ICI **Plant Protection Division**

Report Series

PJ9/WG12/87/01C

# ABSORPTION OF PARAQUAT WORK GROUP - MINUTES OF THE MEETINGS HELD AT CTL ON 17 NOVEMBER 1986 AND 18 FEBRUARY 1987

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J R Heylings

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The Minutes of the meetings of the Absorption of Paraquat Work Group held on 17 November 1986 and 18 February 1987 at CTL are listed in this document.

Copies of the slides used in presentations to these meetings are issued separately as Appendices in "Absorption of Paraquat Work Group - Appendices to the Minutes", PJ9/WG12/87/02C. The Appendices to the Minutes will only be routinely sent to those who attended the meetings.

MINUTES OF A MEETING OF THE ABSORPTION OF PARAQUAT WORK GROUP HELD AT CTL ON MONDAY 17 NOVEMBER 1986

| | | |
|---|---|---|
| Present: | L L Smith (Chairman) | CTL |
| | R C Scott | |
| | C Rhodes | |
| | I Wyatt | |
| | S C Watson | |
| | D C Taylor | Pharms |
| | D Leahy | |
| | Th F Tadros | JH |
| | C Sales | |
| | D K Lawrence | |
| | E J T Chrystal | |
| | P J Bramley | Fernhurst |
| Apologies: | W D McClellan | JH |

## 1. WORK GROUP OBJECTIVES (LLS)

The Absorption of Paraquat Work Group is sponsored by PPD with L L Smith (CTL) and W D McClellan (JH) as co-leaders. The Group's objective is to progress and evaluate novel approaches to either reducing the toxicity of paraquat formulations or providing a treatment for paraquat poisoning.

Bi-monthly meetings of the Work Group will usually be held at CTL. The objectives of these meetings are:

i) To review progress.

ii) To provide a mechanism for information exchange.

iii) To provide an opportunity to present, develop and discuss ideas, without initially considering cost limitations.

## 2. PARAQUAT BACKGROUND

### 2.1 Business (PJB)

Paraquat was launched in 1961. Current sales are 15,000 ton ai per year in 140-150 countries worldwide. Annual sales are valued at £200m, which represents 30% of the plant protection business and provides 30% of the profit. The Autumn Business Review predicted a decrease in business growth in the mid 1990s to reach sales of 20,000 ton ai per year, contributing 20-25% of the total sales. This prediction did not allow for any major loss of product registration.

Glyphosate will soon be off patent, subsequently there will probably be several producers and price erosion in the broad spectrum sector of the herbicide market will occur. Registration has been lost in Germany because of environmental issues. A switch to a paraquat-diquat mixture has been required in other countries, reducing the margin. Thus paraquat sales will be under pressure from both commercial competition and review by the registration authorities.

PSAC at Fernhurst actively supports research into the reduction of the acute oral toxicity of paraquat (PQ).

2.2 Toxicology (LLS)

The aim of toxicological research in PQ is to reduce the movement of PQ from the gastrointestinal tract (GIT) into the bloodstream. PQ is subject to product abuse. There are 2000 deaths per year due to PQ poisoning and greater than 95% are a result of suicidal ingestion. (NB In Japan, there are 800-1000 suicides per year using PQ.)

In large doses, PQ causes rapid multiorgan damage and death; in low doses, the lungs and kidneys actively accumulate PQ resulting in lung damage, anoxia, fibrosis and eventually death. Approximately 50% of suicides are high dose cases.

The peak plasma level of PQ develops within one hour of swallowing. Thus any treatment must reduce the intrinsic toxicity of PQ, or prevent the absorption of PQ from the GIT, or remove PQ from the lungs and kidneys or inhibit organ/tissue damage, or facilitate the repair of damage.

