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ICI Agrochemicals

[illegible] UK

[illegible] 387C

SAFER PARAQUAT FORMULATIONS

TRC 5th March 1990

Editor(s): [illegible] Swaine

Date of issue: February 1990

ICI AGROCHEMICALS
28 FEB 1990
[illegible] HILL
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SAFER PARAQUAT FORMULATIONS : TRC 5th MARCH 1990

Editor: H Swaine

ABSTRACT

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

KEYWORDS

PARAQUAT
SAFER FORMULATIONS
EMETIC
PURGATIVE
MULTIPLE EMULSIONS
GELLING AGENTS

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RECOMMENDATIONS

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

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

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

   Action: Formulation R&D

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

   Action: Formulation R&D

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

   Action: Patents Section

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

   Action: Products/Development Departments

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

   Action: Products Department

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SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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TOXICOLOGY OF NEW FORMULATIONS OF PARAQUAT

Jon R Heylings and Lewis Smith

SUMMARY

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

1. High Concentrations of Emetic in GRAMOXONE

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

2. Multiple Emulsion Formulations

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

3. Magnesium Sulphate and Trisilicate Formulations

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

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Introduction and Objectives of the Safer Paraquat Formulation Programme

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

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

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

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

1. HIGH CONCENTRATIONS OF EMETIC IN GRAMOXONE

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

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

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

Studies in the Dog with High Emetic Concentrations

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

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

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

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

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

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

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

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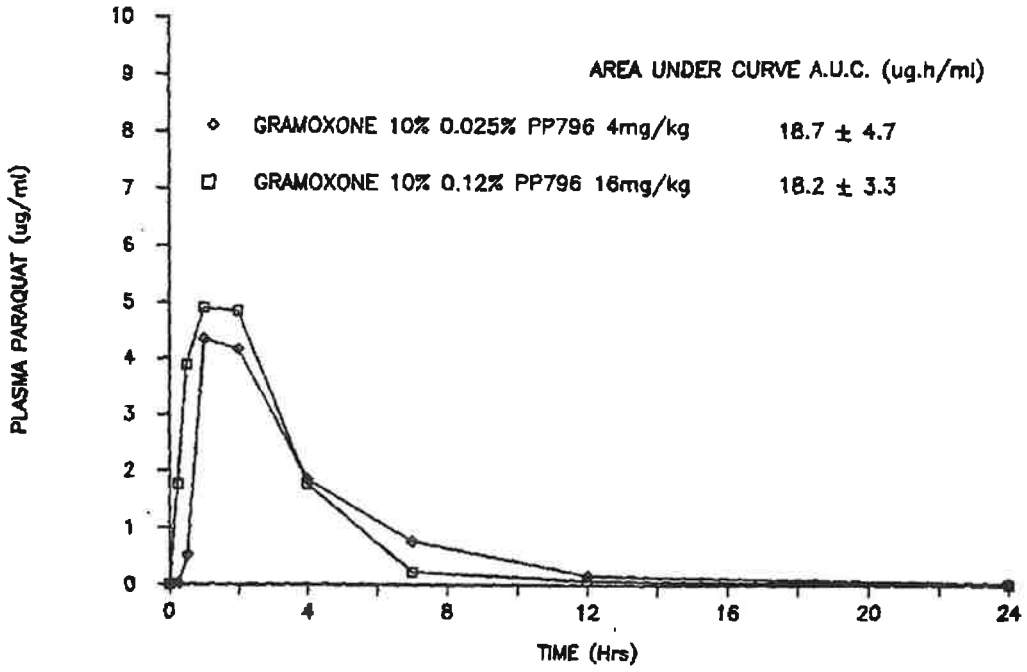

Fig 1. Effect of two formulations of GRAMOXONE in the conscious dog. Both formulations contained 10% paraquat dosed by capsule. Increasing the emetic (PP796) by 5 fold resulted in a very low plasma paraquat profile which was equivalent to a 4mg/kg dose of standard GRAMOXONE. Mean values are shown for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

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2. MULTIPLE EMULSION FORMULATIONS OF PARAQUAT

Rodent Studies

All new Emulsion formulations are tested in rats before any dog studies are undertaken. In rats, the median lethal dose (MLD) for GRAMOXONE is about 90mg/kg paraquat ion. A minimum of a 2-fold safety factor with a new formulation is our minimum criteria to further investigate a new formulation in dogs. From experience, we have set our dose levels in rats within the 150-250mg/kg range where dose level represents the mg of paraquat ion in the 10% (or 100g/l) Emulsion formulation per kg bodyweight. Neat concentrate is dosed orally by gavage to five male rats per dose level. Clinical observations are carefully monitored for 10 days. Formulations which are non-toxic to rats at twice the lethal dose level of GRAMOXONE are deemed to be acceptable for further study. During the programme from over 300 different Emulsions approximately 10% of these have proceeded to dog studies for further evaluation.

Dog Studies

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

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

Progress from 1987-1990

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

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

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

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

Other methods for reducing flocculation were investigated during the latter half of 1989. In particular, E140 which maintains the 'safe' factors of system E90 in terms of volume fraction and magnesium content, but also contains polyvinyl alcohol (PVA) which reduced post-dilution flocculation. Our first example of system E140 gave a 4X safety factor in dogs (Figure 3). Subsequent batches of this Emulsion have given different degrees of safening and sprayability when prepared by different processes. Fortunately, safening and sprayability were not paradoxically related with this formulation. Emulsion E140 has an MLD in rats of 250mg/kg. In the dog only mild clinical observations were observed at 32mg/kg. Plasma paraquat profiles for E140 in dogs dosed at 8, 16 and 32mg/kg did not exceed a standard AUC for GRAMOXONE at 8mg/kg. The predicted MLD in dogs is 48mg/kg based on extrapolation of the AUC curve. This represents a 4X safety factor over GRAMOXONE. Thus, batches of this Emulsion which have both adequate safening and Field trial acceptability have been produced.

Toxicology of Multiple Emulsions E171 and E181

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

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(PP796) would interfere with the Emulsion process in any way as we move closer towards a commercially viable product which would contain safeners. We found that the emetic (0.12% w/v) in a 100g/l Multiple Emulsion formulation of paraquat had no effect on the emulsification process or the toxicity of paraquat Emulsion formulations in rats. Indeed, since the emetic partitions into oil well, it is possible that it will be delivered to the absorptive sites of the intestine at a faster rate than the paraquat which is retained inside the Emulsion droplets. Emulsions 171 and 161 were compared directly with a 100g/l GRAMOXONE formulation containing an identical concentration of emetic (0.12%). Thus, the intrinsic safening of Emulsion could be compared directly with GRAMOXONE under conditions of equal volumes of dosing solution and equal concentrations of both paraquat and emetic.

Rodent Studies

As shown in Figure 4, the rat survival profile following a single oral dose of paraquat as GRAMOXONE compared to paraquat as Emulsion were quite different. The median lethal dose (MLD) for GRAMOXONE was between 50 and 100mg/kg, which is in agreement with previous data. In contrast, the MLD for both Emulsion 171 and 161 was >150mg/kg. All animals received identical doses of paraquat ion and emetic. Rats have no vomit centre in the brain and as a consequence cannot remove the herbicide via emesis. This study clearly demonstrates that both Emulsion 171 and 161 have an intrinsic safening over GRAMOXONE which exceeds 2-fold in the rat. Further work is in progress at higher dose levels in order to determine the actual MLD of these Emulsion formulations in the rat.

Dog Studies

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

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

As shown in Figure 5, using equal doses of paraquat (16mg/kg), the GRAMOXONE treated group absorbed a significantly greater amount of paraquat from the gastrointestinal tract compared to Emulsion 181. The mean AUC for GRAMOXONE was 18.7 ± 4.7µg.h/ml, n=3. All peak paraquat plasma levels were higher in the GRAMOXONE group. All 9 dogs vomited following dosing but the time to vomit was significantly delayed and more variable with GRAMOXONE compared to Emulsion 181. The mean time to first vomiting was 19 ± 4 minutes for GRAMOXONE. Dogs treated with Emulsion 171 had a relatively low peak plasma value, but a very similar plasma paraquat AUC (mean 18.9 ± 7.4µg.h/ml, n=3) compared to GRAMOXONE. All animals had vomited within 20 minutes (mean time = 15 ± 3 min). Dogs dosed with E171 displayed few clinical signs and were normal by 24 hours. Emulsion 181 gave a very promising result. The plasma paraquat AUC for Emulsion 181 was very low (11.0 ± 0.8µg.h/ml, n=3). This represents a significant reduction in paraquat absorption compared to the GRAMOXONE group. Peak plasma paraquat values were also very low for this dose level and paraquat levels had returned to baseline within 4 hours of dosing. All dogs dosed with E181 vomited within 10 minutes of dosing (mean time = 9 ± 0.6 min) and showed no further symptoms thereafter. Indeed, all nine dogs in the study not only survived a lethal dose of paraquat but were feeding normally within a few hours of dosing. This study suggests that a level of 0.12% emetic in GRAMOXONE probably results in at least a 2 fold safety factor compared to GRAMOXONE EXPORT. Emulsion 181 has a further intrinsic safety factor of at least 2 fold on top of this. The AUC value obtained with E181 is the lowest ever value observed during the course of the Emulsion programme at this dose level in dogs.

Based on a very large database of Emulsion formulations studied at CTL over the last 3 years we would suggest that Emulsion 181 would achieve our safety margin of 5X. Obviously, until higher dose levels are tested we cannot extrapolate with exact certainty how safe this Emulsion will be. However, the AUC value obtained at 16mg/kg (11.0 ± 0.8µg/ml) is

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significantly lower than a 4mg/kg dose of GRAMOXONE EXPORT (18.2µg.h/ml, n=3) which is a 4 fold difference in paraquat dose. Therefore, Emulsion 181 is likely to be at least four times safer on a volume basis than an equivalent concentration of paraquat as GRAMOXONE.

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

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

Skin studies with Multiple Emulsion Formulations of Paraquat

(i) Emulsions diluted to spray strength

The skin irritation potential of spray strengths of three Multiple Emulsion formulations of paraquat (E26, E82 and E90) have been compared to GRAMOXONE. The Emulsions all contain B246, Diesel oil and NPE 1800. The external water phase of Emulsions 26, 82 and

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

(ii) Emulsions as neat concentrates

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

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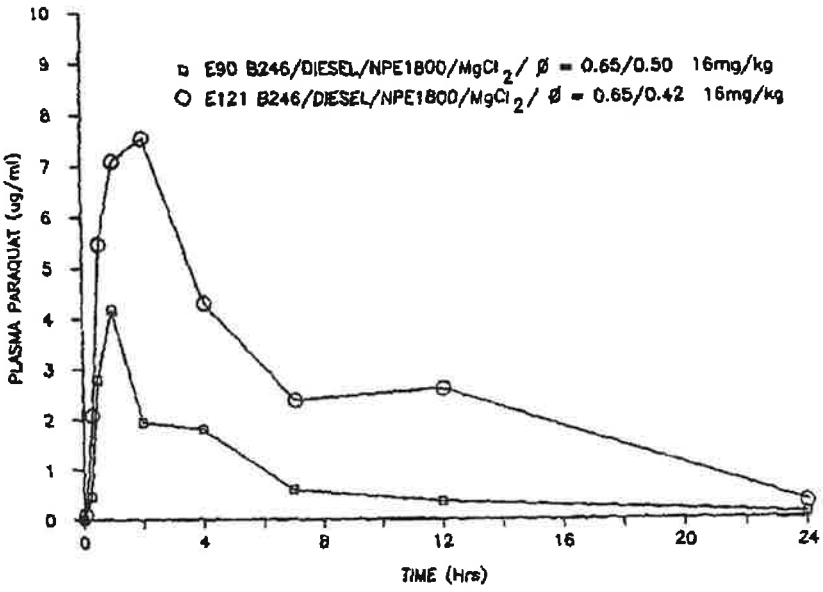

Fig 2. Effect of a single oral dose (16mg/kg) of two Multiple Emulsion formulations of paraquat in the conscious dog. Plasma paraquat levels are very different when the secondary volume fraction is altered. Emulsion 90 contains more oil and gave a much lower plasma AUC (13.7 ± 4.0µg.h/ml) compared to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

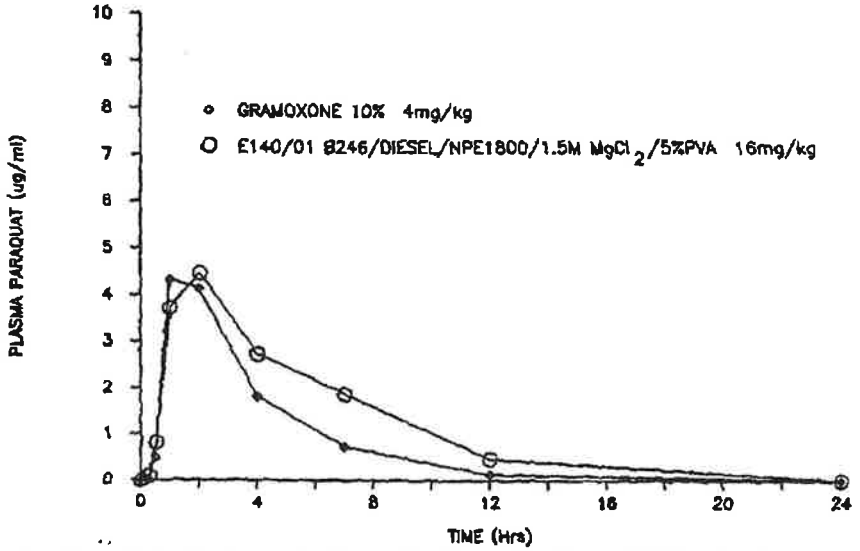

Fig 3. Effect of a single oral dose of the Multiple Emulsions formulation E140 at 16mg/kg in the conscious dog. For comparison a contemporary GRAMOXONE control at 4mg/kg gave a similar plasma profile despite the four-fold difference in paraquat dose.

