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PARAQUAT MULTIPLE EMULSION PROJECT - TRC REVIEW
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ABSTRACT

This document reports on the current commercial position of paraquat and reviews the progress made by the paraquat multiple-emulsions project, development of other formulations and further advances made in the understanding of paraquat uptake in the gastrointestinal tract.

KEYWORDS

PARAQUAT TRC REVIEW COMMERCIAL POSITION MULTIPLE EMULSION FORMULATIONS TWIN-PACK TOXICOLOGY REGISTRATION SAFETY PATENTS SDS PRODUCT

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SUMMARY

Paraquat remains a major product for ICI Agrochemicals with sales of 15,000 tonnes in 1987; generating a sales worth £192 million.

The product is coming under increasing pressure from regulatory authorities all over the world, particularly Western Europe, Japan and Malaysia.

In October 1986, the TRC agreed to a proposal by the Paraquat Research Workgroup to set up a project to develop a safer formulation of paraquat based on the principle of a multiple emulsion. The following requirements were set for such a formulation:

a) should have a 5-fold reduction in toxicity relative to 'Gramoxone',
b) have 90% biological efficacy of paraquat AC
c) a minimum concentration of 100 g ion/l
d) total incremental cost of formulation not to exceed £1000/tonne paraquat ion.

The TRC also approved further research into paraquat antidotes and alternative chemistry for a broad-spectrum contact herbicide.

A new project within the Biochemical Toxicology Section at CTL was set up at the end of 1986 to study the gastrointestinal absorption of paraquat with the view to understanding the basic mechanisms of paraquat absorption in various species and to use this knowledge to produce a less toxic formulation.

The results of these studies, together with clinical data obtained from poisoned victims, clearly established that on the basis of plasma levels, the dog is the most predictive species for human toxicity.

Multiple emulsion paraquat formulation based on diesel oil, B246, Synperonic NPE1800 and 2M NaCl gave more than 5x reduction in toxicity. However, initial formulations proved unstable and have poor dispersion properties, although freshly prepared products gave equivalent biological efficacy as 'Gramoxone' in the glasshouse. Replacing diesel oil with Isopar M improved dispersability, but failed to improve on toxicology. Significant advances have been made to the sprayability of the diesel oil-based formulation and several M-E formulations will be field tested in autumn 1988.

Priority specification describing the multiple emulsion formulation of paraquat 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 this UK application were filed in December 1987. Until the final form of the formulation is settled, it is not possible to make definitive search for patents which may dominate the formulations.

Bioefficacy and sprayability tests were also conducted on the SDS paraquat granules during 1987/88. The granules gave poor sprayability at low to medium spray volumes and exhibited electrostatic properties which poses the problem of safety-in-handling.

The research effort into paraquat antidote and treatment are included for reference in the appendices.

The current and proposed 1989 level of resources employed for paraquat multiple emulsion research and for research into paraquat antidote/treatment and alternative chemistry are as follows:-

Staff Level :	Formulation (TFT)	Chemistry (EJTC)	Weed Science/FTS (MRP)	CTL (LLS)
TO/SRO/RA	0.3	0.1	0.1	2.0
Post Doc.	-	1.0	-	-
EO/EA	3.5	0.1	0.3	3.5
Res. Student	1.0	-	-	-

Field trials resource sufficient for 12 trials is required for Japan, Malaysia and WER in 1989.

CONCLUSIONS

The technical feasibility of producing a commercially acceptable paraquat Multiple Emulsion formulation based on W/O/W emulsion appears very high. Several systems have been progressed, but the best of these are 10% paraquat M-E based on diesel, B246 and Synperonic NPE 1800 with $CaCa_2$ or $MgCl_2$ in the outer phase.

These paraquat M-E formulations are between 6x to 10x safer than paraquat AC in toxicological tests in the rat and the dog. Biological efficacy and sprayability parameters look good, but these need further testing under commercial use situations in the key paraquat market territories during 1989.

Whilst patents applications have been filed for the process, its likely infringement and the validity of Exxon's US patent needs further examination.

SDS solid paraquat formulations have not met sprayability and safety in handling requirements and should not be further progressed.

RECOMMENDATIONS

1. The current level of resources at CTL and Formulation Chemistry be maintained through 1989 to ensure maximum possibility of success for the Multiple Emulsion Project.

2. A proper study be conducted on the packaging, pack types/sizes and market acceptability of the twin-pack product incorporating the primary and secondary emulsions in separate compartments within the same pack.

3. The cost/benefit analyses and proposal for the development of a preferred paraquat M-E formulation be made in 4Q 1989.

4. A field evaluation resource sufficient for up to 12 trials be made available in 1989 to fully test the preferred formulation in Malaysia, Japan and WER.

5. The potential infringement and validity of the Exxon US patent 4244816 (expiry date 13 January, 1998) should be quickly examined once the final paraquat M-E formulation is decided.

6. The level of resourcing and future direction of the paraquat multiple emulsion research programme be critically reviewed at a TRC in 4Q 1989.

7. The current level of research activity into understanding the gastrointestinal absorption of paraquat by continued at CTL through 1989; and be reviewed at the 4Q 1989 TRC.

8. Other formulations of paraquat as and when they appear, should be evaluated for improvements in toxicology, sprayability and biological efficacy.

9. The SDS solid paraquat formulations should be rejected on grounds of its poor sprayability and safety-in-handling properties.

1. INTRODUCTION

In 1987, 15,000 tonnes of paraquat were sold, generating sales worth £192 million. However, the product is coming under increasing pressure from regulatory authorities all over the world, and particularly in West Europe, Japan and Malaysia. In business terms, regulatory restrictions/withdrawals have led to a significant reduction in the Group profit generated by paraquat, either through reduced sales or increased costs or both (e.g., Japan, Germany, Switzerland, Egypt and now France from mid 1988).

The problem lies in the very small amount of paraquat needed to cause death by ingestion (LD_{50} is around 15-20ml, while one mouthful is approximately 40ml). Safening additives (blue dye, stench, emetic) were introduced in the mid 1970's into 'Gramoxone' to prevent accidental abuse of the product, and 95%+ of current incidents are believed to be of suicidal intent. The emetic is effective in cases involving 'marginal' doses, especially with 'Weedol', but has not had any significant impact on survival rates at suicide dose levels. 'Gramoxone' poisoning cases display only a 20% survival rate compared with a more normal 80% survival rate for other chemical poisoning cases. There is thus a strong media/regulatory lobby (often fuelled by deep concern within the medical profession) for the withdrawal of paraquat sales in many countries. Frequently the lobby has little appreciation of the agricultural benefits accruing from the usage of paraquat. This deficiency is being countered by the preparation of a series of leaflets on the proven benefits obtained from using paraquat in various crops. These are targetted at regulatory officials and non-agricultural critics who do not appreciate the value of paraquat to world agriculture.