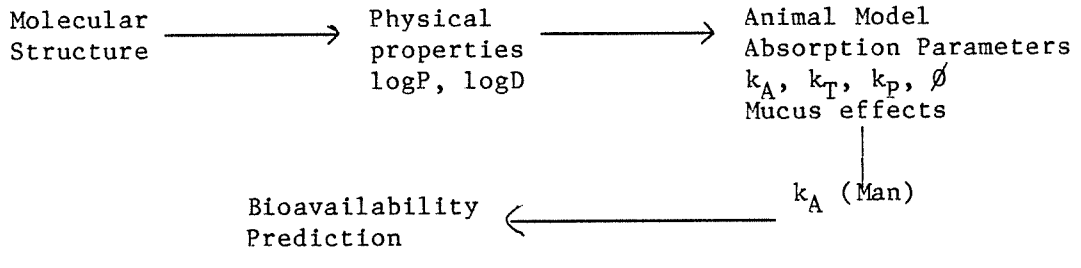
## 3. ABSORPTION PROJECT TEAM (DT)

The work of the Pharmaceuticals Division Absorption Project Team was described. The team's objective is to develop a model of the absorption of drugs from the GIT to aid both drug and dosage form design.

The fraction of drug absorbed is related to two parameters, ka, the rate of absorption and the residence time. The rate of absorption depends on the physical properties of the drug and intestinal mucosa; residence time depends on the site specificity of absorption and gut motility. For PQ in low doses, 5% is absorbed by the rat and 40% by the dog. (In the dog, there is an early peak plasma level as in man but not as in rat.)

The approach to developing a model is outlined in Scheme 1.

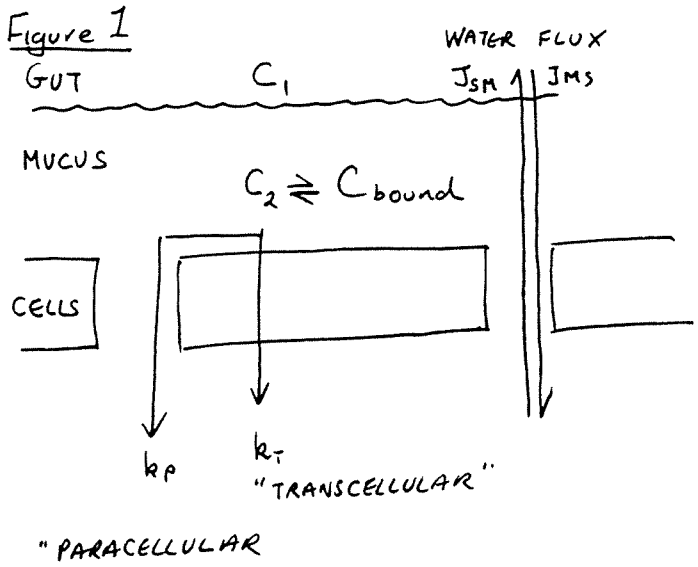
Scheme 1



Over seventy compounds with known absorption kinetics were studied. Their partition coefficients, logP and logD (logP corrected for ionisation) were determined in both octanol-water and PGDP-water systems. The absorption parameters were determined from in vivo animal models using in situ perfused intestinal loops. Two types of absorption process were identified.

For lipophilic compounds, rate of absorption was dependent on logD. (At high values of logD, the value of $k_A$ was constant due to the effect of the unstirred layer of water adjacent to the mucosa.) For hydrophilic compounds, $k_A$ was independent of logD. These observations are consistent with two types of absorption process, a transcellular process via the cellular lipid and a paracellular process via intercellular pores (see Figure 1).

Figure 1




The main conclusions of these studies are:-

1) Absorption of hydrophilic molecules is mainly paracellular and depends on water flux.

2) Absorption of lipophilic molecules is mainly transcellular.

3) For hydrophilic molecules, rate of absorption is highly dependent on molecular weight; for lipophilic molecules rate of absorption is highly dependent on logD up to a limiting value.

4) Anions are not readily absorbed.

5) Cations are readily absorbed.

6) Site specific absorption is greatest in the small intestine.

Good correlations were observed for $k_A$ (rat) and $k_A$ (man). However, PQ absorption depends on concentration and in rats a toxic dose causes water movement into the gut.

## 4. MULTIPLE EMULSIONS (CS)

Copies of the slides for this presentation are included in Appendix A.

The test multiple emulsions are prepared by emulsifying a concentrated aqueous paraquat solution into oil with an appropriate emulsifier, emulsifier 1, and subsequently emulsifying the oil emulsion into an aqueous solution of sodium chloride, using a different emulsifier, emulsifier 2. The sodium chloride is required to maintain the osmotic balance of the system. The final system is an emulsion of oil in water. The oil drops, however, contain droplets of aqueous PQ solution.