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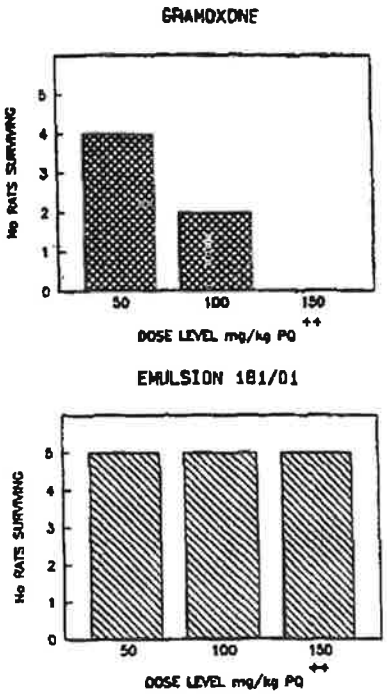

Fig 4. Effect of a single oral dose of paraquat (50-150mg/kg) as GRAMOXONE and Emulsion 181 in the rat. Survival rates are shown for groups of 5 animals per dose level over a 10 day period. Both formulations contained 10% paraquat and 0.12% PP796. The median lethal dose (MLD) for GRAMOXONE was 50-100mg/kg.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

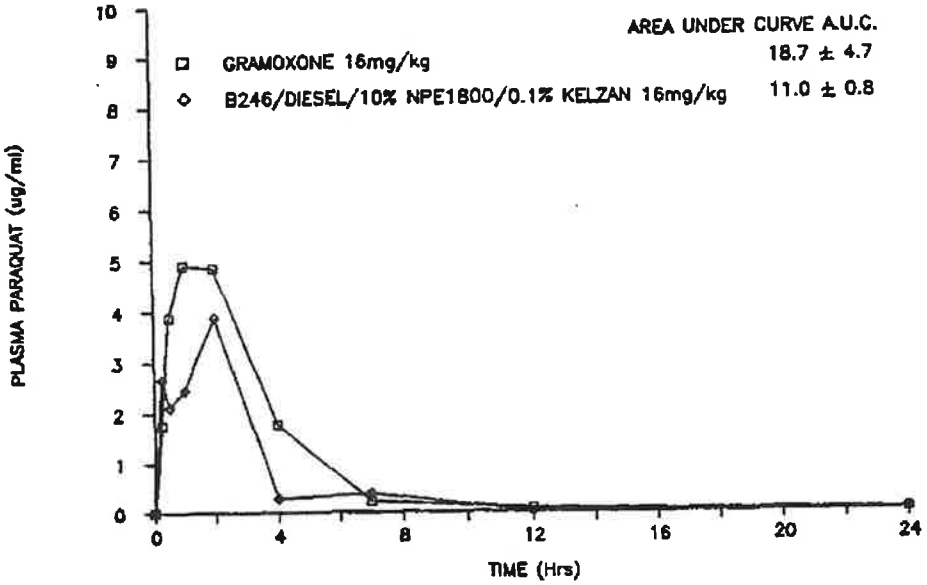

Fig 5. Effect of a single oral dose (16mg/kg) of paraquat as GRAMOXONE and Emulsion 181 in the conscious dog. The mean AUC value for Emulsion 181 was significantly lower than the GRAMOXONE control. Both formulations contained identical concentrations of paraquat (10%) and PP796 (0.12%). Mean values for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

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3. MAGNESIUM SULPHATE AND TRISILICATE FORMULATIONS OF PARAQUAT

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

Aqueous Paraquat Concentrates Containing Magnesium Sulphate

The acute toxicity of a single oral dose of GRAMOXONE containing various salts in the rat is summarised in Figure 6. Generally, Mg-based systems were least toxic with the sulphate producing the best safening in rats. In 1988, we demonstrated that GRAMOXONE containing calcium salts increased toxicity of paraquat. Most Ca uptake processes are antagonised by Mg. Furthermore, Mg salts were less irritant to the mucosa compared to other salts of equal tonicity. Acute toxicity studies in rats were used to characterise the GRAMOXONE-$MgSO_4$ formulation. A dose related reduction in toxicity occurred between 0.5-1.5M $MgSO_4$, where the formulation remained as an aqueous solution. Concentrations above 1.5M (40%) $MgSO_4$ began to salt out of solution. GRAMOXONE containing 1.5M $MgSO_4$ gave an MLD of 190mg/kg in the rat. This compares with 90mg/kg for GRAMOXONE alone.

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In the rat, plasma paraquat analysis following GRAMOXONE-$MgSO_4$ gave a significant reduction in plasma paraquat levels from 4-48 hours after dosing. In dogs, the same GRAMOXONE $MgSO_4$ formulation was dosed orally to 3 animals at 8, 16 and 24mg/kg on three separate occasions one month apart. Although the lethal dose of GRAMOXONE alone in dogs is about 12mg/kg there were no clinical signs of paraquat intoxication at any dose. A common feature throughout was emesis within 30 minutes of dosing and a watery diarrhoea by 2-3 hours in all cases. Since a lethal plasma AUC for paraquat in the dog is around 50µg/ml.hr., we would predict that addition of $MgSO_4$ results in a formulation which is at least 2-3 times safer than GRAMOXONE. The plasma profile for paraquat following oral dosing with GRAMOXONE-$MgSO_4$ in dogs is shown in Figure 7.

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

Aqueous Paraquat Concentrate Containing Magnesium Sulphate and Trisilicate

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

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purgation and emesis to be overcome. As a result more paraquat (as gel) would probably be removed by emesis and any formulation which enters the small intestine (the site of paraquat absorption) would be rapidly cleared by purgation. Studies in the dog at 24mg/kg paraquat ion have confirmed that a formulation of GRAMOXONE containing a combination of Magnesium Sulphate and Trisilicate is safer than GRAMOXONE plus $MgSO_4$ alone (Figure 7). This formulation probably has a minimum of a 3 fold safety factor over GRAMOXONE. Higher dose levels are planned to determine if such a formulation will achieve our intrinsic 5 fold safety factor objective.

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

Future Studies

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

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

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

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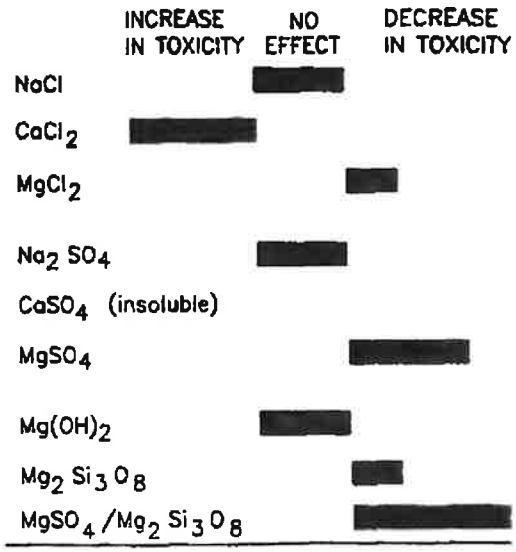

Fig 6. Effect of a various electrolytes on the oral toxicity of GRAMOXONE in the rat. Equimolar solutions (1.5M) of each salt were added directly to 10% GRAMOXONE and dosed over the range 100-300mg/kg paraquat. Magnesium based salts reduced the oral toxicity of GRAMOXONE.

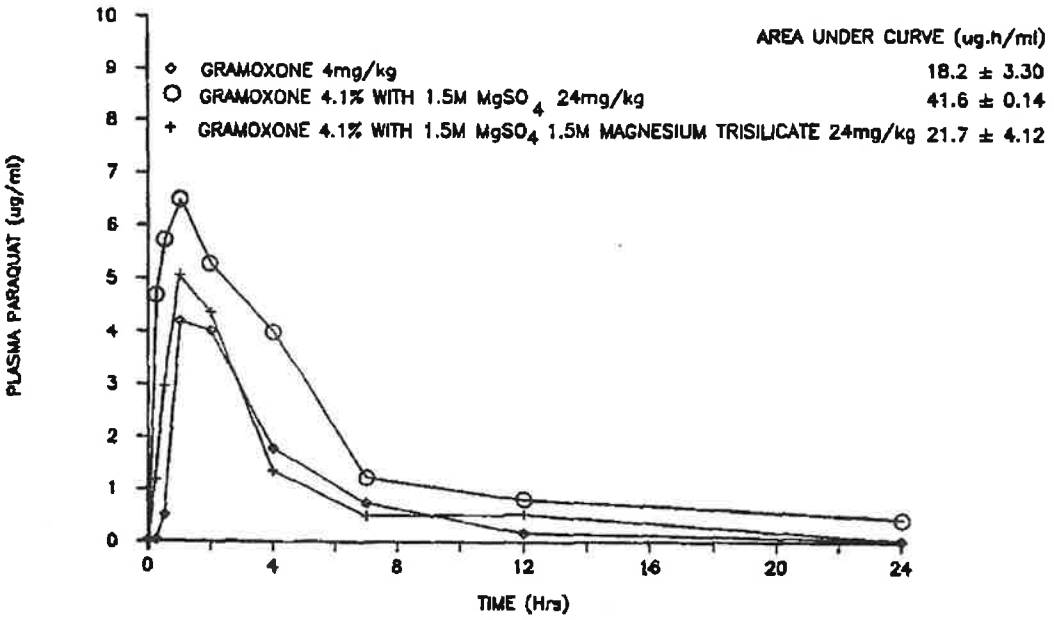

Fig 7. Effect of a single oral dose (24mg/kg paraquat) of GRAMOXONE containing $MgSO_4$ alone or in combination with Magnesium Trisilicate in the conscious dog. The gelling, emetic and purgative properties of the combination of both salts resulted in a reduction in plasma paraquat AUC to values which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

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

2.1 Introduction

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

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

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

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

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

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

2.2 Formulation Research Progress

2.2.1 Reduction of the amount of oil

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

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

2.3.2 Adjustment of secondary emulsification process

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

2.3.3 Secondary emulsion interface variation

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

2.3.4 Addition of Polyvinylalcohol (PVA)

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

2.3.5 Use of a static mixer

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

2.4 Alternative Secondary Emulsifiers

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

2.4.1 Increased NPE1800 concentration

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

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

2.5 Incorporation of the emetic

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

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

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

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

2.6 Twin Pack Concept

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

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

2.7 Further Safening Aids

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

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

2.8 SUMMARY

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

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

3. PATENTS

3.1 Multiple Emulsion Formulations : ICI Case PP34163

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

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

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

3.2 Aqueous Concentrates containing Magnesium Sulphate, Magnesium Trisilicate and Emetic

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

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

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

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

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

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

UK FIELD TRIAL GB01-90H130

PARAQUAT MULTIPLE EMULSION EFFICACY SCREEN

% CHLOROSIS AVERAGED ACROSS ALL THE WEED SPECIES

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

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5. COMMERCIAL OVERVIEW

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

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

Strategy

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

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

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

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

Denmark :