We see no reason to change pro-actively from our current formulations. However, the Executive in July 1985 re-affirmed the need for the reactive strategy of developing alternative formulations to a commercial state 'on the shelf'. This would provide a 'basket of options' to offer Regions/ Regulatory Affairs Section when faced with a paraquat regulatory crisis. None of the alternative formulations currently available offer an economically acceptable solution to the suicide problem either to ICI or to the farmer: significant dilution leads to vastly increased formulation and packing costs, while the solid formulations available so far are prohibitively expensive and can still be used for suicidal ingestion. None of the alternative formulations available reduce ai toxicity per se, so suicidal abuse could not be eliminated. The addition of diquat in a mixture with paraquat gives a modest reduction in overall toxicity. However, diquat is more expensive than paraquat to manufacture and is less effective on grass weeds.

The primary objective of the multiple emulsion project is to develop a formulation which will maintain existing registrations for paraquat by producing a significant increase in the survival rate from poisonings (from whatever cause). This must be done without incurring an excessive cost penalty to the business (a formulation and packing VPC penalty to ICI of less than £1000/te ion), without reducing product effectiveness/ convenience/safety for the farmer. Such an 'economic solution' to the business problem described above would then be available as part of the current reactive strategy for paraquat regulatory crises or possibly even for consideration as the basis of a pro-active strategy for formulation change.

It is recognised that the research programme may result in formulations that do not meet all the primary objectives. A secondary objective is therefore to produce a formulation which, although not fulfilling all criteria, can be regarded as a candidate for the 'basket of options'. Using a twin-pack could circumvent technical problems, which could allow us to develop a product that in all other respects meets the primary objectives. Such a solution would not replace the primary target, but could be regarded as an adequate fall-back in some markets.

The need for viable alternative formulations becomes increasingly more pressing. Further restrictions/bans are possible towards the end of 1988.

2. FORMULATION RESEARCH

2.1 Research Hypothesis

In the last TRC report we postulated that formulation of paraquat dichloride as a multiple emulsion should, in principle, reduce toxicity. Basically a concentrated paraquat dichloride solution (of the order of 300-400g/l) is emulsified into an oil (e.g. paraffinic oil, diesel oil, soyabean oil, white oil etc.) using an emulsifier with low hydrophile - lipophile balance HLB (Emulsifier I) and a high speed stirrer to produce a water in oil (W/O) emulsion (with a volume fraction of 0.45-0.65). The primary W/O emulsion is further emulsified into an aqueous solution of NaCl (to maintain the osmotic balance and aid emulsification) using an emulsifier with high HLB (Emulsifier II) and a low speed stirrer to produce a water in oil in water (W/O/W) multiple emulsion (with a volume fraction of 0.4-0.6). The success of producing a multiple emulsion depends to a large extent on the proper choice of emulsifiers, oil, volume fractions and stirring rates.

Initially, we set outselves the following targets :

(a) five fold reduction in toxicity of the formulation relative to Gramoxone;

(b) at least 90% of the biological efficacy of Gramoxone;

(c) a minimum paraquat ion concentration of 100g/l;

(d) total cost of formulation ingredients not to exceed £1000 per tonne of paraquat.

In order to achieve the above objectives we set ourselves a research programme using an iterative procedure. The initial stage was to investigate whether formulation of paraquat as a multiple emulsion type could reduce toxicity. Before embarking on any in vivo testing, we used simple physical testing methods to investigate the concept. We started with the following hypothesis. If paraquat is contained in a water droplet that is entrapped in an oil drop, it will need to cross the oil barrier, before being released into the outer aqueous phase. Moreover, Emulsifier I which surrounds the water droplet will produce another barrier to transport. By making the osmotic pressure of the solution outside the multiple emulsion drop equal to or slightly less than that of the paraquat concentrate we will also reduce transport significantly. Any material in the oil phase, e.g. high molecular weight polymers, may also reduce

transport. All these effects when combined together should, in principle produce a delayed release sufficient to reduce toxicity. On the other hand, when the formulation is diluted with water during application, the osmotic balance is sufficiently disturbed to allow release of the paraquat ions. Moreover, when the drops impinge on the leaf surface, the oil may spread, and multiple emulsion drops will coalesce at the surface thus maintaining biological efficacy through paraquat availability.

Initial research was carried out in an attempt to investigate the parameters that affected multiple emulsion formation and leakage. The following parameters were thought to be important:

2.1.1 Nature of Emulsifier I

To produce a good barrier the film needs to be coherent and possibly viscoelastic. In this respect polymeric surfactants (ex ICI Speciality Chemicals) are probably the most suitable. Of these two main surfactants were investigated, namely B246 (a block copolymer of polyhydroxystearic acid (PHS) and polyethylene oxide (PEO)) and E475 (a copolymer of polyisobutylene (PIB) succinic anhydride and monoethanolamine). The basic principle is to have part of the molecule soluble in the paraquat dichloride (the anchoring groups) and part soluble in the oil phase (the stabilising chains). For B246 the anchoring groups are PEO whereas the stabilising chains are PHS. For E475, the ethanolamine group is the anchoring part while the PIB is the stabilising chain. It should be mentioned that for good stability and film coherence, the stabilising chain has to be strongly solvated by the medium.

2.1.2 Nature of the Oil Phase

This is important for two reasons, namely as an effective barrier for paraquat transport and for solvating the stabilising chain. For PHS it was thought that an oil that contains some aromatic component should be preferable, e.g. diesel oil. For PIB, an aliphatic oil such as isoparaffinic oils would be preferable. Bearing that in mind we have investigated three oils, namely Isopar M, diesel oil and soyabean oil.

2.1.3 Nature of Emulsifier II

As mentioned above this has to be a high HLB emulsifier with sufficient hydrophilic groups to stabilise the large oil drops (that contain the paraquat dichloride solution dispersed in them). Various emulsifiers were investigated of which the Synperonic NPE1800 (nonyl phenol propylene oxide (13 units) ethylene oxide (27 units) and its analogues containing 48, 79 and 174 moles of EO to be referred to as Synperonic NPE A, B and C respectively) proved to be the most promising. Clearly the EO content that is necessary for stabilisation and subsequent dispersion is important. Moreover, it was thought that another "gel" barrier would be required to prevent coagulation of the large multiple emulsion drop. This barrier could also delay paraquat ion transport. If a polymer coating can be engineered that "gels" under acid conditions (pH in the stomach can be as low as 1) improved safety will be realised.