Any acceptable emulsion must:-

1) Possess a stable oil film to prevent movement of PQ from internal droplets to aqueous media.

2) Be stable when concentrated to prevent PQ release in GIT.

3) Be unstable on dilution to permit PQ release on the leaf surface.

4) Possess an adequate shelf life.

5) Contain a PQ concentration of 100g/l.

6) Be economic and easy to produce.

7) Possess a toxicity one fifth of Gramoxone.

To achieve these properties several parameters may be varied:

1) The primary water/oil emulsifier.

2) The secondary oil/water emulsifier.

3) The sodium chloride concentration.

4) The oil used to form the emulsions.

5) Other additives.

6) The process used to prepare the multiple emulsion.

Several multiple emulsions have been prepared and evaluated by physical studies:

| | | |
|---|---|---|
| 1) | Microscopy: | To determine the size of the multiple emulsion capsules. |
| 2) | Centrifugation: | To determine the percentage free uncapsulated PQ. |
| 3) | Dialysis: | To determine the leakage of PQ. |

The best three systems tested so far are:-

1) E475, 1% NPE 1800, Paddle stirring

2) E475:B246 (1:1), 0.5% NPE 1800, Paddle stirring

3) B475:B246 (7:3), 1% NPE 1800, Elado - ½ minute

Future work will be directed towards:-

1) Thickening the oil phase to reduce PQ movement.

2) Increasing the PQ concentration.

3) Generating a film around the oil drops.

4) Testing secondary emulsifiers from Paints Division.

## 5. ALTERNATIVE ANIONS - MORWET (IW)

Samples of morwet-paraquat formulations containing different ratios of Naphthalene sulphonate sub units to PQ have been prepared at JH and supplied to CTL for toxicology studies. In the morwet formulation studies (see Table 1) all formulations contained 10% weight/volume paraquat dication.

Table 1 - Morwet/PQ Formulation - Survivals for Groups of 2 Rats

| Ratio of PQ:Naphthalene | PQ Dose (mg/kg) 100 | 200 | 250 | 300 |
|---|---|---|---|---|
| 1:1.5 | 2 | 0 | | |
| 1:2 | 1 | 0 | | |
| 1:3 | 2 | 1 | | |
| 1:3 + 1% PUA | 2 | 1 | | |
| 1:3 + 1% NPE 1800 | 2 | 2 | 1 | 1 |

In comparison, $LD_{50}$ for undiluted Gramoxone is ca 50mg/kg, for 10x diluted Gramoxone is 120mg/kg and Paraquat Chromotropate is 700mg/kg.

## 6. CHEMISTRY

### 6.1 Alternative Anions

No further work is being undertaken in synthetic chemistry at JH.

### 6.2 Dextrins

Derivatised dextrins are required for further studies on intraperitoneal dialysis as a treatment for paraquat poisoning. Initial work is directed towards attaching chromotropate or H acid to a dextrin. Attempts at using

cyanuric chloride as the coupling agent have been unsuccessful. Future studies will explore the use of bis epoxides to couple H acid to initially sepherose and subsequently dextrins.

6.3 Crown Ethers

Work on crown ethers is directed towards the design of highly specific chelators for PQ which may act as antidotes to PQ poisoning.

A student is in place and work on the Sheffield collaborative project has begun. Initial synthetic targets will include crown ethers which incorporate dihydroxynaphthalenes as the aromatic spacing unit.

Work has begun at JH on the resynthesis of the Lehn crown ether for further studies at CTL.

6.4 Alternatives to Paraquat

The possibility of identifying a 4,4'-bipyridylium molecule of similar activity to paraquat but with reduced toxicity and without adverse environmental properties is being reviewed. A limited synthetic programme may be required after targets have been identified.

Examples of the Shell pentalenes with the same mode of action as PQ will be prepared to test this claim.

## 7. ACTIONS

1) LLS to arrange meeting dates for 1987.

2) CTL to test Absorption Project Team models to establish a suitable model for PQ absorption.

