subsequent flocculation. This was achieved in part by replacing NaCl with $MgCl_2$ which removed the gelation on storage, and reduced flocculation on dilution, whilst maintaining safety. To satisfy the ultimate criteria for dispersibility the level of flocculation had to be reduced further. This was achieved by addition of polyvinylalcohol (to 5%). Initial dog results showed a four fold safening (E140). However this was not substantiated by further rat testing and storage.

An alternative approach was to replace by NPE1800 by other block copolymers eg. P123 or T908. However, these gave very high leakage. Increasing NPE1800 to 8% and adding 0.1% Kelzan reduced the flocculation to acceptable levels, and also gave low dialysis values and good safety in rats, but were toxic to dogs (E173). By incorporating the emetic to that formulation (at 1.2 g/l), the safety was markedly increased (six fold in the dog). This formulation gave good sprayability but it was thought that the flocculation had to be almost removed. This was achieved by reducing the oil content to 13% (E121). A formulation was then developed based on this concept and containing 10% NPE1800, and 0.1% Kelzan and 1.2 g/l emetic (E181 - JF12255). This so far showed the most promising tox results, whilst being sprayable, and was applied successfully in field trials. This formulation, we believe, could be taken forward to development as a commercial product.

3. PATENTS

3.1 Multiple Emulsion Formulations : ICI Case PP34163

A priority specification describing the formulation of an aqueous solution of paraquat into a multiple emulsion was filed in the UK on 13 January 1987.

Overseas applications claiming the formulation process and the emulsions made by the process and claiming the priority of the UK application were filed in over 40 countries. The patent has been granted by the United States Patent Office and is proceeding normally in other Patents Offices.

A further filing is in progress to claim the synergistic benefits of the use of magnesium chloride as osmotic balancing agent, the use of gelling agents which are activated on contact with gastric juice, and the use of emetics.

3.2 Aqueous Concentrates containing Magnesium Sulphate, Magnesium Trisilicate and Emetic

A priority specification is in progress describing the use and combined benefits of aqueous concentrates of paraquat containing purgatives, preferably magnesium sulphate, gelling agents, preferably magnesium trisilicate and emetic, preferably PP796.

Consideration is being given to publication (Research Disclosures) of the observed safening due to magnesium chloride and emetic alone as these are not protectable by patents.

4. HERBICIAL ACTIVITY OF MULTIPLE EMULSIONS (Mark H Williams and David Thomas)

Early glasshouse and preliminary method development field screen demonstrated that paraquat multiple emulsions showed equivalent herbicidal activity to 'Gramoxone'.

The major constraint to extensive field testing was the poor sprayability experienced with the majority of these early formulations.

The current lead and back-up formulations E181 (JF 12255) and E171 (JF 12254) were tested for efficacy against a range of grasses and broadleaved weeds in a 1990 UK trial. No differences in efficacy were seen between the formulations at either 3 or 7 DAA, and their performance was similar to that obtained by paraquat dichloride used as a standard. No spraying problems were encountered with either formulation.
See Appendix II.

UK FIELD TRIAL GB01-90H130

PARAQUAT MULTIPLE EMULSION EFFICACY SCREEN

% CHLOROSIS AVERAGED ACROSS ALL THE WEED SPECIES

FORMULATION	RATE	3 DAT	7 DAT
JF12254	62.5	15	23
	125	26	38
	250	35	54
	500	43	67
	1000	50	78
JF12255	62.5	14	25
	125	20	33
	250	33	49
	500	43	71
	1000	48	75
YF6219	62.5	13	21
	125	23	35
	250	35	55
	500	51	67
	1000	55	75

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5. COMMERCIAL OVERVIEW

In 1989, 17,000 tes paraquat were sold, generating sales of £190 million. The total non-selective contact herbicide market is continuing to grow in volume and value, although paraquat's share is declining. The major factor in this market growth, and the decline of paraquat's overall share has been the reduction in the glyphosate price prior to patent fall. Paraquat sales are still forecast to rise through the 1990's as manual labour continues to be replaced by chemical weed control methods.

As a result of glyphosate price erosion the commercial environment has clearly changed since the Safer Formulations Project commenced. Regulatory pressures however have remained constant with increasing concerns over paraquat's soil persistence has being added to concerns over toxicity.

