

Report Series

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	ABSORPTION OF PARAQUA METTING HELD AT CTL O	T WORK GROUP - MI N 10 APRIL 1987	INUTES OF THE	
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The Minutes of the Meeting of the Absorption of Paraquat Work Group held on 10th April 1987 at CTL are listed in this document.

Copies of the slides used in presentations to this meeting are issued separately as appendices to the Minutes, PJ9/WG12/87/04C. The Appendices to the Minutes will only be routinely sent to those who attended the meetings.



MINUTES OF THE THIRD MEETING OF THE

ABSORPTION OF PARAQUAT WORK GROUP

HELD ON 10 APRIL 1987 AT CTL

Those present: L L Smith - Chairman

J R Heylings) R C Scott ١ I Wyatt) CTL E A Lock 1 S C Watson) R S Morrod)



) Pharms

1. MULTIPLE EMULSION STUDIES

1.1 CTL Studies in Rat and Dog.

Data was presented by JRH on the recent studies at CTL involving new multiple emulsion formulations of paraquat (PQ). Since the last meeting held on February 18th, 1987 a further 10 emulsions have been tested in rats at 200mg/kg PQ ion (about twice the LD50 when formulated as Gramoxone). The percentage of emulsifier I (E471 or B246) was varied (from 5-10%) together with the nature of the oil phase (isopar, soyabean, diesel, white or paraffin oil). At 200mg/kg the survival time for groups of 5 rats following gavage dosing with E471 was increased compared to controls but there was no significant improvement over previous formulations containing this emulsifier. In contrast, a formulation containing 5% B246 in diesel oil proved to be non toxic to rats at this dose level, and may be an improvement on our previous formulation of B246 in soyabean oil. Altering the percentage of emulsifier did not alter the toxicity of the PQ formulation in rats. Several repeat studies with B246 identified that the LD50 of formulations containing this emulsifier in either soya or diesel oils is between 200 and 250mg/kg PQ ion in the rat. Contemporary data on Gramoxone controls dosed at 45, 68, 91 and 182mg/kg suggests the LD50 is around 100mg/kg PQ ion.

Two emulsion formulations (B246 and E471) have been tested in the dog since the last meeting. Animals were dosed (by gavage) with formulation containing 8mg/kg PQ ion. B246 was formulated in soya oil and E471 in isopar oil. Both contained 5% of the primary emulsifier. Control animals received TAL GRAMOXONE at the same dose. Following dosing all nine animals remained active and alert. Blood levels of PQ were measured by Radioimmunoassay and HPLC over 48 hours following dosing. Two animals vomited (one following E471 and one of the GRAMOXONE controls). On the basis of area under the curve (AUC) the emulsion formulation B246 was about one half that of the controls. Both methods of analysis gave similar results.

It is concluded that in dogs (which is in agreement with the rat data) we have achieved about a two-fold safening with our first two multiple emulsion formulations tested. On the present evidence, emulsion B246 (in either soya or diesel oils) is the safer formulation.



(ICI) Plant Protection Division



ACTION : JRH to test formulations of B246 at twice the previous dose level in dogs (ie 16mg/kg) and to investigate new emulsion formulations in the rat.

1.2 Phytotoxicity.

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Data was presented by WDM on glasshouse tests of the same 10 emulsion formulations tested at CTL. Normal glasshouse 4-6 leaf seedlings but no perennials were used. Only freshly prepared emulsions were used due to problems of disperal in water with samples which had been stored for two months. The 4 days after treatment and 17 DAT were essentially similar. In general there were no major differences between any of the double emulsions and GRAMOXONE. There was nevertheless an indication that formulations 1 and 4 (5% E475 and 5% B246 in soyabean oil) were slightly less active than the other double emulsions. All the formulations were adequately herbicidal in the glasshouse. However it would be preferable to avoid those of types 1 and 4 in favour of the others. Immediate attention is required to solve the problems of water dispersion of formulations after storage.

ACTION : WDM to arrange further glasshouse trials if and when safer multiple emulsion formulations are identified by CTL studies.