2.1.4 Electrolyte Nature and Concentration in the External Phase

As mentioned above one needs to balance the osmotic pressure of paraquat dichloride in the internal water droplets. However the activity constant of paraquat dichloride concentrate is not known. In the case of NaCl, the concentration was thought to lie between 1 and 2 moles/litre. Experiments were carried out whereby the NaCl concentration was varied between the two limits. Unfortunately, formulations based on NaCl did not disperse into water after aging for two days. For that reason we have systematically investigated the effect of the nature of the electrolyte on dispersibility. After a screening exercise using various electrolytes at various concentrations, we arrived at the conclusion that $CaCl_2$ and $MgCl_2$ are probably the best candidates. Formulations prepared based on $CaCl_2$ or $MgCl_2$ (0.75 and 1.5mol dm^{-3}) remained fluid on storage at room temperature for more than three months. The dispersion is certainly better than that using the NaCl, but by no means entirely acceptable. Improvements are still needed to reduce flocculation of the system on dispersion.

To measure paraquat leakage from the multiple emulsion we have devised a simple dialysis technique. The multiple emulsion is placed in visking tubes which are placed in distilled water and kept rotating for about 24 hours. The paraquat concentration in the dialysate was determined by U.V. and then the leakage expressed as a percentage of the total paraquat in the formulation. Alternatively, the rate of leakage of paraquat can be determined using a continuous dialysis method. In this case, the dialysis tube is placed in a thermostated glass tube through which water continuously flows. The aqueous solution is passed through a spectrophotometer cell to monitor the paraquat concentration as a function of time. Our aim is to prepare multiple emulsions that leak less than 10% before sending them for in vivo testing by CTL. The multiple emulsion drops are also assessed using optical microscopy.

2.2 Progress Made to Date

From the above first stage research programme the following trends were obtained:

a) A multiple emulsion prepared using B246, diesel oil, Synperonic NPE1800 and 2 molar NaCl in the outside phase (100g paraquat ion/l) gave more than 5x reduction in toxicity (based on measurement of absorption in the blood of dogs, see below). As mentioned above this formulation does not disperse, gels on storage and we believe is not a practical solution. Increasing the EO chain length of NPE1800 e.g. NPE C gave improved dispersion, and preliminary in vivo testing showed a reasonable reduction in toxicity (probably not as high as 5x). However, the most promising line is to replace NaCl with $CaCl_2$ or $MgCl_2$.

b) A multiple emulsion prepared using B246, Isopar M, Synperonic NPE1800, 2 molar NaCl showed better dispersion but higher leakage of paraquat. The latter was reduced by incorporation of a small amount of E475 (7:1 B246 to E475). Unfortunately, this

formulation does not satisfy the toxicity criterion. Work is now in progress to reduce the leakage of this formulation by incorporation of another oil with some aromatic character. Exxsol D80 and Escaid 100 are being investigated alone and in combination with Isopar M. Preliminary data shows some reduction in leakage with increase of the aromatic component in the oil.

c) Gel Coatings As mentioned above incorporation of a gel should, in principle, reduce paraquat leakage. Various polyelectrolytes were investigated using the Isopar and Diesel oil systems. Of these polymethacrylic acid (PMe) in combination with Al^{3+} seems to be the most promising. When added to the formulation at pH 5-6 it does not produce any gel since the polyelectrolyte is sufficiently dissociated. However, when dispersed into a pH 1-2 medium gelation occurs. The paraquat is only released slowly from the gel structure. Work is in progress to optimise the system by changing the following parameters : PMe concentration and molecular weight, Al^{3+} ion concentration, pH of the final formulation, various polymers in combination with PMe e.g. Poly (vinyl alcohol), poly (vinyl pyrrolidone) poly (ethylene oxide) etc.

2.3 The Twin Pack Concept

At the request of Fernhurst we have investigated the possibility of using a twin pack, one containing the primary emulsion and the other containing a surfactant solution that can rapidly disperse the primary emulsion forming a multiple emulsion. Various systems were studied of which the following is the most promising. For the primary emulsion, the diesel oil system based on B246 (which is intrinsically 5x less toxic than Gramoxone) was found to be the most suitable.

For the surfactant solution, a mixture of Synperonic NPE1800 with alkyl glucoside or ether sulphate prove to be promising. Work is in progress to optimise this formulation and to increase the paraquat concentration (to 200g l^{-1}) in the primary emulsion.

2.4 Management Overview

Results to date suggests that a W/O/W paraquat M-E formulation which is stable on storage, readily miscible in water and sprayable can be produced by mid-1989. Significant advances have been achieved both in the diesel oil and Isopar M + gelling agent fronts. Future work aims at incorporating emetic colour and stench. The Project Team is confident of progressing a preferred formulation into development in 1989.

The twin-pack concept offers a further alternative to the "basket of options", but its technical feasibility and cost of packaging and user acceptability are suspect. These issues must be examined as soon as possible by Development Department and the Regions and a decision on whether to proceed should be made by 1Q 1989.

2.5 Future Work

Future work is envisaged in the following stages:

a) To improve the present formulations based on diesel oil and Isopar M. For the diesel oil formulation, we will look into possible ways of improving dispersion e.g. incorporation of Kelzan, polyvinylalcohol, while maintaining some safety (we may have to be contented with a factor of 3x reduction (6x based on 10% product) in toxicity at this stage). For the Isopar formulation we will attempt to reduce leakage while maintaining dispersion at a reasonable degree. This could be achieved by modification or replacement of the primary emulsifier and/or changing the oil. Our objective is to produce 2-3 formulations for field trials during the Autumn of 1988. Limited storage stability and process evaluation should be available on such formulations at this time.

b) Continue the research on the above formulations to reduce toxicity and improve dispersibility. This may require some major changes in the primary and secondary emulsifiers, together with the use of a redesigned antisettling system. Research will also be carried out using diesel oil precursor (i.e., without the additives) as a starting point and evaluate the relative importance of each additive. The latter may also be incorporated in the Isopar M formulations.

c) A programme designed to build in colour, stench and emetic as appropriate will be initiated as soon as a field testing candidate formulation is finalised. Building in the emetic into the outer aqueous phase may have considerable toxicological advantages over the current Gramoxone system. Studies on the incorporation of diquat into multiple emulsion formulations will also be attempted.

d) It should also be noted that collaborative work is ongoing with staff at Richmond Research Centre. The concept of using microencapsulation as an alternative to liquid membranes in the W/O/W system is being investigated by Dr Scher and his team. This may further reduce/delay paraquat uptake in the gastrointestinal tract.

A consultative agreement will be developed in the future with Professor Florence at the University of Strathclyde. This will support an ongoing research programme investigating the fundamental properties of multiple emulsions and their application to other pesticide formulations.