Strategy

A proactive approach would demand promotion of the safer formulation in all markets. Price erosion has ensured that this is not now possible for the multiple emulsion formulations without loss of significant markets.

Development of a PQME formulation was always intended as part of a reactive formulation strategy. This was affirmed by the Executive in 1985 as a need for "on the shelf" formulations available to counter the threat of deregistration on toxicologial grounds. This need remains unchanged. The PQME will provide a fall-back option to help maintain registrations under toxicological pressure in more sophisticated markets. It can be used to react to the imposition of specific tox requirements which would otherwise prevent access to certain markets.

The PQME project has however opened up other potentially cheaper options. The commercial case for introducing a conventional aqueous concentrate using magnesium sulphate, magnesium trislicate and emetic to confer safety, needs to be assessed. Such a formulation might allow a proactive approach to be followed, if it proves to be lower cost.

The following examples demonstrate where a safened paraquat formulation from a basket of "on the shelf" options might currently be considered.

Denmark :

The Danish authorities have imposed toxicological criteria against which products are judged. Paraquat fails the criteria for the sub-chronic study. The court case continues, but the registration is clearly threatened.
Sales 1989 : 35,000 litres

Austria :

Paraquat sales are small, but likely to diminish altogether, without deregistration. Paraquat is now in the highest tox category - restricted to use by licensed contractors only, highly inconvenient to the majority of small farmers. The product is being squeezed out of the market.

Conclusion

There is still a commercial need for an "on-the-shelf" safer paraquat formulation. E171 and E181 seem to largely be within the original criteria but there are issues which need to be addressed prior to commencing work on further tox or a registration package.

- the safety and application properties need to be confirmed and maintained during scale up and storage of the formulation

- the process technology and the costs of large scale manufacture need to be defined

The case for the magnesium sulphate/magnesium trisilicate formulation option, especially if the anticipated safety margin of 5-fold improvement relative to 'Gramoxone' is realised, is financially more attractive.

The original incremental cost target of £1000 needs to be revisited in the light of (i) more formulation options now being available, (ii) continuing glyphosate price erosion.

APPENDIX I

PARAQUAT : STUDIES ON THE MECHANISM OF GASTROINTESTINAL ABSORPTION

Jon R Heylings

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.

JMPBMISC4
EMULFORM (21.2.90)