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

Austria :

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

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Conclusion

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

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

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

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

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

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APPENDIX I

PARAQUAT : STUDIES ON THE MECHANISM OF GASTROINTESTINAL ABSORPTION

Jon R Heylings

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

Rat Isolated Mucosa

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

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

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

Dog Isolated Mucosa

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

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

Mucus as a Barrier to Paraquat Diffusion

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

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

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

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

Future Paraquat Research

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

JMPBMISC4
EMULFORM (21.2.90)

FIGURE 1

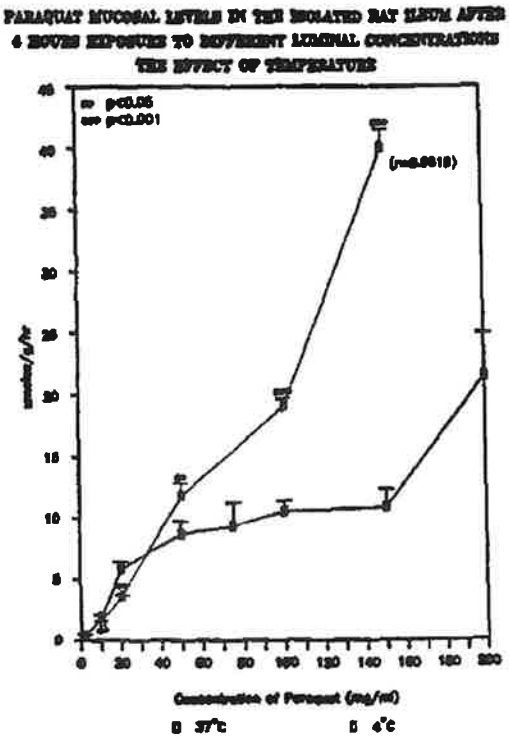

FIGURE 2

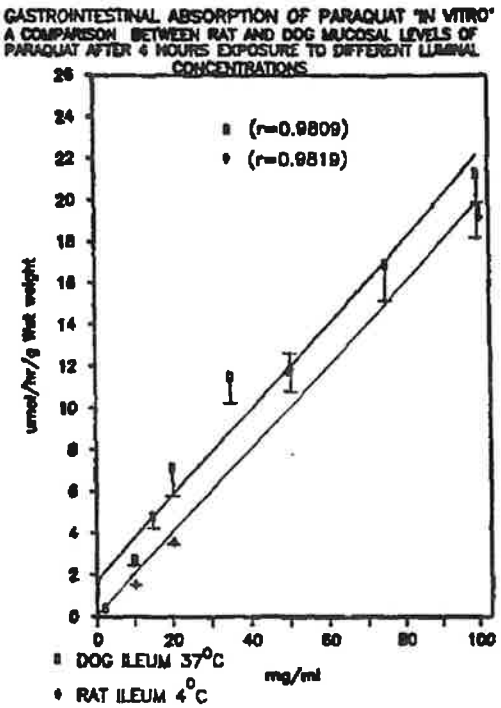

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FIGURE 3

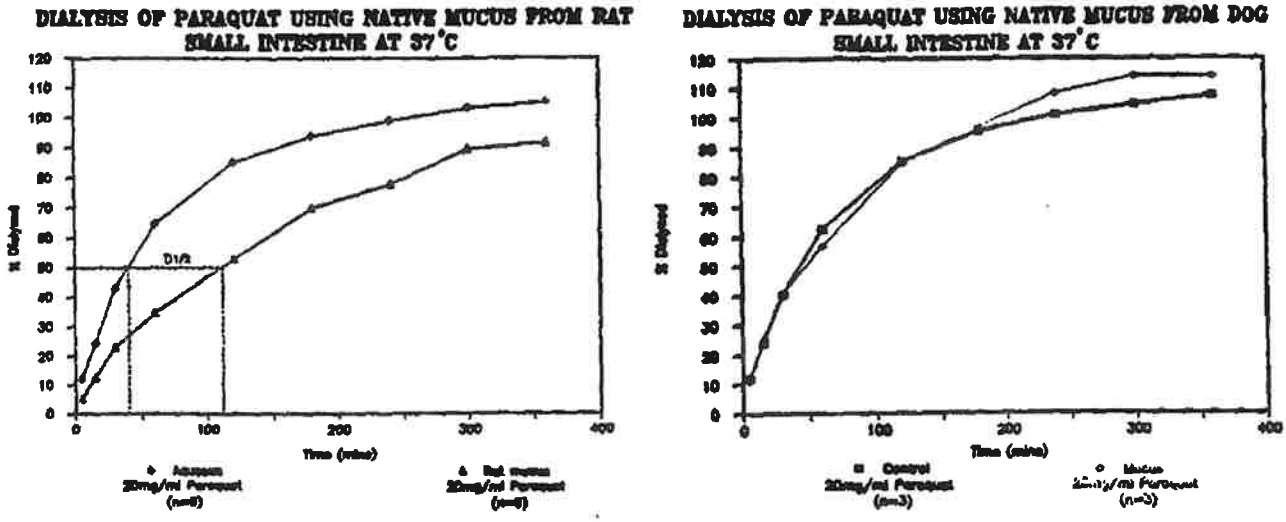

TABLE 1

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

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

*p<0.001

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APPENDIX II

TRIAL NUMBER: GB01-90-H130

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

AUTHOR: M.H.WILLIAMS

LOCATION: HYDE FARM

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

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

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

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

No spraying problems were encountered with any formulation.

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

63110194

TRIAL NUMBER: GB01-90-H130

OBJECTIVES:

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

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

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

CONCLUSIONS:

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

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

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

63110195

TRIAL NUMBER: GB01-90-H130

INTRODUCTION:

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

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

METHOD:

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

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

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

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

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TRIAL NUMBER: GB01-90-H130

RESULTS:

(See Tables 1-3)

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

Control of pansy at this stage was very poor.

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

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

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

Control of pansy was also very poor.

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

'ABLE 1.

:B01-90-H130

ARAQUAT MULTIPLE-EMULSION EFFICACY SCREEN

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

IMPLE MEANS

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

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

SPRAYED - 09/02/90

GROWTH STAGES:

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

63110198

ABLE 2.

301-90-H130

ARAQUAT MULTIPLE-EMULSION EFFICACY TRIAL

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

IMPLE MEANS

	TRZAW	AVEFA	LOLPE	VIOAR	MATPE	STEME
JF12254 @ 62.5 g/ha + AGRAL 0.1%	27.5	30	22.5	5	15	35
JF12254 @ 125 g/ha + AGRAL 0.1%	47.5	50	30	12.5	27.5	60
JF12254 @ 250 g/ha + AGRAL 0.1%	55	60	37.5	47.5	45	80
JF12254 @ 500 g/ha + AGRAL 0.1%	60	70	45	77.5	65	82.5
JF12254 @ 1000 g/ha + AGRAL 0.1%	80	85	62.5	80	75	82.5
JF12255 @ 62.5 g/ha + AGRAL 0.1%	22.5	32.5	20	15	25	35
JF12255 @ 125 g/ha + AGRAL 0.1%	32.5	47.5	27.5	10	22.5	55
JF12255 @ 250 g/ha + AGRAL 0.1%	52.5	60	37.5	30	37.5	75
JF12255 @ 500 g/ha + AGRAL 0.1%	67.5	70	57.5	72.5	70	87.5
JF12255 @ 1000 g/ha + AGRAL 0.1%	72.5	80	62.5	77.5	75	85
PARAQUAT DICHLORIDE @ 62.5 g/ha + AGRAL 0.1%	30	37.5	17.5	10	7.5	25
PARAQUAT DICHLORIDE @ 125 g/ha + AGRAL 0.1%	37.5	47.5	25	20	30	52.5
PARAQUAT DICHLORIDE @ 250 g/ha + AGRAL 0.1%	60	60	37.5	40	57.5	75
PARAQUAT DICHLORIDE @ 500 g/ha + AGRAL 0.1%	65	77.5	47.5	57.5	72.5	82.5
PARAQUAT DICHLORIDE @ 1000 g/ha + AGRAL 0.1%	80	75	50	75	77.5	92.5

RAYED - 09/02/90

OWTH STAGES:

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

63110109

From
Dr J R Heylings
Biochemical Toxicology

ICI Central Toxicology Laboratory

Alderley Park
Macclesfield Cheshire SK10 4TJ

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

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

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

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

FRENCH FORMULATION OF PARAQUAT

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

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

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

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

Cont...

Heylings Dec Exhibit 25

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

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

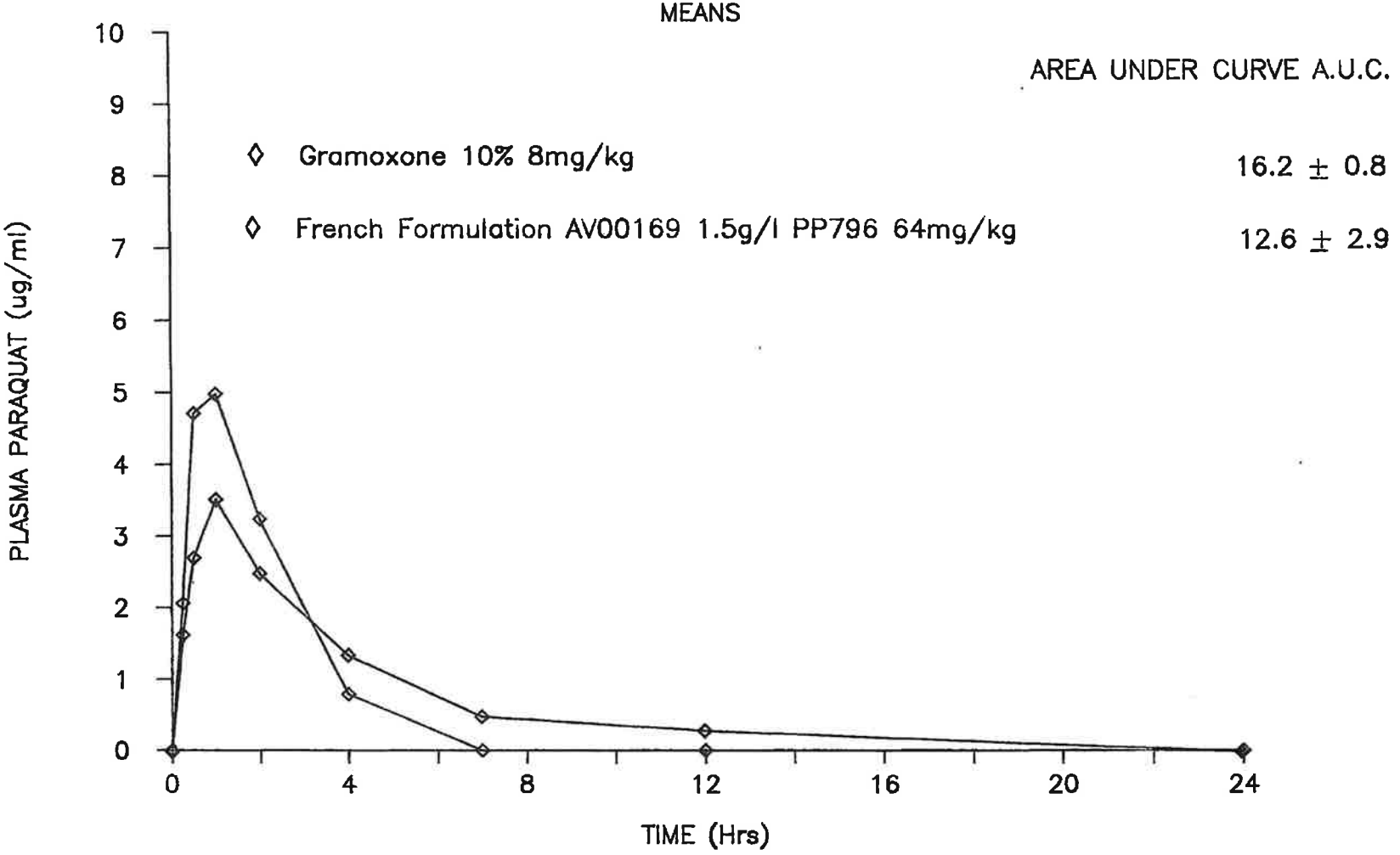
I am unable to find evidence that paraquat poisoning in France since introduction of a paraquat formulation containing 1.5g/l emetic has had no effect on reported poisonings or reported deaths attributed to the herbicide. Indeed, the number of cases appears to be very low. If increasing the level of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

Jon R. Heylings

J R HEYLINGS
Biochemical Toxicology

FORMULATION STUDIES XD1328

MEANS



Heylings Dec Exhibit 25

SYNG-PQ-03709697_R

From: **Andy Cook** Date: **25-Feb-1995 15:34**
COOK AR@A10FHVAXC

TO: **Bob Scott - CTL** **(SCOTT RC@A10APVXC1)**
CC: **Jon Heylings** **(HEYLINGS JR @ A1 0 APVXC1)**

Subject: **RE: PQ/EU/Emetic**

Bob,

Thanks for your note.