1.3 New Formulations.

The progress on the multiple emulsion formulations was presented by CS. It was explained that various simple in vitro tests were being used at Jealott's Hill to determine the stability of the formulations. Such methods include dialysis and centrifugation. For example E471 was stable over two months whereas E475 breaks down. This is supported by CTL studies in rats which show E471 to be much less toxic than E475. Attempts are being made to thicken the external phase with various agents. Carboxymethylcellulose (CMC) is a possibility which has been introduced into the formulation. Morwet is also worthy of investigation. Thicker oils are less stable and high molecular weight additives do not improve stability when added to the oil phase. To date the secondary emulsifier has always been synperonic (1% NPE 1800). Use of a new emulsifier R6777/280 may give larger droplets. Other formulations which will be investigated will include variations in the outer aqueous phase which is at present 2M NaCl.

THT added to the discussion the different permutations of emulsifiers, oils, additives etc and it was agreed that although we want to keep our options as open as possible, that a rational approach must be adopted. For instance a blank emulsion should be tested in the dog as a positive control. It was agreed that CTL will continue to test new emulsion formulations in the rat prior to any dog studies and that an increase in the current throughput of ten formulations every month could be handled by CTL.

ACTION : THT to submit a new batch of emulsion formulations for toxicity testing at CTL during May.

ACTION : PJB to check the patent situations with multiple emulsions in particular the EPA list and Japanese regulations.

2. DEXTRAN SULPHATE FORMULATIONS.

2.1 CTL Studies.

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

Binding Studies.

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

Rat Studies.

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

2.2 Phytotoxicity.

WDM described a single glasshouse experimental programme on Dextran Sulphate carried out at Jealott's Hill. No further glasshouse work is scheduled but a single field test is to be done in the UK.

Test plants were 4-6 leaves with no perennials included. Plants were sprayed at 2001/ha with all components tank-mixed with 0:1% Agral 90 added to all treatments. There were no major differences between types of DS. Further, DS alone dose not cause visible phytotoxicity. Increasing the rate of DS decreases the efficacy of paraquat. Thus, at a ratio of 1:1 PQ to DS there was no depression of PQ activity. At a 1:2 ratio the depression of plant damage was significant only on Avena Fatua. However, at 1:4, there was a depression of PQ activity, in

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general to half that acheived in the absence of dextran sulphate.

2.3 Binding Studies/Other Sulphated Polysaccharides.

EJTC explained that each paraquat molecule binds to only one sulphate on the Dextran sulphate molecule and that each sulphate acts independently. The binding study data is consistent with the Biology.

An outline of the various types of polysaccharides which may be utilized in a similar programme as Dextran sulphate is discussed in Section 6 below.

2.4 Future Work

LLS summarised the various findings with Dextran sulphate formulations and it was generally agreed that this area will be given lower priority compared to the multiple emulsion and SDS formulation programmes. DS formulations will now not be tested in the dog as previously agreed. It is likely that such a formulation was developed it would require reactivation and thus use of a twin pack.

The use of DS as an antidote was again discussed. It may be a useful, but not neccessarily a better oral antidote than Fuller's Earth. Its use as an I.V antidote is probably limited.

ACTION : LLS to communicate to Japan that in view of the limited usefulness of Dextran sulphate to prevent oral toxicity of paraquat in rats, coupled with its relative depression of PQ activity in glasshouse trials, that dextran sulphate formulations be shelved for the moment.

3. SDS FORMULATIONS

Background.

A report received from Japan on March 3 1987 contained evidence that that the SDS paraquat formulation was non-toxic in the dog at about 10 times the LD50 (120mg/kg). Plasma paraquat levels were substantially lower than predicted for this dose level. It was therefore decided to undertake our own in-house study in dogs to verify this claim.

3.1 CTL Studies in Rat and Dog.

JRH presented data from CTL studies with a paraquat WDG formulation provided by SDS of Japan. Effects of this 20% solid paraquat formulation have been studied in both rat and dog.

Rat Studies.

In rats, groups of ten animals were gavage dosed with 200mg/kg PQ ion (about twice the LD50). The solid formulation was prepared at a pourable strength of 1:7 (SDS: Water). There were no surviving animals by day 6 and results were very similar to a Gramoxone control group receiving the same dose. Thus, in rats the SDS paraquat formulation is no less toxic, at least at 200mg/kg PQ, as Gramoxone.