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FIGURE 1

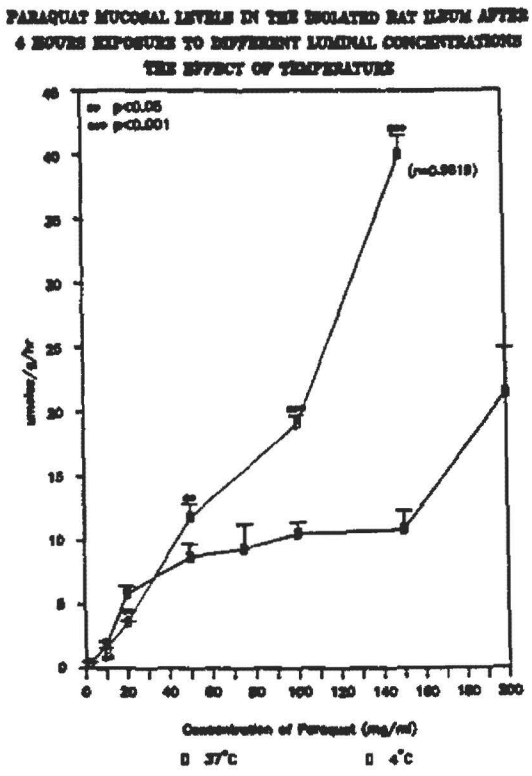

FIGURE 2

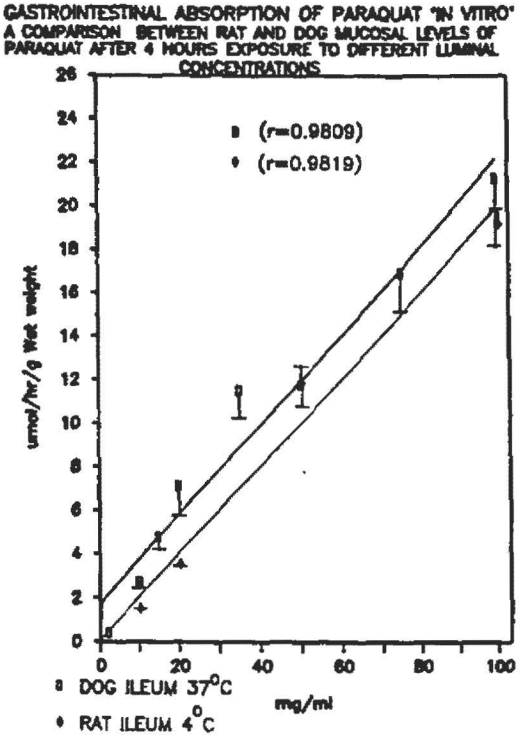

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FIGURE 3

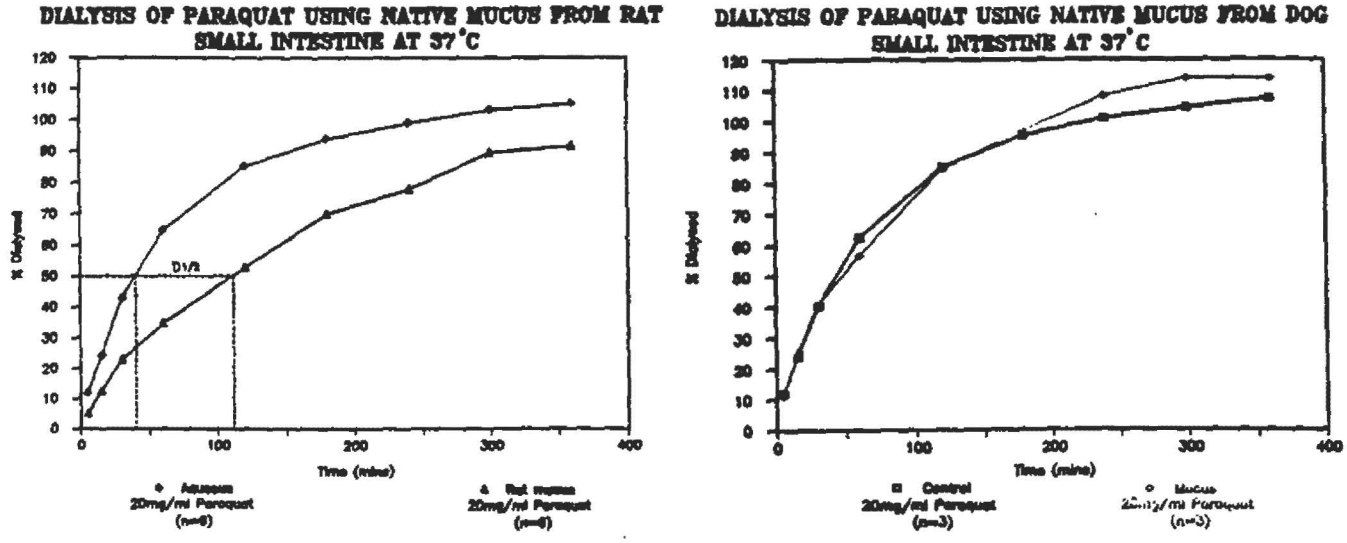

TABLE 1

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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APPENDIX II

TRIAL NUMBER: GB01-90-H130

TITLE: TO COMPARE THE EFFICACY OF TWO PARAQUAT MULTIPLE EMULSION FORMULATIONS FOR THE CONTROL OF A RANGE OF GRASSES AND BROADLEAVED WEEDS.

AUTHOR: M.H.WILLIAMS

LOCATION: HYDE FARM

ABSTRACT: This trial was a "look-see" screen for possible future development of paraquat multiple emulsion (PQME) formulations.

Two PQME formulations were tested (JF12254 and JF12255) for efficacy against a range of grasses and broadleaved weeds. Comparisons were made to a 10% solution of paraquat dichloride + emetic (YF6219).

It was also necessary to monitor the sprayability of these formulations.

No differences in efficacy were seen between the formulations at either 3 or 7 DAA, and their performance was similar to that obtained by paraquat dichloride used as a standard.

No spraying problems were encountered with any formulation.