3) TT to select and provide several samples of multiple emulsions for testing for both herbicidal activity and reduced PQ toxicity for the end of January.

4) EJTC to progress dextrin/dialysis programme.

5) EJTC to progress the review of alternative 4,4'-bipyridiliums.

# MINUTES OF THE SECOND MEETING ON THE ABSORPTION OF PARAQUAT - SAFER FORMULATIONS

## HELD ON 18 FEBRUARY 1987 AT CTL

| | | | | |
|---|---|---|---|---|
| Those present: | L L Smith - Chairman | | J R Heylings) | |
| | | | R C Scott ) | |
| | W D McClellan ) | | I Wyatt ) | CTL |
| | E J T Chrystal) | | S C Watson ) | |
| | T F Tadros ) | PPD | T Auton ) | |
| | C Sales ) | | | |
| | P J Bramley ) | | A Garner ) | |
| | | | D Leahy ) | Pharms |
| | | | D C Taylor ) | |

1. FUTURE MEETINGS.

LLS introduced the meeting and discussed the organisation of the ABSORPTION meetings and the PQ RESEARCH WORK GROUP meetings planned for 1987.

It was agreed to keep the Management meetings separate from the technical sessions. It was stressed that in view of the need to make rapid progress in the area of safer formulations that the meetings arranged every two months to exchange technical information would go ahead as planned.

2. ANIMAL MODEL FOR MAN.

2.1 IRI Dog Studies.

Data was presented by RCS from the recent dog study carried out at IRI with four formulations of PQ. Mortality data and plasma profiles were presented for Gramoxone (20% PQ), Gramoxone (9% PQ), Preeglox (4.5% PQ and 4.5% DQ), and Preeglox without wetters. Neat formulations were used at an appropriate dose volume. Mortality data was similar between all four groups with LD50 values around 12mg/kg. Plasma levels of PQ generally were at a peak around 1hr, this declined rapidly and was virtually zero at 24hr. Profiles of Preeglox with and without wetters were very similar. Modelling mortality with area under the curve (AUC) may improve predictability of survival compared with the more variable parameter of peak plasma PQ concentration.

2.2 CTL Rat Studies.

Data was presented by LLS on the effect of surfactants with 45 and 70mg/kg PQ ion in rats. At 70mg/kg six of ten animals died in the group with surfactant whereas in the absence of surfactant animals though subdued and showing piloerection, all survived. The lower dose showed a similar difference in the extent of paraquat toxicity with and without surfactant. In contrast to the dog, the plasma PQ levels did not show early peaks in the first hours after dosing. At 45mg/kg there was a slow followed by a more progressive rise in plamsa PQ concentration in the presence of surfactant. Without surfactant plasma levels remained low and fairly constant over 50hrs. At 70mg/kg with surfactant present, the surviving animals had relatively low plasma PQ levels. A similar picture was obtained with diquat. Data was also presented on the paraquat and diquat levels in urine and faeces with and without surfactant.

2.3 Method Development.

A research group has been set up at CTL to study the absorption of bipyridyls from the gastrointestinal tract. JRH discussed some of the techniques which will be used to examine this in vitro and in vivo. Although the objective of this programme is to understand the difference between species with respect to PQ absorption and the possible mechanisms involved, an important area of study will be to investigate the absorption profiles of various new paraquat formulations which are currently being evaluated in acute oral toxicity studies. Discussion of the methodologies suggested that in vitro techniques may have limitations but may be useful as a primary screen to compare formulations. Effort will be concentrated on the development of in vivo animal models to study bipyridyl absorption. It was agreed that a pivotal aspect of this programme is to investigate the difference between the plasma paraquat profiles in rat and dog following oral dosing, in the light of the similarity of dog and human data.

2.4 Discussion and Actions.

In the general discussion around animal models for man, the use of the best animal model (rat vs dog) was considered. The rat may be good for extrapolation to man in the case of certain drugs, but it was agreed that with paraquat the dog is the more appropriate model. However the rat may be a useful primary screen so both species will be used to study formulations until we have a clearer picture of this species difference.

**ACTION: JRH to prepare a table of differences/similarities of rat, dog and human with respect to paraquat absorption.**

The effect of wetters and their enhancement of paraquat toxicity was discussed in the light of the present findings with Preeglox in the dog and the rat CTL studies.