3. TOXICOLOGY OF MULTIPLE EMULSION FORMULATIONS

3.1 Introduction and Objectives

In man, following oral ingestion of a lethal amount of paraquat, plasma levels of the ion reach a peak usually within 2 hours. If there is no therapeutic intervention in these early hours following ingestion, then the usual sequence of events is that lethal concentrations of paraquat are

accumulated in the epithelial cells of the lung and death results from pulmonary failure. One approach to reduce the oral toxicity of the herbicide is to modify the formulation in order to reduce its intrinsic absorption from the Gastrointestinal tract. Furthermore, by gaining detailed knowledge of the mechanism and site of paraquat absorption, current therapeutic approaches may be improved.

To investigate potential safer formulations of paraquat a new project within Biochemical Toxicology Section at CTL was set up at the end of 1986 to study the Gastrointestinal Absorption of the herbicide. One objective of the programme is to understand the basic mechanisms of paraquat absorption in various species and then ultimately to use this knowledge to produce a formulation of paraquat which is less toxic to man.

There are important considerations in the use of animal models to predict the toxicity of paraquat formulations and their likely toxicity in man:

a) Extensive pharmacokinetic studies with paraquat in experimental animals together with clinical data obtained from poisoned victims has very clearly established that, on the basis of plasma paraquat levels, the dog is the most predictive species for human toxicity. Thus, judgement of likely safening in man with a new formulation of the herbicide has to be based primarily on dog data.

b) The ultimate evaluation of safening of an alternative formulation of paraquat can only be achieved once the product is sold and human poisoning cases are subsequently monitored.

The majority of the effort over the last two years has been devoted to the toxicology of Multiple Emulsion formulations of paraquat. The principle behind this approach is to encapsulate a paraquat concentrate in tiny droplets by a mixing process using a series of primary and secondary emulsifiers and an oil. The formulation, which has a final paraquat concentration of 100g/l, is a water/oil/water emulsion. Successful emulsification is usually assessed by dialysis. Formulations which demonstrate a low paraquat leakage are then evaluated toxicologically and for herbicidal efficacy. The ultimate goal of this approach is to provide a new paraquat formulation which has reduced bioavailability (and thus less paraquat absorption from the Gastrointestinal tract), but once diluted to a workable spray strength, will release paraquat by osmotic disruption of the emulsion droplets thus restoring the activity of the herbicide.

A strategy for evaluating animal toxicity and phytotoxicity has been developed between CTL and Jealott's Hill. New Multiple Emulsion formulations are first tested in rats to assess acute toxicity. Promising formulations using identical batches of Emulsions are evaluated by Formulation and Weed Science Section in order to test both sprayability and herbicidal efficacy. Following these assessments, more extensive bioavailability studies are carried out at CTL in dogs. A brief outline of the CTL studies is described below. More detailed descriptions of methodology and results, together with minutes of the meetings of the workgroup can be found in Reports (Series PJ9/WG12/87/01C-10C).

3.2 Primary Evaluation of new Paraquat Emulsions in the Rat

The plasma profile following a single oral dose of paraquat in rats is different to that in dogs and man. Indeed, the LD_{50} in rodents is considerably higher (75-100mg/kg) when compared to dog and man (10-20mg/kg). These acute toxicity studies in rats are useful as qualitative estimates of toxicity prior to more accurate assessments of human risk which can only come from dog experiments. Furthermore, as a safeguard against dogs receiving an unstable Emulsion, this preliminary testing in rats has proved essential. All the Multiple Emulsion formulations of paraquat tested have contained 100g/l of paraquat ion. In this part of the toxicity evaluation five adult male rats are gavage dosed with this concentrate following an overnight fast. The standard dose levels chosen are 150-200mg PQ ion/kg which is about twice the LD_{50} for conventional paraquat formulations such as Gramoxone in rats. Animals are monitored for signs of toxicity for 10 days. Since the beginning of 1987, we have evaluated more than 100 different Emulsion formulations in rats, in addition to various control Emulsions and paraquat standards. Of these, about 15 have shown minimal acute toxic effects in rats at the paraquat LD_{50} x 2 dose level. The composition of the Emulsion formulation is complex. Each constituent is being systematically evaluated in an attempt to optimize the safening and sprayability properties of the Multiple Emulsion formulation. The rat studies are being continuously developed to meet the needs of the programme. Thus, by varying the dose levels and group sizes a more accurate assessment of the LD_{50} dose for each new Emulsion sent to CTL in 1988 has been instigated, to hopefully detect smaller differences in toxicity between formulations.

3.3 Secondary Evaluation of Paraquat Emulsions in the Dog

Multiple Emulsion formulations of paraquat showing a significant reduction in toxicity in rats are evaluated in the dog in more detail. A dog colony comprising 18 adult male beagles has been established. Animals are randomized as far as possible with three chosen to serve as Gramoxone controls. As in our rat studies, the neat paraquat concentrate is adminstered by gavage or capsule following an overnight fast. Clinical signs are monitored and blood samples taken over the first 24 hours after dosing. Plasma paraquat is measured by Radioimmunoassay. All control animals receive sub-lethal doses of paraquat (8mg/kg) as Gramoxone. Extensive studies with the same dogs have shown intra- and inter-animal variation to be low with respect to plasma paraquat area-under-curve (AUC) data (Figure 1). Using this data-base, and commencing at the same dose level (8mg/kg) with promising Emulsion formulations, direct comparisons of plasma paraquat levels can be made with Gramoxone. A minimum of 3 animals are used at any given dose level with a new Multiple Emulsion. Only those formulations which show a significant reduction in absorption of paraquat into the bloodstream at this low dose level are repeated at higher doses on subsequent occasions.

To date, we have assessed 15 different Emulsion formulations in the dog and dose levels have been increased in separate studies from 8, 16, 32, 48, 64 to 80mg/kg. By this experimental design the more toxic formulations can be detected (by high plasma paraquat levels) at doses which are non-toxic to

ABSORPTION OF PARAQUAT (GRAMOXONE) IN THE CONSCIOUS DOG 'IN VIVO'

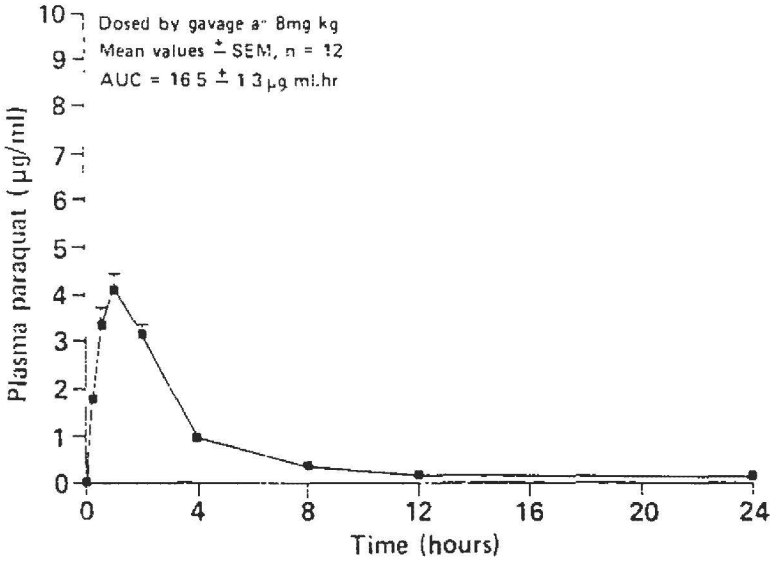

Fig 1. Effect of a single oral dose of paraquat (Gramoxone) at 8mg/kg in the conscious dog. Plasma levels reach a peak within two hours. Data represents the Mean ± SEM of three dogs dosed on four separate occasions one month apart.