Re. the emetic, it would seem entirely appropriate to use CTL/T/2471 in answering part of the question from Germany.

However, I propose that we do not prepare an EU Tier I summary of this study report. I propose to address the issue of the emetic in an Appendix to the paraquat Tier II document on toxicology (Document M-II, Section 3). Since all of the studies to be submitted on the emetic are 'supplementary' in that they are not strictly required for EU review/approval of the active substance I believe that we can submit without corresponding Tier I summaries of the individual studies. This approach obviously suits us in that many of the studies are research-orientated and do not follow specific guidelines. However please bear in mind that there is no guarantee of success and we may find ourselves compelled to produce Tier I summaries at a later date (post-submission). Given the commercial importance of PP796 I will offer (at the time of submission) to supply Tier I summaries of the key PP796 studies on request.

I have now completed a first draft of the document to be submitted to the EU on the emetic (minus the contributions from yourself and Martin on the 'exam questions'). I attach a copy for urgent review by yourself and Jon (Heylings). ALL comments gratefully received, in particular whether or not I have included the most appropriate references.

Thanks.

ANDY

P.S. I do not require a Tier I summary for the rabbit plasma modelling report.

From: Jon Heylings
HEYLINGS JR

Date: 27-Feb-1995 18:03

TO: Bob Scott - CTL (SCOTT RC)

Subject: Emetic document

Bob,

As I am sure you are aware I will have to vent my concern over the validity of the Rose (1977) conclusions which are cited in Andy's report.

I am surprised that he is unaware of the issue. Martin Wilks certainly is aware of the issue around the human emetic data and it may be time to re-open the case and get a thorough independent review.

Jon

CONFIDENTIAL

From: Jon Heylings
HEYLINGS JR

Date: 01-Mar-1995 10:40

TO: Andy Cook (COOK AR@A1@FHVAXC)
CC: Bob Scott - CTL (SCOTT RC)
CC: Martin Wilks (WILKS MF @ A1 @ FHVAXC)

Subject: PQ/EU/Emetic review

Andy,

Following your request for me to give comments on the EU/Emetic document and my discussions with yourself and Martin Wilks my response is as follows:

Section 1 is fine. It is along the lines of Peter Slade's paper (EDC 729).

Section 2. Page 2, line 8 cites human as being "particularly sensitive" to the emetic compared to the pig, dog and monkey. I do not agree. I carried out an extensive review of the human volunteer data at Pharmaceuticals in 1990 (PH20992, Bayliss). In the first trial with normal healthy volunteers there was no emesis at the 5 doses below 0.06mg/kg, yet CTL/R/390R (Rose,1977) quotes an emetic response of 11% at 0.03mg/kg. Only 2 out of 12 subjects actually vomited in the whole study. The one subject who was given the top dose of 0.1mg/kg did not even fulfil the suggested criteria for the emetic in paraquat of "emesis within 1 hour". In fact, emesis occurred at 2 hours.

Overall, the 0.1mg/kg dose is a threshold response in man - not an effective dose. This is consistent with the inclusion of the emetic in PQ products having had some discernable improvement in survival, but clearly not as good as had been anticipated back in 1977.

Given the fact that we have human data and sound animal data with the emetic and that the emetic response curves are steep and parallel across species, basic pharmacological principles tell us that a 3-5 fold increase in emetic concentration will markedly improve the efficiency of emesis in man. By extrapolation this would suggest a 5 fold improvement in oral toxicity.

I do agree with the animal data presented in the document. Indeed, dog studies conducted by my research group with Gramoxone containing different levels of emetic (as I presented to the TRC in 1991) are in full agreement with the Brammer and Robinson data. Here we demonstrated that dogs could tolerate 5 lethal doses of Gramoxone by increasing the emetic from 0.5g/l to 2.4g/l.

(Magnoxone contains 1.5g/l emetic plus other safening ingredients balanced out to trade off the commercial penalty of a 2.4g/l emeticized Gramoxone).

In view of this background information, the rationale for including the emetic at a concentration of 0.5g/l in Gramoxone based on "greater sensitivity in humans" is unsubstantiated. Thus, the second paragraph in Section 4.1 needs to be changed including the "within one hour" statement.

I fully understand the sensitivity of this whole issue and regard this as highly confidential within Zeneca. However, as a matter of scientific integrity, having been asked to comment on the document, I feel I should share these views with you.

Regards,

Jon

From: Bob Scott - CTL
SCOTT RC

Date: 01-Mar-1995 14:41

TO: Andy Cook (COOK AR@A1@FHVAXC)
CC: Jon Heylings (HEYLINGS JR)
CC: Martin Wilks (WILKS MF @ A1 @ FHVAXC)

Subject: PQ/EU/Emetic

Andy,
Thanks for the opportunity to comment on Review of the PP796 data.
I have made hand-written comments on your document and these will be Fax'd to you.
I believe you have presented the facts as they appear in the relevant reports accurately and you have not altered the conclusions of these reports.

I am sure you realise that some of these reports are in the vintage category and might not stand firm under a thorough 1995 QA-type interogation.

You will see I have attempted to 'soften' some of you statements re PP796 so we do not appear to be too up-beat about its merits and effect.

I am sure Jon and Martin Wilks will send you more comments.

Bob.

Message

From: Ashford Emma EJ [/O=ZENECA/OU=AGUK/CN=RECIPIENTS/CN=EMMA.ASHFORD]
Sent: 9/29/2000 9:33:03 AM
To: Heylings Jon GBAP [/O=NOVARTIS-AG/OU=GBRGCP01P/CN=RECIPIENTS/CN=802690]; Farnworth Mike GBAP [/O=NOVARTIS-AG/OU=GBRGCP01P/CN=RECIPIENTS/CN=802676]
Subject: RE: Paraquat emetic info

Dear Jon/Mike,

Thank you both very much for putting all that information together for me. It is very much appreciated.

Kind regards,
Emma

-----Original Message-----
From: Heylings Jon JR
Sent: 28 September 2000 16:04
To: Ashford Emma EJ
Cc: Shaunak Richa R; Farnworth Mike MJ
Subject: RE: Paraquat emetic info

Emma

I have a few comments for you on the emetic PP796. Mike has also dug out some old studies we did back in the early 1990s.

PP796

Originally known as ICI63197. Molecular formula C9H13N5O with a MW of 207.2. I do not have its octanol:water partition coefficient (log P) but it is soluble in 500parts of water in 12 parts of chloroform and in 170 parts of alcohol.

Effective dose
Effective dose rate i.e. vomiting within 30min (ED50) in dog, monkey, marmoset and pig is 0.5mg/kg. Shown to be safe in dogs at 20mg/kg. Effective dose rate in man is also circa. 0.5mg/kg (ICI Pharms report PH20992B) when it was tested as a drug in human volunteers. The shape of the dose response curves in all species are remarkably similar and particularly steep over the 0.5-1.5mg/kg range.

Assuming a 70kg man an effective dose is 70X0.5=35mg PP796 in a lethal dose of Gramoxone which is widely agreed to be 15ml. This indicates that a concentration of 2.3mg/ml PP796 would cause vomiting within 30min in a minimally lethal dose of Gramoxone. We currently put 0.5mg/ml in the product. The 2.3mg/ml emetic version of Gramoxone provided a 5-fold safety factor in the dog (CTL/R/1250). Based on the similarities in dose response curves of the 5 vomiting species studied I would expect this to give a 5X safening in man.

Physical state and uptake
Physical state in the stomach really depends on its thermodynamic interaction with the gastric juice (pH2-3) and electrolyte composition both of which can shift solubility. The normal rule is if the PP796 is unionized at the prevailing pH it is more likely to diffuse into the lipid rich mucosal membrane and be absorbed. If it remains ionized (like paraquat itself) it will be poorly absorbed. Blood kinetics for PP796 and the vomiting response suggest it is rapidly absorbed and therefore may be difficult to boost. It would be geat, however, if we could, by formulation.

Gastric emptying
PP796 is a phosphodiesterase inhibitor and as such can affect GI motility. High doses have been shown to inhibit gastric emptying (which is good for T-gels). From our research it was concluded that over 2 hours gastric emptying itself does not seem to effect plasma emetic (PP796) concentrations in the rat, using an anaesthetised starved rat model in which the pylorus was ligated. However, at 4 hours the plasma concentrations were significantly increased when the stomach was unligated compared to ligated (138 compared to 54 ng/ml) indicating further absorption in the small intestine. In the ligated rat increasing the emetic three fold from 0.5 to 1.5 g/l in a Gramoxone formulation resulted in a similar increase in plasma concentrations to unligated animals, however the higher dose was not cleared as rapidly. On balance it would suggest that PP796 can be absorbed by the gastric mucosa.

Intestinal transit
PP796 only really effects intestinal transit at a dose of paraquat that is equivalent to 10 lethal doses at a concentration of

1.5 g/l PP796 in a 200 g/l paraquat formulation. This was concluded from a study in which an oral dose of emetic (12mg/kg) was given 1 hour prior to a charcoal bolus in the mouse, significantly ($P<0.001$) reduced the distance travelled by the bolus in 1 hour compared to control.

Absorption and excretion in vivo

In the rabbit when orally dosed at 40 mg/kg PQ with a similar paraquat concentration (Gramoxone formulation) containing 0.5 and 1.5 g/l resulted in a similar 3 fold increment in the peak plasma concentration and in the rate of absorption over the first 15 minutes. The emetic was rapidly cleared from the system by 24 hours post dosing irrespective of the dose of emetic.

In the dog the emetic is cleared rapidly from the plasma following a 32 mg/kg (twice lethal) dose of Gramoxone containing 1.5mg/kg emetic with emesis occurring before 10 minutes in animals with plasma concentration above 100 ng/ml at 15 minutes. The emetic plasma concentration profiles were similar to those observed with an oral dose 40 mg/kg PQ ion of Gramoxone containing the 0.5g/l emetic.

As far as I am aware there is no data generated in-house that investigated the effect of food on emetic absorption although there is no effect of removing food for a 24 hour period prior to dosing on the toxicity of paraquat in both rat and dog. Food can slow absorption of drugs particularly if it binds the drug or interferes with its delivery into the absorptive small intestine.

I hope this is of some use.

Improvement in Survival after Paraquat Ingestion Following Introduction of a New Formulation in Sri Lanka

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Funding: See section at end of manuscript.

Competing Interests: MFW and DJB are employees of Syngenta, the study sponsor. JAT is a paid consultant to the study sponsor, RF, PA, ME, NAB, SJ, DG, and AD received travel expenses from the study sponsor for attending meetings of the SG and SAP.

Academic Editor: Mervyn Singer, University College London, United Kingdom

Citation: Wilks MF, Fernando R, Ariyananda PL, Eddleston M, Berry DJ, et al. (2008) Improvement in survival after paraquat ingestion following introduction of a new formulation in Sri Lanka. PLoS Med 5(2): e49. doi:10.1371/journal.pmed.0050049

Received: June 12, 2007
Accepted: January 9, 2008
Published: February 26, 2008

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Abbreviations: CI, confidence interval; HR, hazard ratio; IQR, interquartile range; PH, proportional hazards

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ABSTRACT

Background

Pesticide ingestion is a common method of self-harm in the rural developing world. In an attempt to reduce the high case fatality seen with the herbicide paraquat, a novel formulation (INTEON) has been developed containing an increased emetic concentration, a purgative, and an alginate that forms a gel under the acid conditions of the stomach, potentially slowing the absorption of paraquat and giving the emetic more time to be effective. We compared the outcome of paraquat self-poisoning with the standard formulation against the new INTEON formulation following its introduction into Sri Lanka.