KEYWORDS: Paraquat multiple emulsion
Paraquat dichloride
Broadleaved weeds
Grasses
JF12254
JF12255
YF6219

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TRIAL NUMBER: GB01-90-H130

OBJECTIVES:

1. To assess the sprayability of the paraquat multiple emulsion formulations JF12254 and JF12255 under field conditions.

2. To compare the efficacy of JF12254 and JF12255 for the control of a range of grasses and broadleaved weeds.

3. To compare the efficacy of the experimental formulations with paraquat dichloride (YF6219).

CONCLUSIONS:

1. No problems were encountered in spraying any of the formulations.

2. JF12254 and JF12255 performed similarly across the rates tested at both 3 and 7 DAA.

3. The control exhibited by the experimental formulations closely matched the control achieved by the standard paraquat dichloride + emetic, when averaged across the weed species.

TRIAL NUMBER: GB01-90-H130

INTRODUCTION:

This trial was carried out as a "look-see" screen for two new PQME formulations, which were hoped to overcome previous problems of sprayability, and at the same time exhibit control of a range of grasses and broadleaved weeds.

The formulations tested were JF12254 and JF12255. These were compared to a 10% solution of paraquat dichloride + emetic (YF6219).

METHOD:

This trial was sprayed on 09/02/90 using a hand-held 3 jet boom sprayer pressurised by CO2. The spray volume was 200 l/ha.

The screen was situated in Block E at Hyde Farm. The weeds were sown in September, and at the time of spraying were at the following growth stages:

Winter wheat	6-7 tillers, 1 node detectable
Wild oats	3-5 tillers, 1 node detectable
Perennial ryegrass	5 tillers, no nodes, 25 cm tall
Field pansy	13 leaves, 6 cm diameter, 3 cm height
Mayweed	18 stalks, 10 cm diameter, 2 cm height
Chickweed	4-5 stalks, 25 cm diameter, 10 cm height

Visual assessments of % chlorosis were made at 3 DAA and 7 DAA.

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TRIAL NUMBER: GB01-90-H130

RESULTS:

(See Tables 1-3)

JF12254 At 3 DAA the maximum control averaged across the species was achieved by the 1000 g/ha rate which gave 50% control.

Control of pansy at this stage was very poor.

The overall performance of JF12254 3 DAA was similar to YF6219 at the lower rates. However, at 500-1000 gai/ha the standard appears to be twice as active, achieving 51% with 500 g/ha, as apposed to 50% with JF12254 at 1000 g/ha, but this was not carried through to 7 DAA.

Control had improved considerably by 7 DAA (particularly with pansy) with an average of 78% chlorosis reached with 1000 g/ha, and no differences were seen between the levels of control attained by JF12254 and the standard YF6219.

JF12255 The results from the 3 DAA assessment show similar levels of control to JF12254. At 1000gai/ha, 48% chlorosis was recorded, averaged across the weed species.

Control of pansy was also very poor.

At 7 DAA, the levels of control were better, reaching an average of 75% chlorosis at 1000 g/ha. Across the rates JF12255 performed similarly to YF6219.

ABLE 1.

B01-90-H130

ARAQUAT MULTIPLE-EMULSION EFFICACY SCREEN

SSESSMENT - VISUAL % CHLOROSIS
12/02/90
3 DAA

IMPLE MEANS

	TRZAW	AVEFA	LOLPE	VIOAR	MATPE	STEME
JF12254 @ 62.5 g/ha + AGRAL 0.1%	10	27.5	10	0	12.5	30
JF12254 @ 125 g/ha + AGRAL 0.1%	22.5	42.5	20	0	35	37.5
JF12254 @ 250 g/ha + AGRAL 0.1%	30	45	25	7.5	45	57.5
JF12254 @ 500 g/ha + AGRAL 0.1%	37.5	65	35	7.5	55	60
JF12254 @ 1000 g/ha + AGRAL 0.1%	52.5	62.5	42.5	12.5	60	70
JF12255 @ 62.5 g/ha + AGRAL 0.1%	12.5	20	15	0	12.5	22.5
JF12255 @ 125 g/ha + AGRAL 0.1%	15	30	22.5	0	22.5	32.5
JF12255 @ 250 g/ha + AGRAL 0.1%	35	47.5	30	5	37.5	45
JF12255 @ 500 g/ha + AGRAL 0.1%	45	62.5	40	7.5	52.5	52.5
JF12255 @ 1000 g/ha + AGRAL 0.1%	55	70	45	12.5	42.5	62.5
PARAQUAT DICHLORIDE @ 62.5 g/ha + AGRAL 0.1%	15	17.5	17.5	0	10	15
PARAQUAT DICHLORIDE @ 125 g/ha + AGRAL 0.1%	25	32.5	20	5	20	32.5
PARAQUAT DICHLORIDE @ 250 g/ha + AGRAL 0.1%	45	35	30	10	42.5	50
PARAQUAT DICHLORIDE @ 500 g/ha + AGRAL 0.1%	57.5	65	40	12.5	62.5	70
PARAQUAT DICHLORIDE @ 1000 g/ha + AGRAL 0.1%	67.5	62.5	42.5	20	67.5	72.5