**ACTION: PJB to discuss this aspect of inclusion of wetters with Peter Slade.**

One physiological difference between rat and dog which may affect the absorption profile of PQ is the control of gastric emptying. AG suggested the use of compounds which are good inhibitors of gastric emptying to study this possible influence. These compounds originally from Organics Division are being used by Pharms Division.

**ACTION: JRH and AG to discuss the use of such agents in our CTL studies.**

Absorption studies at Pharms Division involve administration of compound directly to the lumen of the small intestine. These studies in rats are predictable for man with certain drugs. If PQ is administered in this way, would the plasma profile be different from conventional oral dosing in rats?

**ACTION: JRH to discuss running such experiments in conjunction with DCT's group.**

3. MULTIPLE EMULSION STUDIES.

3.1 Formulation progress.

CS described the preparation and progresss in the area of multiple emulsions. The differences between the various formulations being tested at CTL were considered. Stability of the emulsions is an important consideration and various methods of acheiving stable and higher paraquat concentrations in the formulation were discussed. Tests on the emulsions include dialysis centrifugation, rheology and photomicrography. It was found that E475 was less stable than E471. Old and fresh emulsions have been submitted to CTL for toxicology testing. LLS asked how many emulsions could be submitted to CTL for toxicological evaluation and what was the rate limiting factor involved. TFT stated that there are so many variables that feedback is required before a strategy can be developed.

**ACTION: TFT to meet with JRH and LLS to discuss future work in this area and to arrange a manageable number of formulations which will be tested at CTL. The next batch would have to be tested in mid-March in order to report findings at our April 10th meeting.**

3.2 Phytotoxicity Progress.

WDM stated that this is still in progress.

3.3 Toxicity Testing Progress.

The effect of 10 emulsion formulations in rats was presented by JRH. Animals were dosed with 188mg/kg PQ ion and compared with a contemporary Gramoxone control. Formulation E475 which had been prepared two months prior to the study was no less toxic than the Gramoxone control. However, freshly prepared E475 showed less toxicity. Formulation E471 was less toxic than Gramoxone with all animals surviving three days. B246 was also less toxic than Gramoxone and was even more promising as a combination with E475. This data supports the stability data of the various emulsion formulations. JRH went on to discuss future work with the multiple emulsions in the dog.

**ACTION: Two formulations are to be tested at non toxic doses in the dog along with an equivalant Gramoxone control. Plasma PQ profiles will be thus compared. This will be co-ordinated by JRH.**

4. DEXTRAN SO4.

4.1 Paraquat Binding Studies.

Based on the rationale that co-administration of paraquat with Dextran sulphate results in reduced toxicity of the bipyridyl, in-house data is required to substantiate this claim. This polymer of glucose comes in a range of MWts (5000-500 000). EJTC described its properties and informed the group that he is awaiting results of the binding studies. It was agreed that its phytotoxicity would be investigated and that CTL would study dextran sulphate with various doses of paraquat.

**ACTION: EJTC to obtain binding study results for dextran sulphate.**

4.2 Phytotoxicity Studies.

WDM stated that data will be available by mid-March on the herbicidal activity of dextran sulphate.

**ACTION: WDM to update LLS on progress.**

4.3 Toxicity Testing.

LLS expressed an urgency for testing dextran sulphate/paraquat combinations in the rat and dog. The Japanese material has not arrived to date but we will proceed with material obtained through Sigma.

**ACTION: JRH to arrange studies in rats and dogs to investigate the oral toxicity of dextran sulphate.**

**IW to run an antidote study with paraquat/dextran sulphate combinations.**

**LLS will co-ordinate the reporting of all the dextran sulphate data to ICI Japan.**

5. DEXTRIN CHROMOTROPATE.

EJTC described the methods of synthesis of dextrin chromotropate. Cost effective aspects vs dextran were discussed. This part of the research effort has one full-time worker. At the moment an affinity chromatography model is being tested. The triazine method has problems associated with it.