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Dog Studies.

The acute oral toxicity and absorption profile of paraquat with a PARAQUAT WDG formulation from SDS was investigated in the conscious dog The SDS formulation was prepared at two different strengths. The material supplied from SDS (Y00061/120/001) was analysed on 23/3/87 and found to contain 19.9% paraquat ion. The formulation would not flow at a 1:5 strength when diluted with water, therefore we used 1:7 and 1:10 dilutions for gavage dosing to dogs. Three animals were given each dilution at a final dose of 50mg PQ ion/kg. A further 3 dogs received TAL GRAMOXONE at 8mg PQ ion/kg. This dose of TAL is expected to be sub-lethal and provides an internal comparison. Clinical observations were recorded and plasma paraquat levels measured by Radioimmunoassay over the following 24hrs.

Clinical Observations.

All animals receiving the SDS PARAQUAT WDG vomited. Emesis occurred between 20 and 80 min after dosing. Two out of three animals receiving TAL GRAMOXONE also vomited during the first hour. (In our last series of experiments with the same dose of GRAMOXONE, one of three dogs vomited). All nine dogs showed no further clinical signs and were active and feeding normally 24hrs after dosing.

Plasma Paraquat Profiles.

Despite the six-fold difference in dose of PQ ion, plasma profiles for the SDS formulation were similar to TAL GRAMOXONE. Peak plasma concentrations were 3.56, 4.74 and 3.92 for TAL, SDS (1:10) and SDS (1:7) respectively, and all occurred at one hour. Plasma concentrations decreased over the next 12 hours at the same rate in all three groups.

Conclusions.

The SDS PARAQUAT WDG formulation showed no clinical signs of toxicity when fed to dogs at 50mg PQ ion/kg which is more than four times the LD50 value for GRAMOXONE formulations. Plasma profiles of the two strengths of SDS formulation were similar to that observed with 8mg/kg TAL GRAMOXONE. Based on these preliminary findings the SDS PARAQUAT WDG formulation may offer around a five fold safening factor for paraquat ingestion, assuming that the findings in dogs relate to man.

3.2 Future Work.

It was agreed that we will continue to investigate the SDS formulation in the dog. Details on the original Japanese study are limited but features such as emesis and dose volume will be investigated in our neat dog study. More information on the effects of SDS formulation in rats is also required.

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ACTIONS:

JRH to investigate the mechanism by which the SDS formulation affords protection against paraquat toxicity in dogs.

RCS to arrange further studies in the rat to determine an accurate LD50 for the SDS formulation.

IN to investigate the binding of SDS formulation in vitro by dialysis.

PJB to supply CTL with SDS formulation without emetic.

PJB to obtain more detailed information on the Japanese dog study with the SDS formulation.

4. MORWET

4.1 CTL Studies in Rats.

IW presented data on recent studies with Morwet in the rat. Three groups of five animals were dosed with Morwet containing 200, 250 and 300mg/kg PQ ion. At the lowest dose 4 out of 5 animals survived. However, at 250 and 300mg/kg none of the animals survived the 10 day study. The middle dose prolonged survivability but doses above 200mg/kg, at least in rats, do not show any safening.

It was agreed that although Morwet is relatively inexpensive, since it only offers about a two-fold safening (and would also need a twin pack) that its usefulness may be limited to a combination formulation with say a multiple emulsion.

5. COMBINATIONS OF DIFFERENT FORMULATIONS.

LLS raised the issue of combining one or more of the formulation types to produce an even safer paraquat formulation. At the moment we are pursuing multiple emulsions, SDS gel formulations, sulphated dextrans etc, in addition to our older approaches such as Morwet. THI explained the latest ideas of his group and how the emulsion formulations could probably be improved. One approach is to use a gel layer outside the primary emulsifier such as carboxymethylcellulose (CMC) instead of the emulsifier II. This may help the cross-linking and give a more stable formulation. Other approaches include using agents such as alginates, gums or modified starch either with or without cross-linking agents. A gel network either round the droplets or in the outer phase look promising and such an example should be available soon for testing. Other approaches such as emulsion formulations with Morwet outside may also be beneficial.