CHLOROSIS SYMPTOMS:
BROWNING - TRZAW, LOLPE, MATPE, VIOAR.
BLEACHING - AVEFA, STEME, VIOAR.
VEINING - VIOAR.

SPRAYED - 09/02/90

GROWTH STAGES:

TRZAW	6-7 TILLERS, 1 NODE DETECTABLE
AVEFA	3-5 TILLERS, 1 NODE DETECTABLE
LOLPE	5 TILLERS, NO NODES, 25 cm TALL
VIOAR	13 LEAVES, 6 cm DIAMETER, 3 cm HEIGHT
MATPE	18 STALKS, 10 cm DIAMETER, 2 cm HEIGHT
STEME	4-5 STALKS, 25 cm DIAMETER, 10 cm HEIGHT

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ABLE 2.

301-90-H130

ARAQUAT MULTIPLE-EMULSION EFFICACY TRIAL

SSESSMENT - VISUAL % CHLOROSIS
16/02/89
7 DAA

MPLE MEANS

	TRZAW	AVEFA	LOLPE	VICAR	MATPE	STEME
F12254 @ 62.5 g/ha + AGRAL 0.1%	27.5	30	22.5	5	15	35
F12254 @ 125 g/ha + AGRAL 0.1%	47.5	50	30	12.5	27.5	60
F12254 @ 250 g/ha + AGRAL 0.1%	55	60	37.5	47.5	45	80
F12254 @ 500 g/ha + AGRAL 0.1%	60	70	45	77.5	65	82.5
F12254 @ 1000 g/ha + AGRAL 0.1%	80	85	62.5	80	75	82.5
F12255 @ 62.5 g/ha + AGRAL 0.1%	22.5	32.5	20	15	25	35
F12255 @ 125 g/ha + AGRAL 0.1%	32.5	47.5	27.5	10	22.5	55
F12255 @ 250 g/ha + AGRAL 0.1%	52.5	60	37.5	30	37.5	75
F12255 @ 500 g/ha + AGRAL 0.1%	67.5	70	57.5	72.5	70	87.5
F12255 @ 1000 g/ha + AGRAL 0.1%	72.5	80	62.5	77.5	75	85
ARAQUAT DICHLORIDE @ 62.5 g/ha + AGRAL 0.1%	30	37.5	17.5	10	7.5	25
ARAQUAT DICHLORIDE @ 125 g/ha + AGRAL 0.1%	37.5	47.5	25	20	30	52.5
ARAQUAT DICHLORIDE @ 250 g/ha + AGRAL 0.1%	60	60	37.5	40	57.5	75
ARAQUAT DICHLORIDE @ 500 g/ha + AGRAL 0.1%	65	77.5	47.5	57.5	72.5	82.5
ARAQUAT DICHLORIDE @ 1000 g/ha + AGRAL 0.1%	80	75	50	75	77.5	92.5

RAYED - 09/02/90

OWTH STAGES:

TRZAW	6-7 TILLERS, 1 NODE DETECTABLE
AVEFA	3-5 TILLERS, 1 NODE DETECTABLE
LOLPE	5 TILLERS, NO NODES, 25 cm TALL
VICAR	13 LEAVES, 6 cm DIAMETER, 3 cm HEIGHT
MATPE	18 STALKS, 10 cm DIAMETER, 2 cm HEIGHT
STEME	4-5 STALKS, 25 cm DIAMETER, 10 cm HEIGHT

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