**ACTION: LLS to discuss with Don Davis the possible use of dextrin sulphate in peritoneal dialysis instead of dextrin chromotropate.**

6. CROWN ETHERS.

EJTC decribed the effort in the crown ether area, and in particular the formation of charge transfer complexes. Detailed analyses and molecular graphics are carried out at PPD. At the moment most of the effort is through collaboration with Sheffield (One student, one postdoc) and does not represent a lot of EJTC's time. There are problems with this area and other synthetic routes are being considered.

**ACTION: It was agreed that the crown ether programme (like dextrin chromotropate) should not recieve much emphasis in the total work effort.**

7. CTL ANTIDOTE STUDIES.

LLS and IW discussed the effort in this area and in particular the iron chelator desferrioxamine. Previous studies in Israel had demonstrated a reduction in PQ toxicity with the iron chelator but this could not be repeated at CTL.

**ACTION: IW to follow up a methodological approach to study this with Bernard Loveday.**

8. ANY OTHER BUSINESS.

The status of Morwet was raised by LLS and it was agreed to update the group at the next meeting on planned studies at CTL.

**ACTION: IW to run acute studies in rats to evaluate Morwet and update the group in April.**

Actions arising from the Absorption of Paraquat - Safer Formulations Meeting held on 18 February 1987 at CTL.

| | | |
|---|---|---|
| 1. | Preparation of a table of paraquat absorption profiles and differences in gastrointestinal function between rat, dog and human. | **J R Heylings** |
| 2. | Discussion with Peter Slade on the aspects of inclusion of wetters in paraquat formulations. | **P J Bramley** |
| 3. | To make arrangements with Andrew Garner to examine the effect of agents which inhibit gastric emptying. | **J R Heylings** |
| 4. | Investigate the absorption profile of paraquat in rats using the method employed at Pharms Division. | **J R Heylings** |
| 5. | Arrange a meeting between TFT, LLS and JRH to plan and discuss the preparation and testing of new multiple emulsions. | **T F Tadros** |
| 6. | Test the most promising emulsion formulations in the dog. | **J R Heylings** |
| 7. | Report the phytotoxicity data of dextran sulphate with LLS. | **W D McClellan** |
| 8. | Investigate the effect of dextran sulphate in the rat and dog. | **J R Heylings** |
| 9. | Obtain binding study results for dextran sulphate. | **E J T Chrystal** |
| 10. | To run an antidote study with paraquat and dextran sulphate combinations. | **I Wyatt** |
| 11. | Reporting of dextran sulphate data to ICI Japan. | **L L Smith** |
| 12. | Discussion with Don Davies on the potential use of dextran sulphate in peritoneal dialysis instead of dextran chromotropate. | **L L Smith** |
| 13. | To follow up an approach to test disferrioxamine. | **I Wyatt** |
| 14. | To update the group on the status of Morwet. | **I Wyatt** |

Distribution

| | | |
|---|---|---|
| 1. | E J T Chrystal | Jealott's Hill |
| 2. | J E Downes | |
| 3. | D K Lawrence | |
| 4. | C F MacBean | |
| 5. | W D McClellan | |
| 6. | M R Parham | |
| 7. | K P Parry | |
| 8. | S Sales | |
| 9. | H Swaine | |
| 10. | Th F Tadros | |
| 11.-15. | Report Centre | |
| 16. | P J Bramley | Fernhurst |
| 17. | T B Hart | |
| 18. | P Slade | |
| 19. | C Spinks | |
| 20. | G A Willis | |
| 21.-23 | Report Centre, Fernhurst | |
| 24. | A Garner | Pharmaceuticals Div |
| 25. | D Leahy | |
| 26. | D C Taylor | |
| 27. | T Auton | CTL |
| 28. | P L Batten | |
| 29. | J R Heylings | |
| 30. | S E Jagger | |
| 31. | E A Lock | |
| 32. | R S Morrod | |
| 33. | R C Scott | |
| 34. | L L Smith | |
| 35. | S C Watson | |
| 36. | I Wyatt | |
| 37.-39. | Report Centre, CTL | |

Circulation

| | |
|---|---|
| 40. | T E M Fraser/D A Griffin |
| 41. | J R Hadfield/C N E Ruscoe |
| 42. | D H Brooks/G M Farrell |
| 43. | C A Manley/A K Stapleton |