Methods and Findings

Clinical data were prospectively collected on 586 patients with paraquat ingestion presenting to nine large hospitals across Sri Lanka with survival to 3 mo as the primary outcome. The identity of the formulation ingested after October 2004 was confirmed by assay of blood or urine samples for a marker compound present in INTEON. The proportion of known survivors increased from 76/297 with the standard formulation to 103/289 with INTEON ingestion, and estimated 3-mo survival improved from 27.1% to 36.7% (difference 9.5%; 95% confidence interval [CI] 2.0%–17.1%; $p = 0.002$, log rank test). Cox proportional hazards regression analyses showed an approximately 2-fold reduction in toxicity for INTEON compared to standard formulation. A higher proportion of patients ingesting INTEON vomited within 15 min (38% with the original formulation to 55% with INTEON, $p < 0.001$). Median survival time increased from 2.3 d (95% CI 1.2–3.4 d) with the standard formulation to 6.9 d (95% CI 3.3–10.7 d) with INTEON ingestion ($p = 0.002$, log rank test); however, in patients who did not survive there was a comparatively smaller increase in median time to death from 0.9 d (interquartile range [IQR] 0.5–3.4) to 1.5 d (IQR 0.5–5.5); $p = 0.02$.

Conclusions

The survey has shown that INTEON technology significantly reduces the mortality of patients following paraquat ingestion and increases survival time, most likely by reducing absorption.

The Editors' Summary of this article follows the references.

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Introduction

Self-poisoning with pesticides is a major public health problem in many developing countries, accounting for up to one-third of all suicides worldwide according to recent estimates [1]. While organophosphorus insecticides are by far the leading cause of morbidity and mortality in these self-poisonings, other pesticides are important in specific regions and countries [2,3]. Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) is a nonselective contact herbicide that has been widely used in many countries since the 1960s. Following ingestion of large amounts of concentrated formulation, the rapid development of multi-organ failure and cardiogenic shock is almost universally fatal. When smaller amounts are ingested, paraquat is actively taken up into pulmonary epithelial cells where redox cycling and free radical generation trigger a fibrotic process that may lead to death [4–7].

Survival after acute paraquat poisoning is related to the ingested amount, the circumstances of poisoning, and the formulation ingested [8]. While intentional ingestion of paraquat concentrate accounts for most recorded fatalities, the problem of unintentional ingestion prompted the introduction of formulation changes (a blue colour, a stenching agent, and an emetic) to the liquid concentrate in the late 1970s and early 1980s [9]. This change is believed to have made a major contribution to the decrease of unintentional paraquat ingestion in many countries [9,10]. However, mortality following intentional ingestion remains high, and a beneficial effect of these early formulation changes on the survival rate has not been demonstrated [11].

GRAMOXONE INTEON is a novel paraquat formulation specifically developed to decrease toxicity through a reduction in the amount of paraquat absorbed from the gastrointestinal tract following ingestion [12]. A natural alginate that immediately gels when entering the low-pH environment of the stomach has been incorporated into the formulation and the amount of emetic has been increased. These changes are designed to improve efficacy of emesis after gelling of the formulation in the stomach. An osmotic purgative, magnesium sulphate, has also been added to the INTEON formulation to help speed up the passage of remaining paraquat through the small intestine, the main site of paraquat uptake, thereby reducing overall absorption.

We carried out an observational study to compare the 3-mo survival of patients admitted to hospital following paraquat ingestion before and after the introduction of the new INTEON formulation in Sri Lanka.

Methods

Patients

The study was conducted in nine large hospitals (in Galle, Hambantota, Anuradhapura, Polonnaruwa, Colombo, Gampaha, Ratnapura, Kandy, and Peradeniya), covering the main agricultural areas in Sri Lanka, with the exception of the northern and eastern regions. The protocol (Text S1) was approved by four separate Ethical Committees (Text S2–S5) in Sri Lanka with responsibility for surveys/studies conducted in the nine hospitals. Patients were recruited by study physicians into the survey if they reported that they had ingested products containing paraquat or, if the pesticide ingested was unknown, the patient had clinical signs typical of paraquat poisoning (mouth lesions and/or blue colouration around the mouth). Oral informed consent to participate in the survey was sought from patients or their relatives in their native language.

Procedures

Data on the exposure, treatment, and outcome of patients ingesting paraquat were collected prospectively from December 2003 to January 2006. Following review and approval of the registration package by the Office of the Registrar of Pesticides, the new INTEON formulation was introduced in October 2004 and stocks of the existing formulation were actively withdrawn from distributors and retailers. The pesticide, bottle, and label were similar to the standard formulation, the only differences being that the INTEON formulation was slightly more viscous, and the batch numbers differed. INTEON also included a tracer compound (500 ppm diquat) that could be detected in blood and urine following oral ingestions.

Data were collected by trained research assistants using a standardised questionnaire. Upon admission, demographic data (age, sex, and weight) were recorded together with information relating to previous treatments and transfer from a primary hospital. Details relating to the ingestion were taken: time of exposure; circumstances (intentional self-harm, accidental, homicide, or occupational); time to emesis; and number and force of vomiting episodes. The patient was asked to state the ingested volume from a range of quantities (<5 ml to >150 ml) with a variety of measuring schemes (millilitres, fluid ounces, or various-sized spoon/cup measures).

A plasma and/or urine sample was taken soon after admission, where possible. Samples were stored frozen and sent to Syngenta CTL (Alderley Park, Macclesfield, Cheshire, UK) for determination of paraquat ion concentration and detection of the tracer compound diquat ion to classify the case as either standard formulation or INTEON. Analysis was conducted using HPLC, LC-MS-MS, and LC fluorescence [13].

Details of treatments and clinical observations throughout the patients' stay in hospital and clinical outcome were recorded; if the patient was discharged from hospital, study doctors visited the patient at home at least 3 mo after the initial exposure to ascertain survival.

Cases were initially recorded on paper and then transferred to a Microsoft Access database. For quality control, a separate database was created from data collected from the medical notes by an auditor (this was not possible in two of the hospitals where permission for access to the medical records archives was refused). The two databases were compared to assess completeness of case ascertainment and to highlight differences in recording of details.

To find out whether the pattern of patient admissions to, and referrals from, hospitals not participating in the survey had changed over time, the study team contacted 147 hospitals and care units towards the end of the survey in the provinces where the study hospitals were located. Using a structured questionnaire, information was obtained from physicians who were in charge of admitting patients, or, in the case of central dispensaries, from the pharmacists.

Case Definition and Power Calculation

Both standard and INTEON formulation cases were classified as 'confirmed' on the basis of blood or urine

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Table 1. Categorisation of Cases into Standard Formulation and INTEON Formulation Groups

Formulation	Category	Before 1 October 04	After 1 October 04	Confirmation of Product Identity			Recorded after Washout Period[b]
				Paraquat Present	Diquat Present	Bottle or Label Confirmation	
Standard formulation	Confirmed	+	−	n/a	n/a	n/a	n/a
	Confirmed	−	+	+	−[a]	n/a	n/a
	Probable	−	+	n/a	n/a	+	n/a
INTEON formulation	Confirmed	−	+	+	+	n/a	n/a
	Probable	−	+	n/a	n/a	+	n/a
	Possible	−	+	n/a	n/a	−	+

[a]Provided there was sufficient paraquat present to ensure the detectability of diquat.
[b]Defined individually for each hospital as the time from 1 October 2004 until the recording of the first two consecutive confirmed INTEON cases.
+, yes; −, no; n/a, not applicable or not available.
doi:10.1371/journal.pmed.0050049.t001

analysis and as 'probable' when bottle or label were presented (Table 1). The recording of the first two consecutive confirmed INTEON cases at each hospital was taken to indicate that INTEON use had become common in the area, and a washout period was defined for each hospital from 1 October 2004 until that time point. Cases after the washout period without sample confirmation or evidence from the bottle/label were classified as 'possible' INTEON cases.

The power calculation was based on the Mantel-Haenszel risk ratio estimate stratified for three ingestion groups and indicated that a total of 210 cases would give > 85% power to detect a 2-fold reduction in potency for a two-sided test with significance level of 5%. It was decided to use the number of confirmed INTEON cases to close the survey in order to achieve adequate power for the sensitivity analyses. The number of confirmed cases fell below 210 after some patients were identified with admission records at more than one hospital after transferring between hospitals and other patients had to be excluded because they did not meet the study entrance criteria. However, the total number of INTEON cases (confirmed, probable, and possible) included in the analyses exceeded 210.

Statistical Analysis

Means and proportions for baseline variables were compared between the two ingestion groups using Student's t test for continuous variables and the χ^2 test for categorical variables. The primary analysis compared survival among standard formulation cases before 1 October 2004 with survival among confirmed, probable, and possible INTEON formulation cases after the washout period. In sensitivity analyses, survival among all confirmed and probable standard formulation cases was compared with survival among all confirmed and probable INTEON formulation cases.

Time to death analyses were performed using both nonparametric analysis methods (Kaplan–Meier survival curve estimates and the Mantel–Cox log rank test) and semiparametric methods (Cox proportional hazards [PH] regression models). Standard errors for 3-mo survival estimates were obtained using Greenwood's method [14]. All statistical analyses were performed using Stata version 9.

Cox PH regression models were used to estimate unadjusted and adjusted hazard ratios for the INTEON formulation. Adjusted analyses always included terms for the following covariates: (a) sex, age, and weight of participant; (b) treatments received; (c) use of adsorbent; and (d) time from ingestion to presentation at a medical centre.

Estimated ingestion amount was an important factor influencing survival, but information was not available for a number of cases. Consequently, unadjusted and adjusted hazard ratios were also derived for the subset of patients who had ingestion information. Adjustment was performed with and without estimated ingestion amount in the regression model. Ingestion amount was included as a categorical variable (eight levels) but also as a continuous variable using the logarithms of the midpoint of ingestion categories. Models were also fitted to examine whether the relationship with ingestion amount differed between the two groups. Estimates of relative potency were derived using the slope of the relationship with the logarithm of ingestion amount and term for formulation group in the Cox PH model.

Variation in survival characteristics between the nine study hospitals was investigated using a gamma frailty model (proportional hazard functions with random scaling factors). In addition, evidence of nonproportional hazard functions was assessed by visual methods and by testing the significance of the interaction with the logarithm of survival time. Stratification was used to account for nonproportionality of the hazard functions.

Results

Information was collected by the nine study hospitals on 774 patients over the study period. The numbers of participants eligible for the primary analysis and sensitivity analyses broken down by formulation are given in Table 2. The primary study population included 297 confirmed cases of standard formulation ingestion admitted before 1 October 2004 and 289 confirmed, probable and possible cases of INTEON ingestion. For sensitivity analyses all confirmed or probable cases were used (382 standard formulation and 206 INTEON cases).

The two primary study populations were similar for demographic and ingestion variables at baseline (Table 3). Most patients had ingested paraquat deliberately (93.7% of all cases). Information on ingestion volume was not available for a higher percentage of standard formulation than INTEON

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Table 2. Survey Participants

Category	Patients	*n*
Total cases (1 December 2003 to 26 January 2006)	—	774[a]
Exclusions	—	97
	Non-oral exposure	30
	Consent refused	8
	Incomplete record	5
	Unintentional poisoning with illicit alcohol[b]	36
	Washout period cases without sample confirmation or bottle/label information	18
Standard formulation cases	—	382
	Before 1 October 2004[c]	297
	Washout period—confirmed with plasma/urine analysis	38
	Washout period—probable (bottle or label information)	—
	Post washout period—confirmed with plasma/urine analysis	47
	Post washout period—probable (bottle or label information)	—
INTEON cases	—	295
	Washout period—confirmed with plasma/urine analysis	6
	Washout period—probable (bottle or label information)	—
	Post washout period—confirmed with plasma/urine analysis[c]	195
	Post washout period—probable (bottle or label information)[c]	5
	Post washout period—possible[c]	89

[a]Includes five patients with records at two centres.
[b]In a single incident, 36 patients ingested kassipu (illegally brewed alcoholic drink) to which a small amount of paraquat had been added. It was not possible to establish which formulation had been used or how much paraquat had been ingested.
[c]Dataset used in the primary analysis.
doi:10.1371/journal.pmed.0050049.t002

cases, and the distribution of cases among the ingestion subgroups was different between the two formulations.

The clinical characteristics of the two groups were generally similar (Table 4), but a significantly higher proportion of INTEON patients vomited within 15 min of ingestion. Just over half of all patients were treated at a primary hospital before being referred to a study hospital and this proportion was higher for patients who had ingested INTEON formulation (57.8% versus 45.5%). Lavage, intravenous fluids, and prednisolone were the only treatments for which there was a significant difference between the two groups. Fewer INTEON patients received these treatments than patients who had ingested the standard formulation paraquat.