Plasma Paraquat Levels with Multiple Emulsion Formulations

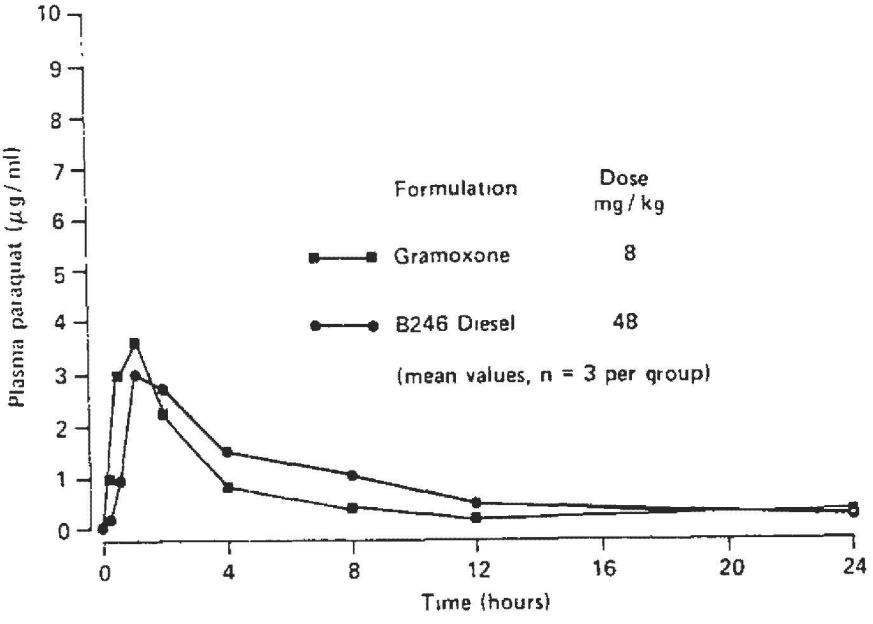

Fig 2. Effect of a single oral dose of the Multiple Emulsion formulation comprising:5% B246, Diesel, 1% NPE 1800 at 48mg/kg in the dog. For comparison, a contemporary Gramoxone control (8mg/kg) gives a similar plasma paraquat profile despite the six-fold difference in dose.

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the animals. Thus, dog mortality rates in the programme has been very low (<10%). Different degrees of safening have been demonstrated between different Emulsions. For instance, the most promising formulation in terms of toxicology comprises the primary emulsifier 5% B246 (primary emulsifier), Diesel oil, and 1% NPE 1800 (secondary emulsifier). This formulation of paraquat has been demonstrated to be non-toxic to dogs at 48mg/kg. Plasma paraquat profiles are comparable to an 8mg/kg dose of Gramoxone (Figure 2), and animals showed no signs of toxicity. This six-fold safening factor is put into perspective when compared with contemporary toxicity data generated last year at Inveresk Research International which gave an LD_{50} dose of 12mg/kg in the dog with conventional commercial formulations of the herbicide such as Preeglox and Gramoxone. Other closely related Emulsions where the oil phase contained Soya oil or Isopar M in place of Diesel oil have demonstrated only about a 2-3 fold safety factor in dogs when the plasma paraquat AUC was compared with Gramoxone (Figure 3). Furthermore, when the dose level of the Diesel based formulations was pushed even higher, significant levels of paraquat only reached the blood at a dose of 80mg/kg, and even at this dose level there were no fatalities.

3.4 Toxicology Overview

The hypothesis that paraquat, protected by a lipophilic phase, is less available to active uptake in the gastronitestinal tract is confirmed. Degree of 'safening' depends on the type of oil used and the stability of the W/O/W phases in the gut. More recent studies suggest that in toxicological terms, $MgCl_2$ is a better electrolyte than $CaCl_2$ in the outer aqueous phase for diesel oil-based formulations. The Isopar M M-E formulation is improved by the addition of Kelzan, which gels at low pH and delayed paraquat uptake over time. With further refinements of these formulations, we should have a paraquat M-E formulation which is at least 6x safer than the AC formulation.

3.5 Current and Future Work

During 1988, the major objective in the overall Emulsion programme has been to improve the sprayability of the Multiple Emulsion without compromising toxicity to unacceptable levels. Three approaches are being followed: Firstly, the present Diesel based system is being systematically modified to aid dilution properties of the formulation. Secondly, the Isopar M systems which have better dispersion properties have been modified by incorporating pH-sensitive gels to hopefully improve their toxicity profiles. Formulations are sent to CTL for toxicological evaluation once judged to have suitable physico-chemical properties in addition to low paraquat leakage rates. A third approach currently under investigation is the separate pack system. The toxicology of a Primary Emulsion based on B246 and Diesel oil has been vrey promising and may well be our safest paraquat-containing Emulsion tested so far. We are in a position to test and report on new formulation within a short period of time. Regular technical meetings will continue to ensure close liaison between CTL and Agrochemicals. In our efforts to discover a safer paraquat formulation, the Multiple Emulsion programme will remain as our priority.