ACTIONS:

THT to rationalize the formulation research and provide the group with strategy to investigate the various approaches discussed.

THT to supply CTL with formulations of paraquat which incorporate multiple emulsions and other potential safening agents eg. Dextran sulphate, chromotropate and Morwet.

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6. CHEMISTRY UPDATE.

EJTC discussed the various sugars which were available and how sulphated products may be useful along the same lines as dextran sulphate. The monosaccharides would be expensive but persulphation of some of the common monosaccharides or disaccharides could be tried eg. D-glucose or sucrose. They may be of use as a combination formulation but unlikely on their own. Of the polysaccharides dextran sulphate has already been investigated but there are others such as cellulose. Also the dextrins which hold water may have different properties than dextrans. There are many naturally occurring polysaccharides which include carrageenan, chondroitin, dermatan, heparin and lignan sulphates.

ACTION : WDM to decide from the list of potential sugars which of them may be useful to follow up.

7. ANY OTHER BUSINESS.

LLS informed the group that the world market for peritoneal dialysis is around \$2.5bn. Would dextrin sulphate for instance be useful in this respect?

ACTION : LLS to discuss this with Pharmaceuticals Division.

WDM was asked to review the Chromotropate Biology data with Arquad mixtures.

ACTION : WDM to arrange a meeting with Martin Parham to discuss this.

10 April 1987. Absorption of Paraquat Work Group Meeting.

Summary of Actions:

- JRH to test higher doses of the most promising emulsion formulations in the dog.
- NDM to arrange further glasshouse trials on any new emulsion formulation which shows substantial improvement in safening in the dog.
- THT to submit a new batch of emulsion formulations for toxicity testing in rats.
- 4. PJB to check the patent situation with our emulsion formulations in particular EPA listings and Japanese regulations.
- LLS to communicate to ICI Japan a summary of the data obtained with Dextran sulphate.
- 6. JRH to investigate the mechanism by which the SDS formulation affords protection against paraquat toxicity in the dog.
- RCS to arrange further studies in the rat to determine an accurate LD50 for the SDS formulation.
- 8. IW to investigate the binding of SDS formulation in vitro by dialysis.
- PJB to supply CTL with an SDS formulation which dose not contain emetic.
- PJB to obtain more detailed information on the Japanese dog study with the SDS formulation.
- 11. THT to rationalise the formulation research and provide the group with a strategy to investigate the various approaches discussed.
- 12. THT to supply CTL with formulations of paraquat which incorporate multiple emulsions and other potential safening agents eg. Dextran sulphate, Chromotropate and Morwet.
- WDM to decide from the list of potential sugars which of them may be useful to follow up.
- 14. LLS to discuss the use of dextrin sulphate in peritoneal dialysis with Pharms Division.
- 15. WDM to arrange a meeting to discuss the Chromotropate Biology data with Arquad mixtures.

OUTSTANDING ACTIONS FROM 18 FEBRUARY 1987.

- 1. JRH to prepare a table of paraquat absorption profiles and differences in GI function between rat, dog and man.
- 2. IN to follow up an approach to test desferrioxamine.

IMPERIAL CHEMICAL INDUSTRIES PLC CENTRAL TOXICOLOGY LABORATORY ALDERLEY PARK MACCLESFIELD CHESHIRE

ABSORPTION OF PARAQUAT - SAFER FORMULATION

Agenda for Meeting to be held at CTL

on Friday 10 April 1987 in Room HG3 at 10.30am

1. Multiple Emulsion Studies

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	i) ii) iii)	CTL studies in rat and dog Phytotoxicity New formulations	(JRH) (WDMcC) (TFT)		
2.	Dext	ran Sulphate Formulations			
	i) ii) iii) iv)	CTL studies in rats Phytotoxicity Binding studies/other sulphated polysaccharides Future work	(IW) (WDMcC) (EJTC) (LLS)		
3.	SDS	Formulations			
	i) ii)	CTL studies in rat and dog Future work	(JRH) (LLS)		
4.	Norwet				
	i) ii)	CTL studies in rats Future work	(IW) (LLS)		
5.	Comb	inations of Different Formulations	(A11)		
6.	Che	ństry Update	(EJTC)		
7.	Any	Other Business	(A11)		

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