Follow-up of patients was generally good (Table 5), but it was not possible to find out whether ten patients (4.4% of those followed up) were still alive at 3 mo. Four INTEON patients were followed up slightly early (a minimum of 11 wk after ingestion) and are described as alive in Table 5. The proportion of known survivors increased from 76 of 297 patients with the standard formulation to 103 of 289 patients with INTEON ingestion, and there was an increase in estimated 3-mo survival (Kaplan–Meier estimates) among the INTEON patients from 27.1% to 36.7% (difference 9.6%; 95% CI 2.0%–17.1%). Kaplan–Meier survival analysis (Figure 1) and log rank test indicated a significant difference between the two survival curves ($p = 0.002$). Median survival time increased from 2.3 d (95% CI 1.2–3.4 d) with the standard formulation to 6.9 d (95% CI 3.3–10.7 d) with INTEON ingestion ($p = 0.002$, log rank test).

The overall improvement in survival among patients who had ingested the INTEON formulation was seen in every ingestion group except the <5 ml group, in which survival was already high. Figure 2 shows summary Kaplan–Meier survival curves for patients categorised into four ingestion groups (<10 ml, 10–30 ml, 30–100 ml, and ≥100 ml) for each formulation. In addition, survival curves are shown for patients for whom ingestion information was not available.

Survival following ingestion of INTEON was significantly better than the standard formulation (hazard ratio [HR] 0.73, 95% CI 0.60–0.89; $p = 0.002$) in an unadjusted analysis (Table 6). There was evidence of nonproportionality of the hazard functions of different hospitals, and stratification was used to account for this. However, HR changed only slightly when

Table 3. Demographic and Ingestion Details of Patients in the Formulation Groups

Category	Group	Standard Formulation Cases before 1 October 04 ($n = 297$)	Confirmed, Probable, or Possible INTEON Cases (after Washout Period) ($n = 289$)
Demographic details	Male (%)	230 (77.4)	233 (80.6)
	Age, y (mean ± SD)	31.0 ± 13.7	29.3 ± 12.4
	Weight, kg (mean ± SD)	55.0 ± 8.1	56.4 ± 9.0
Ingestion details	Deliberate ingestion (%)	282 (94.9)	267 (92.4)
	Ingestion amount known (%)	221 (74.4)	248 (85.8)***
	<5 ml	37 (16.7)	32 (12.9)
	5 to <10 ml	18 (8.1)	25 (10.1)
	10 to <15 ml	24 (10.9)	43 (17.3)
	15 to <30 ml	31 (14.0)	45 (18.1)
	30 to <50 ml	22 (10.0)	30 (12.1)
	50 to <100 ml	26 (11.8)	31 (12.5)
	100 to 150 ml	25 (11.3)	14 (5.6)
	>150 ml	38 (17.2)	28 (11.3)

*** $p < 0.001$. SD, standard deviation.
doi:10.1371/journal.pmed.0050049.t003

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Table 4. Clinical Details of Patients in the Formulation Groups

Detail	Standard Formulation Cases before 1 October 04 ($n = 297$), n (%)	Confirmed, Probable, or Possible INTEON Cases (after Washout Period) ($n = 289$), n (%)
Treated at primary hospital	135 (45.5)	167 (57.8)**
Vomited within 15 min	113 (38.0)	158 (54.7)***
Treated within 4 h of ingestion	175 (58.9)	166 (57.4)
Lavage	208 (70.0)	154 (53.3)***
Lavage—primary hospital only	46 (15.5)	39 (13.5)
Lavage—study hospital only	123 (41.4)	87 (30.1)
Lavage—both	39 (13.1)	28 (9.7)
Adsorbent	254 (85.5)	241 (83.4)
Adsorbent–Fullers Earth only	237 (79.8)	220 (76.1)
Adsorbent—activated charcoal only	13 (4.4)	6 (2.1)
Adsorbent–both	4 (1.3)	15 (5.2)
Intravenous fluids	283 (95.3)	262 (90.7)*
Diuretics	26 (8.8)	33 (11.4)
Antiemetic	38 (12.8)	51 (17.6)
Magnesium	2 (0.7)	6 (2.1)
Prednisolone	50 (16.8)	28 (9.7)*
Cyclophosphamide	34 (11.4)	39 (13.5)

*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.
doi:10.1371/journal.pmed.0050049.t004

stratification was made for treatment centre and when covariates other than estimated ingestion amount were included in the model. Table 6 also shows that HRs were smaller when these analyses were restricted to the group of patients with ingestion information, but the fully adjusted analysis (including ingestion amount) for this latter group of patients gave an HR of 0.67 (95% CI 0.52–0.87), which is similar to that seen in the unadjusted analysis for all participants.

Replacing the eight-level categorical variable for ingestion amount with the logarithm of the midpoint of ingestion in each category made little difference to the fit of the model (change in $\chi^2 = 3.62$, 6 df) and there was no evidence of a different relationship with ingestion amount for the standard and INTEON formulations. The HR for a doubling of ingestion amount was 1.57 (95% CI 1.46–1.69). The strong relationship with the logarithm of ingestion amount enables an estimate to be made of the potency (toxicity) of the INTEON formulation relative to the standard formulation. Based on the subset of patients with ingestion information, the potency of INTEON was estimated to be 0.54 of the standard formulation.

Sensitivity analyses including all confirmed and probable cases gave results that were very similar to those obtained in the primary analysis. There was an increase in estimated 3-mo survival among the INTEON patients from 27.4% to 37.9% (difference 10.5%; 95% CI 2.5%–18.6%) and an HR of 0.64 (95% CI 0.50–0.82) with a potency estimate for INTEON of 0.47 of the standard formulation.

Among patients who died there was an increase in median time to death from 0.9 d (interquartile range [IQR] 0.5–3.4) for the standard formulation to 1.5 d for INTEON (IQR 0.5–5.5); $p = 0.02$. This effect was more pronounced in the sensitivity analysis, restricted to confirmed and probable cases, where the median time to death was 1.1 d (IQR 0.5–3.9) for the standard formulation but 2.5 d for INTEON (IQR 0.8–9.0); $p = 0.001$.

Monthly admissions of patients with paraquat poisoning to study hospitals showed some seasonal variability, related to the use pattern of paraquat in Sri Lanka (Figure 3). However, they also suggest an overall decrease of the number of cases over time. In the separate admission and referral survey of 147 contacted hospitals and care units, 83 (56%) reported having received a total of 541 patients with paraquat

Table 5. Vital Status of Patients at Three Months Following Paraquat Ingestion in the Formulation Groups

Outcome 3 Mo after Ingestion	Standard Formulation Cases before 1/10/04	Confirmed, Probable, or Possible INTEON Cases (after Washout Period)
Dead (%)	215 (72.4%)	182 (63.0%)
Alive (%)	76 (25.6%)	103 (35.6%)
Lost to follow-up (%)	6 (2.0%)	4 (1.4%)
Total	297	289

doi:10.1371/journal.pmed.0050049.t005

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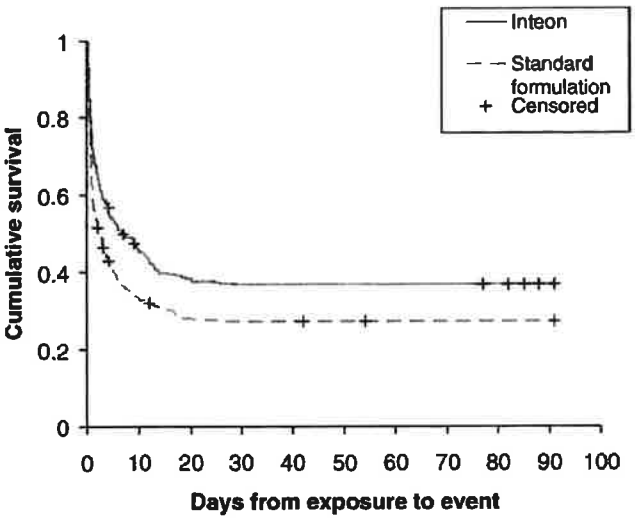

Figure 1. Kaplan-Meier Survival Curves for Patients Ingesting Standard and INTEON Formulation
doi:10.1371/journal.pmed.0050049.g001

poisoning. Nearly two-thirds (63%) of hospitals and care units reported no change in the number of patients seen since the introduction of INTEON, whereas 29% reported a decrease and 8% an increase. Virtually all hospitals that were able to provide information had not changed their referral pattern of paraquat-poisoned patients, and there was no difference between the larger and smaller units.

Discussion

In Sri Lanka, pesticides are the most common means of self poisoning, with case fatality ratios more than 10-fold higher than those from self-poisoning in industrialised countries [15]. Although not the most common cause of pesticide death, paraquat has a higher case fatality ratio than other commonly ingested pesticides [16]. We have shown in this study that the development of a new formulation that turns to a gel in the stomach, slowing absorption and increasing the time available for effective emesis, increases estimated 3-mo survival from 27.1% for patients ingesting the standard formulation to 36.7% with the INTEON product. In individual terms this equates to approximately 30 lives saved within the survey due to the introduction of INTEON.

Despite much research into the mechanism of toxicity and the potential for treatment of paraquat poisoning, no specific therapy has so far been shown to affect outcome in controlled clinical studies [5,6,17]. Consequently, prevention of absorption remains an important approach to reduce paraquat toxicity. For this reason a potent emetic has been included in paraquat formulations since the late 1970s [9]. However, a beneficial effect of this measure on case fatality has not been conclusively demonstrated [11,18–22]. This may be related to the relatively large quantities of product that are often ingested in self-harm cases.

Paraquat causes mucosal damage and increases passive flux across the mucosal barrier at high concentrations [23], and peak plasma levels occur within one hour, since the liquid formulation rapidly reaches the absorptive site in the small intestine [6]. The principle of the INTEON formulation is based on the addition of alginates, which become protonated after contact with gastric acid and transformed into a gelatinous mixture. This technology is used in pharmaceuticals to treat heartburn and acid reflux [24] and to cause satiety in the treatment of obesity, by virtue of the intragastric bulking of alginates [25]. In vitro and in vivo studies have shown that the inclusion of the alginate into the formulation led to a decrease in paraquat absorption [12]. The combination of the alginate with an increased emetic concentration and magnesium sulphate added as purgative is considered to be necessary to achieve an optimum safening effect. The INTEON formulation introduced into Sri Lanka also contained a built-in surfactant system. Some of the

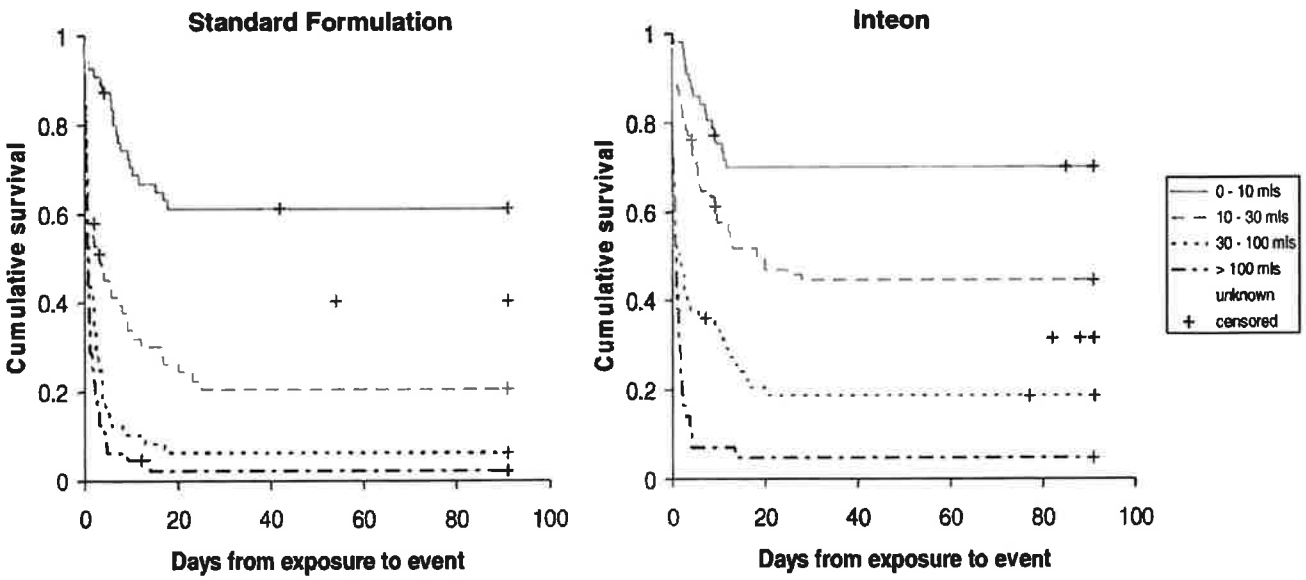

Figure 2. Kaplan-Meier Survival Curves by Formulation Group and Ingestion Amount
doi:10.1371/journal.pmed.0050049.g002