TOXICOLOGY OF MULTIPLE EMULSION FORMULATIONS OF PARAQUAT

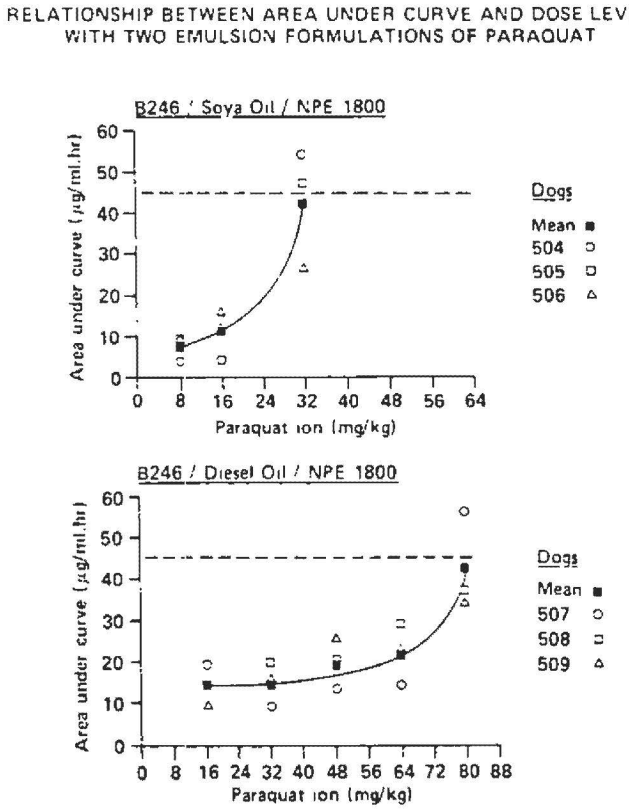

Fig 3. Effect of increasing dose levels of two Multiple Emulsion formulations of paraquat in the dog. Formulations of B246 and NPE 1800 which differ in the nature of the oil show different absorption into the blood. Thus, a Soya oil formulation (upper panel) was toxic at 32mg/kg, but there was much less paraquat absorption from a Diesel formulation which only showed marginal signs of toxicity at 80mg/kg. The dotted line represents the AUC value below which there have been no observable signs of toxicity.

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In conjunction with the Emulsion Programme, a Research Group has been set up at CTL to study the site and mechanism for paraquat absorption across the Gastrointestinal tract. We have developed a number of techniques to study paraquat absorption both in vitro (using isolated tissue) and in vivo (in anaesthetized animals). Studies are underway which are addressing the movement of formulations through the GI tract, and exactly how the herbicide reaches the bloodstream. Mathematical modelling is attempting to target the site of absorption based on this data. Thus, it may be possible in the future to improve therapeutic regimes following paraquat poisoning and to develop less toxic formulations once a better understanding of the absorption process is gained.

4. BIOLOGICAL ACTIVITY OF MULTIPLE EMULSIONS

Multiple emulsion formulations of paraquat undoubtedly show exceptional promise for reduced mammalian toxicity. Herbicidal efficacy can be good. The single factor which prevents progression to the field is the poor sprayability characteristics of these formulations. Unfortunately, those formulations which are most safe to dogs tend to exhibit the greatest sprayability problems.

An extensive screening programme during '87 attempted to improve sprayability characteristics through formulation modification with minimum compromise to mammalian safety. Parallel studies at Jealott's Hill and CTL attempted to correlate trends in sprayability, herbicidal activity and safety with variations in the formualation recipe. A four species herbicidal bioassay was implemented to facilitate high throughput screening of new formulations plus a quantitative, if crude, test for sprayability.

The current conclusions from this work can be summarised as follows:

1. Multiple emulsion formulations have been glasshouse tested in the absence of added surfactant and found to be as active as 'Gramoxone'. Formulations based on 'Isopar' tend to be more effective than those based on diesel oil.

2. In a limited number of cases 'Agral' has been added and has boosted the activity of PQ ME formulations above that of 'Gramoxone'.

3. Four PQ ME formulations have been field tested in a preliminary method development screen and found to be as active as 'Gramoxone'. In the latest field test, the Twin-pack formulation showed the least acceptable dilution properties.

4. The above statements apply only to formulations which have been tested under artificial conditions, i.e., sample < 2 days old and diluted to spray volume < 5 minutes prior to spraying.

5. SPRAY CHARACTERISTICS OF PARAQUAT ME

The behaviour of PQ ME formulations on dilution to spray strength has been a major problem to date. Three aspects have been investigated:

a) the initial dilution characteristics on addition to the water
b) the subsequent stability and behaviour of the diluted formulation
c) the behaviour in spray equipment.

5.1 Initial Dilution Characteristics

Many of the early formulations showed poor initial dilution properties, failing to break up and disperse throughout the water. Considerable agitation was required to prepare any sample for biological testing. These problems have now been overcome by formulation development, and good initial dispersion can be achieved.

5.2 Stability after Dilution

A characteristic of the formulations is that subsequent to the initial dilution a coagulation/flocculation process occurs which can lead to significant phase separation. This was a major problem with early formulations, but has again been significantly improved. It has not been eliminated however and can lead to problems in the spray equipment.

5.3 Behaviour in Spray Equipment

This aspect is considered to be the one requiring the greatest future attention. There is still a problem with the acceptability of the coagulate formed with time. With the latest formulations this tends to be a very soft, creamy form that does not generally block filters or nozzles but tends to leave a 'scum' and gelatinous deposit in the spray tank and lines. Depending on the formulation this can be very difficult to remove by washing and may require the use of solvents. There is evidence to suggest that the effect is worse on 'plastic' surfaces - such as typically used in knapsack sprayer tanks.

The acceptability of any candidate formulation is judged on a 'cascade' basis, in that the requirements for testing by Weed Science are more tolerant than those for Field Testing and those again are more tolerant than those for the farmer. To date the preferred formulations are judged to be viable for field testing providing certain handling criteria are followed, but are not judged to be acceptable to a farmer.

Further work is required on this interface between formulation properties and user acceptability and techniques of assessment improved from the present subjective judgement.

5.4 Twin Pack Emulsion

Early formulations of this concept where the 'safe' primary emulsion and its emulsifers are mixed immediately prior to dilution have been studied and show excellent spray handling characteristics, with no evidence of stability or residue problems. Unfortunately, the concept requires complex pack developments to make it vialble and a more recent batch used in the field tests has given high level of residues.

6. SDS PARAQUAT GRANULES

6.1 Biological Efficacy

A sample of U-83028 was evaluated in the glasshouse at Jealott's Hill. Taken across all grass species and application rates, U-83028 gave equivalent bioefficacy as paraquat AC. Its activity on some dicotyledons were inferior to paraquat AC, particularly at the higher rates of 200 and 400g ion/ha. At these rates, the spray solution appeared gelatinous, (spray volume used was 200 l/ha) and affected the spray pattern, which has given rise to the lower kill observed due to poorer spray coverage.

6.2 Handling Hazard

Apart from the problems of sprayability at low volumes, the granules appear to have electrostatic properties and could have problems of safety in handling the product. This could also pose problems of explosivity, which is currently being checked. The sprayability issue may not be as serious when high volume (1000 l/ha) applications are used, e.g., Japan.

6.3 Sprayability

The latest SDS Bioteck formulation - U83028 - has been tested. This shows much improved handling and gelling characteristics. However, when sprayed through typical Japanese knapsack sprayers with hollow cone nozzles the polymer shows a severe adverse effect on the spray formulation - virtually preventing the formation of the spray cone. This makes it very impractical to use. However, the effect is a function of the nozzle designs and the product is totally acceptable through - for example - a Polijet nozzle. This creates a difficult market situation. However, the present formulation would not be acceptable at more normal volume rates than the Japanese 1000 l/ha, because of too high a polymer content. Thus the required balance between gelling effect for safety and lack of effect on atomisation would have to be found.

6.4 Future Work

On the basis of its poor sprayability and potential hazards in handling, the SDS solid paraquat formulation would have major product stewardship problems. In Japan, cone nozzles are the most commonly used nozzles and it would be unacceptable/inpractical to switch to polijets. The SDS formulation, therefore, should not be further progressed.

7. PATENTS

7.1 Multiple Emulsion Formulations : ICI Case PP 34163

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 this UK application were filed as follows in December, 1987:

a) European Patent Application designating the following countries:

UK	Luxembourg
Austria	Netherlands
Belgium	Sweden
France	Switzerland
W Germany	Spain
Italy	Greece

b) National applications:

USA	South Africa
Canada	New Zealand
Japan	Hungary
Australia	Israel
Portugal	Taiwan
Philippines	Korea
USSR	China
Denmark	Argentina
Egypt	Turkey
Brazil	Pakistan
Malaysia	

The first official action dealing with the patentability of the multiple emulsions has now been received. This has been issued by the United States Patent Office, which considers that patentability has not been demonstrated by the information given in the specification. It will be necessary to submit detailed evidence of the reduced toxicity of the emulsion formulations as shown by experiments with laboratory animals.

7.2 ICI's Freedom to Manufacture and sell Multiple Emulsion Formulations of Paraquat

Until the final form of the formulation is settled, it is not possible to make definitive search for patents which may dominate the formulations. However, if the formulation involves the use of an agent to coat the globules of the emulsion to improve its stability, there is a risk of infringing Exxon's US patent 4244816 (expiry date 13 January, 1998).

Exxon has filed this patent in seven other countries outside the US including Japan. The full implications of their filings will need to be addressed once the preferred paraquat M-E is decided.

8. PARAQUAT TREATMENT AND ALTERNATIVE CHEMISTRY RESEARCH

The 1986 TRC approved for resources to be expended in paraquat antidote and treatment research. The research effort into the paraquat treatment is directed at acquiring a better understanding of paraquat uptake in the gastrointestinal tract, which has been particularly useful to the paraquat M-E project.

The other research effort is in Chemistry; both towards the discovery of a potential paraquat antidote as well as a probable alternative to paraquat.

Progress and level of success in both the toxicological studies and chemical effort are presented in Appendices 1 and 2 respectively.

APPENDIX I

PARAQUAT : ANTIDOTE/TREATMENT RESEARCH EFFORT AND STRATEGY

The toxicity of paraquat to humans depends on the amount ingested. Paraquat is absorbed into the blood from the gastrointestinal tract with the majority of absorption probably occurring from a site distal to the stomach. From the blood, paraquat will diffuse into the cells of most organs. However, the lung has the ability to selectively accumulate paraquat so this organ achieves high concentrations and is consequently more severely damaged in comparison with other tissues. When paraquat is present in the cell, it redox cycles thereby initiating a series of biochemical reactions that leads to cell death, organ damage and the death of the patient.

Given this appreciation of the toxicology of paraquat, there are several steps in its mechanism of toxicity where it ought to be possible to interrupt the process and, in so doing, reduce its toxicity and increase the likelihood that the patient will survive. These can be summarised as:

i) Reduce or prevent the absorption of paraquat from the gastrointestinal tract into the blood.

ii) Remove paraquat from the blood.

iii) Prevent the accumulation of paraquat into the lung.

iv) Remove paraquat from the lung and other organs.

v) Prevent the redox cycling of paraquat in the lung and other organs.

Historically, we have explored all of these areas of research. The current recommended treatment which includes administering Fullers Earth to bind paraquat in the gastrointestinal tract and prevent its absorption, or the use of haemodialysis or haemoperfusion to remove paraquat from the blood, are at least rational in terms of our understanding of the mechanism of paraquat toxicity. To date, no effective measures have been developed to stop the accumulation of paraquat from the plasma into the lung, prevent its redox cycling in cells, or remove it from the body tissue.

Overall, the current recommended treatment makes little difference to the outcome of poisoning cases. This is largely because paraquat is rapidly absorbed into the bloodstream and quickly distributes into the body tissues. It is clear from humans plasma paraquat data that absorbents and haemodialysis/haemoperfusion must be given within a few hours (1-6hrs) of swallowing paraquat if they are to be of any benefit. Since the vast majority of cases of paraquat poisoning involve suicide attempts, it is relatively unusual for the patient to be hospitalised and treated in the first few hours. Furthermore, the quantities of paraquat taken by 'would-be' suicides is usually several times a lethal dose. This makes it even less likely that the marginal benefit that the recommended treatment provides will reduce paraquat fatalities.

It therefore seems logical to concentrate our efforts in reducing the intrinsic toxicity of paraquat formulations rather than continue to develop treatments for poisoning that by our best judgement are unlikely to significantly reduce mortalities. This has led to the search for multiple emulsion formulations (described separately). Despite our conviction that reducing the toxicity of paraquat formulations is the approach which is most rational and most likely to succeed CTL continues to carry out some research into improving the treatment of poisoning cases. This is because:-

i) There is a perceived registration need for antidotes or palliatives treatments of paraquat poisoning.

ii) Even with a safer formulation there will be cases of poisoning that will prove fatal unless an effective treatment is available.

iii) There is a strong product stewardship benefit in being able to state that there is an effective treatment to paraquat poisoning.

PARAQUAT ABSORPTION PROCESS IN ANIMALS

To support the multple emulsion research programme, CTL is attempting to determine the site of paraquat absorption in the gastrointestinal tract and the mechanism that allows it to pass into the bloodstream. Importantly, the comparative ability of the gastrointestinal tract from various species of experimental animals and man will be investigated. This may allow us to establish which species of experimental animal and which conditions of study are required to mimic the situation in man. 1.5 man years (£100,000) is devoted to this area in 1988.

CTL is also investigating claims that other absorbents of paraquat are better able to prevent its absorption than Fullers Earth, e.g., dextran sulphate. This effort is primarily support for ICI Japan since several Japanese researchers claim they have developed improved treatments.

PREVENTION OF ACCUMULATION INTO THE LUNG

It is unlikely that even if a very effective inhibitor of paraquat accumulation into the lung could be found, its use in the majority of cases of paraquat poisoning would prove effective. This is because the distribution of paraquat into the lung is likely to be complete before the inhibitor could be adminstered. However, it may improve the prognosis in some cases of poisoning and it would allow us to state categorically that there was an 'antidote' to paraquat if only it could be adminstered quickly. Consequently, there is still some limited resource devoted to this area.