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Table 6. Hazard Ratios for INTEON Formulation from Cox Proportional Hazards Regression Models

Participants Included in Analysis	No Stratification; no Covariate Adjustment		Stratification for Centre; No Covariate Adjustment		Stratification for Centre; Adjustment for All Covariates Except Ingestion Amount		Stratification for Centre; Adjustment for All Covariates Including Ingestion Amount (Continuous Variable)	
	Hazard Ratio (95% CI)	*p*-Value	Hazard Ratio (95% CI)	*p*-Value	Hazard Ratio (95% CI)	*p*-Value	Hazard Ratio (95% CI)	*p*-Value
All participants[a]	0.73 (0.60–0.89)	0.002	0.77 (0.62–0.95)	0.014	0.71 (0.57–0.87)	0.001	—	—
Participants with ingestion information[b]	0.63 (0.50–0.78)	<0.001	0.66 (0.52–0.85)	0.001	0.61 (0.47–0.78)	<0.001	0.67 (0.52–0.87)	0.002

[a]Standard formulation: $n = 297$; INTEON: $n = 289$
[b]Standard formulation: $n = 221$; INTEON: $n = 248$
doi:10.1371/journal.pmed.0050049.t006

formulation ingredients were found to gradually separate out in the bottle with prolonged storage, creating a surfactant and emetic-rich phase, and one with increased paraquat and alginate concentration. Although the formulation could be easily rehomogenised by light agitation of the bottle the overall safening effect may potentially have been suboptimal.

Although steps were taken to actively withdraw the old product from the market when the new formulation was introduced, we recognised that there would be a period in which the old product would still be with farmers. It was therefore important to unequivocally identify as many cases as possible through analysis of the marker that had been added to the INTEON product in a plasma or urine sample. However, this identification was possible only in two-thirds of the INTEON cases due to a combination of samples not being taken (e.g., in patients who were very ill on admission and died quickly) and samples with plasma paraquat concentrations so low that the diquat marker could not be detected. To reduce the number of standard formulation cases incorrectly included in the INTEON group we introduced washout periods for the centres. During the washout periods only 6/44 (14%) of patients with sample confirmation were INTEON ingestions. In contrast, 195/242 (81%) of patients with samples after the washout period had ingested INTEON. Hence, it is likely that the majority of the 89 possible INTEON cases after the washout period were correctly classified as INTEON cases. Only 18 cases with no sample information or equivocal results occurred during the washout period and had to be excluded from the survival analyses. Importantly, the sensitivity analyses excluding those patients without sample or bottle confirmation gave very similar results to the primary analysis, providing further evidence that our overall classification of cases was largely correct. The possible inclusion of a small number of standard formulation cases in the INTEON group may have had a small impact on the survival rate. However, the effect of not including possible INTEON formulation cases would have been far greater because of (a) missing cases with large ingestion volumes because of the difficulty of collecting samples from very sick patients, and (b) missing ingestions too small for the marker to be detectable in samples.

Ingestion information was not available for 26% of standard formulation cases and 14% of INTEON ingestions. The higher proportion of standard formulation cases with missing ingestion information resulted because information was not routinely collected at the start of the survey at one hospital. Many of the other patients without an ingestion amount were too ill to supply this information. Standard formulation patients with ingestion information tended to have ingested more than INTEON patients, and 29% had ingested more than 100 ml compared with 17% of INTEON patients. However, this difference in ingestion amounts would only explain a small part of the observed improvement in survival since standardising the survival rate of the standard formulation cases with ingestion information to the ingestion amount distribution of the INTEON patients only increased the estimated survival probability of standard formulation cases from 27.1% to 27.7%. Furthermore, standard formulation cases without ingestion information appeared to have ingested less than INTEON patients without ingestion information based on their higher survival rate, and the ingestion distributions of the full groups were probably closer than those of the subgroups with ingestion information.

Since the INTEON formulation was introduced in the whole country at the same time we had to rely on a before-and-after design for the survey. It is therefore possible that changes in treatment, hospital admissions, or referrals may have occurred over the period of the survey. There were some differences between the two groups in terms of treatment, with fewer INTEON patients receiving gastric lavage and prednisolone, but none of the differences were major confounders of the observed beneficial effect of INTEON on survival. Table 6 shows that the hazard ratios with and without covariate adjustment are very similar, suggesting that the differences in treatment explain very little of the group difference in survival. There is a difference in crude survival rate between those who had lavage and those who did not, but the effect disappears when adjustment is made for ingestion amount. The lower rate of lavage in the INTEON group is more likely a consequence of factors such as the higher rate of early emesis and not an explanation for improved survival. There were a number of patients who stated that they had ingested very small amounts of either formulation but had a rapid onset of emesis. It is suspected that some of these patients ingested much more than stated and hence that rapid onset of emesis in the lower exposure groups may be an indicator of misreported exposure.

Heylings Dec Exhibit 28

SYNG-PQ-00038666

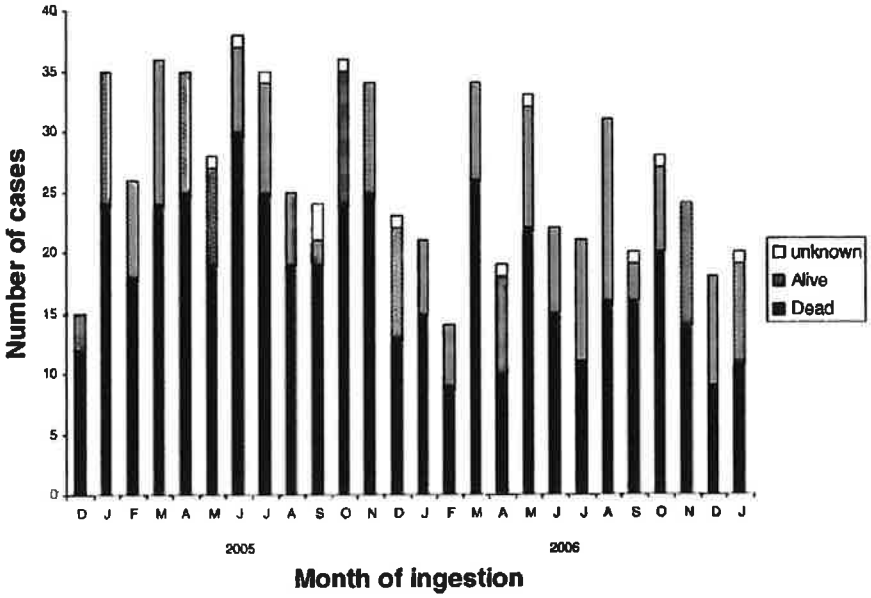

Figure 3. Monthly Admission Rates of Patients with Paraquat Poisoning to Study Hospitals According to Outcome at Three Months
doi:10.1371/journal.pmed.0050049.g003

The monthly admissions over the study period suggest an overall decrease of the number of cases over time. In the survey of peripheral hospitals and care units there was no indication of a change in their referral practices over time. Changes in case ascertainment and management are therefore unlikely to have substantially contributed to the improved survival noted with INTEON. However, many hospitals indicated that the number of paraquat cases had decreased. This change may relate to shifts in the general pattern of self-harm incidents, but it is also possible that fewer patients ingesting the INTEON formulation were seeking health care.

For those patients who did not survive, there was an increase in time to death for INTEON compared to the standard formulation. This difference may become important when trying to achieve improvements in the treatment of paraquat poisoning, as it may allow more time for new or existing therapies to become effective. Our data show that in Sri Lanka self-harm patients reach hospital reasonably quickly (nearly 60% are treated within 4 h), so improved treatment of poisoning cases in addition to the INTEON formulation could have a further positive effect on survival.

While our finding of improved survival of patients in the INTEON group is encouraging the data also show that the beneficial effect of the formulation is limited by the amount of product ingested, since this was the single most important predictor of survival in both groups. It is therefore apparent that formulation changes in themselves will not be sufficient to comprehensively address the problem of mortality from self-harm with paraquat. An integrated approach has recently been proposed including generic measures to reduce self-harm incidents, as well as focusing on reducing access, reducing formulation toxicity (e.g., by reducing formulation strength), and improving the treatment of poisoning [26]. However, there are clear tensions between what is desirable from public health, agricultural, and industry perspectives, and this lies at the heart of the controversy over the benefits and risks of paraquat use, in particular in developing countries. A detailed discussion of this subject is beyond the scope of this paper, but can be found elsewhere [27–29]. Nevertheless, it is evident that, as long as paraquat and other potentially harmful pesticides continue to be widely used, a comprehensive programme to prevention and management of poisoning is needed. This is why the World Health Organization (WHO) has announced a public health initiative with the overall goal to reduce morbidity and mortality from pesticide poisoning, including improved regulatory policies, epidemiological surveillance, improved medical management and mental health-care, training in the safe handling of pesticides, and community programmes that minimise the risk of intentional and unintentional poisonings [1].

In conclusion, this survey shows that the introduction of a new paraquat formulation with INTEON technology has led to a significant improvement in survival of patients with paraquat poisoning. Our statistical analyses indicate that this effect is due to a real difference between the two formulations. Patients who ingested a lethal amount of the formulation survived longer with INTEON, raising the prospect of more opportunities for treatment. These encouraging results were achieved despite suboptimal homogeneity of the formulation, and future improvements in formulation technology may reduce overall toxicity even further.

Supporting Information

Text S1. Survey Protocol
Found at doi:10.1371/journal.pmed.0050049.sd001 (152 KB DOC).

Text S2. Ethics Committee Approval University of Ruhuna
Found at doi:10.1371/journal.pmed.0050049.sd002 (284 KB PDF).

Text S3. Ethics Committee Approval Anuradhapura General Hospital
Found at doi:10.1371/journal.pmed.0050049.sd003 (322 KB PDF).

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SYNG-PQ-00038667

Text S4. Ethics Committee Approval General Hospital (Teaching) Kandy

Found at doi:10.1371/journal.pmed.0050049.sd004 (284 KB PDF).

Text S5. Ethics Committee Approval National Hospital of Sri Lanka (Colombo)

Found at doi:10.1371/journal.pmed.0050049.sd005 (627 KB PDF).

Acknowledgments

We thank the directors, consultant physicians, study physicians, preinterns, and medical and nursing staff of the study hospitals as well as the management and staff of Chemical Industries (Colombo) Ltd. for their support. We are indebted to Justin Perera for local coordination, to Bruce Woollen for paraquat analysis, and to Prof. Keith Hawton for his contribution to the Scientific Advisory Panel (SAP). We thank Nilupa Herath, Dr. Lumbini de Silva, Fahim Mohamed, Dr. Sasanka Gunaratne, Dr. Hasantha Ranganath, and Dr. K Chathurika for supervising the data collection, data entry, quality control checks, and patient follow-up.

Author contributions. MFW designed this study, acted as Principal Investigator, chaired the Steering Group (SG), and wrote the first draft of the report. RF, PLA, and ME had responsibility for data collection in the participating hospitals, acted as co-investigators, and were members of the SG. ME also helped design the study. DJB helped design the study, coordinated the data collection, extracted and checked patients' data for analysis, and acted as Secretary to the SG. JAT carried out the statistical analysis and was a member of the SG. NAB helped design the study and was a member of the Scientific Advisory Panel (SAP). SJ had responsibility for data collection in a participating hospital and was a member of the SAP. DG advised on the epidemiological and statistical methodology, checked the analysis, and was a member of the SAP. AD chaired the SAP and had responsibility for the local study coordinators and the data audit. All authors helped improve the study design and finalise the report.