PREVENTING THE REDOX CYCLING OF PARAQUAT

This area probably offers the most hope for some development in the treatment regime. It seems probable that the toxicity of paraquat is at least in part caused by the catalytic formation of H_2O_2 which in turn reacts with Fe^{2+} to generate hydroxy radical (OH*). This radical (OH*) is

amongst the most reactive identified. By chelating the free ion with desferrioximine (DF) it seems that paraquat toxicity can be reduced. However, at present this can only be achieved by the prophylactic adminstration of DF. This is of no clinical use. However, it appears that the reason DF does not work, after paraquat has been given, is due to its relatively poor adsorption into lung cells. We are interested to evaluate much more liquid soluble chemicals that have DF properties. These are currently being synthesised by a wide range of Institutes for other purposes (they are also very active anti-inflammatory chemicals). This work will be carried out in collaboration with other workers involved in DF research.

APPENDIX 2

PARAQUAT : ANTIDOTE AND ALTERNATIVE CHEMISTRY RESEARCH

CHEMISTRY - E J T Chrystal

1. ANTIDOTES

1.1 Objectives

To devise a compound which could act as an antidote to paraquat toxicity in mammals by either:

a) Aiding the removal of paraquat from the blood stream by intra-peritoneal dialysis.

b) Tightly binding paraquat in either the blood stream or gut to prevent the eventual uptake by lung tissue, but not the excretion of the paraquat complex.

c) Preventing paraquat accumulation in the lung tissue by inhibition of the polyamine/paraquat transport system in this organ.

These objectives are very desirable, but appropriate molecules, although likely to be active *in vitro*, are unlikely to be active *in vivo*.

1.2 Dextrins

1.2.1 Background

A concentrated dextrin solution has been used as an osmotically active medium for intraperitoneal dialysis, a treatment for kidney failure. Work at the Royal Postgraduate Medical School indicates that intraperitoneal dialysis could be used as a treatment for paraquat ingestion. During dialysis, a concentrated dextrin solution, with a high osmotic potential, is placed in the peritoneal cavity. The solution is diluted by a flux of water and solutes from the body tissues. If the dextrin solution is regularly exchanged and the body's water loss is replenished, solutes, such as paraquat, can be removed from the body tissues and fluids.

Chromotropic acid forms a complex with paraquat in solution. Theoretically, chromotropic acid could be attached to the dextrin molecule. Such a dextrin-chromotropate would complex paraquat, reducing the concentration of paraquat in solution and enhancing the removal of paraquat by dialysis from the body tissues.

1.2.2 Status

The attempted synthesis of a dextrin-chromotropate failed. An analogue of chromotropic acid, H acid, was used instead; a dextrin-H acid adduct was prepared using epoxide chemistry. While the adduct could absorb paraquat in an *in vitro* dialysis test, it failed to stop the uptake of paraquat by lung slices. No further work will be done on this topic.

Following the reports of dextran sulphate binding paraquat by Ukai, attempts were made to prepare dextrin sulphate. These have all failed.

1.2.3 Future Actions

No future actions are proposed in this area.

1.3 Paraquat Complexing Agents - Crown Ethers

1.3.1 Background

Recent publications have shown that crown ethers can form complexes with paraquat. From the x-ray chrystallographic structures of these complexes, an understanding of the geometrical requirements for the binding of the cationic centres of paraquat and diquat to selected crown ethers has been developed. It is now feasible to design crown ethers which could specifically bind either paraquat or diquat very tightly. Such crown ethers could offer the possibility of binding paraquat in the blood stream. The paraquat in the paraquat-crown ether complex would then be unavailable for uptake by the polyamine transport system in the lung tissue, but unlike the antibodies used in previous studies, the molecular weight of the complex should be sufficiently low to permit its excretion and the removal of the paraquat from the blood stream. (Certain crown ethers are very toxic. However, the mode of action for this toxicity is well known and it should be possible to design non-toxic crown ethers.)

1.3.2 Current Status

An ICI Agrochemicals wholly sponsored studentship has been funded in Dr Fraser Stoddart's research group at Sheffield University to study the binding interactions of paraquat and crown ethers. The first student left the project after one year. A replacement student has been working for one year and the project will run until October 1990. The current aims of the project are to relate the structures of the complexes in solution to those determined by x-ray in the solid state and to prepare water soluble crown ether complexing agents.

1.3.3 Future Actions

a) To design and synthesise paraquat high affinity water soluble neutral ligands.

b) To test the physical binding parameters of such crowns.

c) To test the ability of such ligands (crown ethers) to prevent the uptake of paraquat into lung tissue slice by the paraquat/ polyamine transport system.

1.4 Inhibition of Transport - Polyamine Analogues

1.4.1 Background

The paraquat dication and polyamines are structural mimics at certain biological binding sites. There is a CASE project in progress between CTL and Professor B T Golding, Newcastle University, to prepare suitable analogues of polyamines to either label the paraquat receptor in the lung, or block paraquat/polyamine uptake into lung tissue.

2. ALTERNATIVE CHEMISTRY

2.1 Objective

To find a compound with a similar herbicidal effect to paraquat but without paraquat toxicity, which is either:

a) An alternative bipyridyl.

b) A non-bipyridyl system, but with the same mode of action as paraquat.

c) A non-bipyridyl system with a different mode of action from paraquat.

Objectives b) and c) are no longer within the remit of this Work Group and are being considered under the Innovation initiative by the Contact and Burn Working Party.

2.2 An Alternative Bipyridyl

2.2.1 Background

Previous work on 4,4'-bipyridyl analogues of paraquat was reviewed in 1984 by Calderbank in TMJ 2299B. In general, herbicidal activity and oral toxicity are strongly correlated, although there are some exceptions. The report identified several possible actions. All these actions are outstanding.

Any work in this area can only generate another bipyridyl paraquat analogue. Such a close paraquat analogue would have potential registration problems and work in this area is only viable if registration is perceived as feasible.

Following the TRC in 1986, a meeting was held to review the feasibility of registration of novel bipyridyls. Two key registration issues, oral toxicity and rate of degradation in soil, were highlighted. Thus any replacement bipyridyl herbicide should not only possess reduced oral toxicity, but also be readily degradable in soil. It was agreed that synthetic chemical effort should be directed into a limited synthetic programme to prepare bipyridinium compounds with potentially degradable cationic centres.

One compound, R213036, the dimethoxymethyl bipyridinium dibromide, has been prepared. It is active on the herbicide primary screen at 0.25kg/ha. Its stability, oral toxicity and effect in the lung slice assay are currently being evaluated. Any further work will depend on the outcome of these studies.

2.2.2 Future Objectives

a) To obtain full data on R213036.