Funding. This study was funded by Syngenta Crop Protection AG, Basel, Switzerland. The study sponsor was involved in study design, data collection, data analysis, data interpretation, and writing of the report. The protocol (Text S1) included a commitment by the study sponsor to present and publish data from the survey in appropriate scientific venues and journals. The corresponding author had full access to all the data in the study and followed the protocol (Text S1) in deciding to submit for publication. ME is a Wellcome Trust Career Development Fellow funded by grant 063560MA from the Wellcome's Tropical Interest Group. The South Asian Clinical Toxicology Research Collaboration (SACTRC) is funded by the Wellcome Trust/National Health and Medical Research Council International Collaborative Research Grant 071669MA.

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Heylings Dec Exhibit 28

SYNG-PQ-00038668

Editors' Summary

Background. Paraquat is a non-selective herbicide used in many countries on a variety of crops including potatoes, rice, maize, tea, cotton, and bananas. It is fast-acting, rainfast, and facilitates "no-till" farming, but it has attracted controversy because of the potential for misuse, particularly in developing countries. Better training of workers has been shown to reduce the number of accidents, and additions to the liquid formulation have contributed to a reduction in cases where paraquat was drunk by mistake—blue color and a stench agent made it less attractive to drink, and an emetic to induce vomiting aimed to reduce the time it is retained in the body.

Why Was This Study Done? Despite the changes made to the formulation, paraquat is still taken deliberately as a poison by agricultural workers in parts of the developing world. Although other pesticides cause more deaths overall, paraquat poisoning is more frequently fatal than other common pesticides. Syngenta, a commercial producer of paraquat, has developed a new paraquat formulation designed to reduce its toxicity. Syngenta introduced the new formulation in Sri Lanka, a country well known for its high level of suicides with pesticides, in 2004. This new formulation includes three components designed to reduce paraquat absorption from the stomach and intestines: a gelling agent to thicken the formulation in the acidic environment of the stomach and slow its passage into the small intestine; an increase in the amount of emetic to induce more vomiting more quickly; and a purgative to speed its exit from the small intestine, the main site of its absorption. The researchers wished to know whether the new formulation could contribute to improved survival in instances where paraquat had been ingested.

What Did the Researchers Do and Find? The researchers gathered information on the time and circumstances of when paraquat was taken, the amount that was taken, the times, and details of any vomiting, treatment, and outcomes for cases of attempted suicide by paraquat poisoning at nine large hospitals in agricultural regions of Sri Lanka from December 2003 to January 2006. In total, 774 patients were tracked in this time. Syngenta introduced the new formulation in Sri Lanka on 1 October 2004. The researchers gathered information on the formulation involved in subsequent cases, by either interview or analysis of samples. After excluding some unusual or less certain cases, they analyzed data on 586 patients, of whom 297 had deliberately taken the standard formulation and 289 the new formulation.

Although the new formulation was still toxic, the data showed an increase in the proportion of cases surviving for at least three months—from 27% (standard formulation) to 37% (new formulation), an effect that was unlikely to be due to chance. More patients vomited within 15 minutes of taking the new formulation of paraquat. Patients who died generally survived longer if they had taken the new rather than the standard formulation. The researchers estimated that the new formulation is just over half as toxic as the standard formulation, meaning that a patient was likely to suffer the same level of ill effects after taking twice as much of the new formulation compared to the standard formulation.

What Do these Findings Mean? This study was designed, funded, and led by Syngenta, the manufacturer of the standard and new formulations of paraquat but the study team included a number of independent Sri Lankan and international scientists. As the researchers observed the effects of the introduction of the new formulation across the entire country at the same time, they could not completely rule out other possible reasons for the differences in outcomes for those who had taken the two formulations, such as differences in treatment.

Despite this inherent drawback, the researchers estimate that during the study the new formulation saved about 30 lives. They conclude that the the new formulation does reduce the amount of paraquat absorbed by the body, although the study does not answer the question whether this was due to the gelling agent, the increased emetic in the new formulation or a combination of factors. The researchers suggest that the new formulation, by keeping patients alive longer, may allow doctors more time to treat patients. As no effective treatment exists at present, this benefit relies on a treatment being developed in the future.

The researchers note that the most important factor in predicting the outcome when paraquat has been taken deliberately is the dose. As a result, they suggest that the new formulation can only be one part of a wider strategy to reduce deaths by deliberate self-poisoning using paraquat. They suggest that such an integrated approach might include generic measures to reduce incidents of self-harm, reduced access to paraquat, reduced formulation strength, and improvements in treatment.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0050049.

- The US Environmental Protection Agency has published its Reregistration Eligibility Decision for paraquat
- The Department of Health and Human Services of the US Centers for Disease Control and Prevention provides a fact sheet on how to handle paraquat and suspected cases of exposure
- The World Health Organisation has recently finished consulting on a draft Poisons Information Monograph for paraquat
- The International Programme on Chemical Safety (IPCS) has published a review of paraquat in its Environmental Health Criteria Series
- MedlinePlus provides links to information on health effects of paraquat

Heylings Dec Exhibit 28

SYNG-PQ-00038669

Paraquat Dichloride: One Sip Can Kill.

The Accidental Poisoning Problem

The California Poison Control System and the Central California Children's Hospital reviewed data from 1998-2009 and identified more than 1,400 cases of accidental poisonings caused by storage of non-food substances in soda bottles, unmarked bottles, cups or glasses. Several of the deaths involved the accidental ingestion of pesticides, including paraquat. [1]

Recent Deaths from the Accidental Ingestion of Paraquat

In 2013, the California Poison Control System and the American Association of Poison Control Centers (AAPCC) sent letters of concern to EPA regarding a series of deaths from accidental ingestion of the pesticide paraquat in the San Joaquin Valley of California. AAPCC cited 50 deaths from paraquat; at least 12 were from accidental ingestion of paraquat from a beverage container.

This is a major concern to EPA because paraquat is a Restricted Use Pesticide that should not be accessible to the general public and, as with all pesticides, should never be placed into a beverage container. Paraquat is highly toxic to humans; one small accidental sip can be fatal and there is no antidote.

The product labels clearly prohibit pouring paraquat into food or beverage containers with the prominently-placed statements:

- "NEVER PUT INTO FOOD, DRINK OR OTHER CONTAINERS" and
- "DO NOT REMOVE CONTENTS EXCEPT FOR IMMEDIATE USE."

Paraquat Use Profile

Paraquat dichloride, commonly referred to as "paraquat," is an herbicide registered in the United States since 1964 to control weeds in many agricultural and non-agricultural use sites. It is also applied as a pre-harvest desiccant on some crops including cotton.

All paraquat products registered for use in the United States are Restricted Use Pesticides (RUPs), which can only be sold to and used by certified applicators (and applicators under their direct supervision). There are no homeowner uses and no products registered for application in residential areas.

EPA Incident Investigation

The fatalities resulting from paraquat products transferred into beverage containers in California prompted EPA to investigate all reported cases. EPA conducted an investigation of all reports of fatal and high-severity paraquat incidents. EPA identified 27 paraquat fatality reports through 2014 in its Incident Data System (IDS). The IDS database contains all registrant submissions of adverse health effects from pesticide products, as required by federal law (FIFRA §6(a)(2)). More than 80% of all identified paraquat fatality cases reported to IDS were due to ingestion of the product.

At least eight of these 27 deaths were due to the accidental ingestion of paraquat. All eight of these accidental deaths involved transfer of paraquat into a beverage container. Several of these cases have occurred recently. A review of the SENSOR-Pesticides data identified additional ingestion cases, including the fatal case of an 8-year-old child who drank the paraquat out of a soda bottle.

True Stories

- In 2013, a 70-year-old female ingested some contents of a re-used iced tea bottle that contained paraquat, unknown to her. She went to the hospital awake and alert with persistent vomiting. Over the course of a 16-day admission, she evolved the classic picture of paraquat ingestion: corrosive gastrointestinal injury plus kidney and respiratory failure leading to death.
- In 2010, a 44-year-old male mistakenly drank paraquat, which he thought was fruit juice. He developed difficulty breathing and vomited blood. He was admitted to the hospital intensive care unit where he died after 20 days of aggressive treatment.
- In 2008, an 8-year-old boy drank paraquat that had been put in a Dr. Pepper bottle, which he found on a window sill in the garage. He died in the hospital 16 days later. His older brother had used the product on weeds around the house and put it in the bottle in the garage. The older brother obtained the product from a family friend who is a certified Restricted Use Pesticide applicator.
- In 2003, a 49-year-old male took a sip from his coffee cup in which he had poured paraquat because the product's bottle was deteriorating. He realized his mistake and went to the Emergency Department. At that time, he was vomiting, cold and sweating profusely. Doses of activated charcoal were administered and his stomach was pumped; morphine was provided for esophageal pain; and he was intubated to support breathing function on the fourth day. Aggressive supportive care continued until he died on the tenth day.

- In 2000, a 15-month-old boy ingested paraquat that had been transferred into a Gatorade container and stored inappropriately. The boy survived in the hospital for 13 days after the ingestion and received aggressive treatment but died after suffering acute kidney and liver failure.
- In 2000, an 18-month-old boy ingested an unknown amount of paraquat solution from a bottle found in his father's landscaping truck. He received multiple-dose activated charcoal treatment two hours after the ingestion. He suffered from lack of oxygen during the first 24 hours followed by progressive liver, kidney, and cardio-pulmonary dysfunction. The boy died 11 days after the ingestion.

EPA Response

See EPA Actions to adopt measures to prevent poisoning and protect workers from paraquat. EPA has warned the applicator community about the high toxicity of paraquat.

As required by EPA's Paraquat Dichloride Human Health Mitigation Decision certified applicators must successfully complete an EPA-approved training program before mixing, loading, and/or applying paraquat. See the training module and paraquat training FAQs.

It is the responsibility of pesticide applicators to ensure that RUP products are used safely and appropriately, including never transferring any pesticide product, including paraquat, into a beverage container.

The Solution is YOU

ONE SIP CAN KILL!

To prevent the severe injury and/or death from paraquat ingestion, a paraquat product must:

- Be used only by a certified applicator or under the direct supervision of a certified applicator. Per new EPA-approved labels (which should begin appearing on products in 2019), paraquat may be used only by a certified applicator.
- Never be transferred to a food, drink or any other container.
- Always be kept secured to prevent access by children and/or other unauthorized persons.
- Never be stored in or around residential dwellings.
- Never be used around home gardens, schools, recreational parks, golf courses or playgrounds.

Paraquat Dichloride Information Resources

- EPA's Paraquat Dichloride Registration Review Docket, EPA-HQ-OPP-2011-0855, for information on EPA's current re-evaluation of paraquat. This docket includes a letter from Dr. Gellar (California Poison Control System), the EPA response, and the AAPCC letter.

- Syngenta's Paraquat Information Center: www.paraquat.com/en/safety

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[1]Epidemiology of Accidental Poisoning Caused by Storage of Non-Food Substances in Food Containers and unmarked Bottles/Containers. Geller RJ, Kezirian R, Bangar P, Strong D, Carlson T. Children's Hospital Central California; California Poison Control System (CPCS). Found online at: http://www.tandfonline.com/doi/pdf/10.1080/15563650903076924.

FAO SPECIFICATIONS AND EVALUATIONS FOR AGRICULTURAL PESTICIDES

PARAQUAT DICHLORIDE[1]

1,1'-dimethyl-4,4'-bipyridinium dichloride

FOOD AND AGRICULTURE ORGANIZATION of THE UNITED NATIONS

[1] Paraquat is the ISO common name for the 1,1'-dimethyl-4,4'-bipyridyldinium dication.

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PARAQUAT DICHLORIDE

Disclaimer[1]

FAO specifications are developed with the basic objective of promoting, as far as practicable, the manufacture, distribution and use of pesticides that meet basic quality requirements.

Compliance with the specifications does not constitute an endorsement or warranty of the fitness of a particular pesticide for a particular purpose, including its suitability for the control of any given pest, or its suitability for use in a particular area. Owing to the complexity of the problems involved, the suitability of pesticides for a particular purpose and the content of the labelling instructions must be decided at the national or provincial level.

Furthermore, pesticides which are manufactured to comply with these specifications are not exempted from any safety regulation or other legal or administrative provision applicable to their manufacture, sale, transportation, storage, handling, preparation and/or use.

FAO disclaims any and all liability for any injury, death, loss, damage or other prejudice of any kind that may be arise as a result of, or in connection with, the manufacture, sale, transportation, storage, handling, preparation and/or use of pesticides which are found, or are claimed, to have been manufactured to comply with these specifications.

Additionally, FAO wishes to alert users to the fact that improper storage, handling, preparation and/or use of pesticides can result in either a lowering or complete loss of safety and/or efficacy.

FAO is not responsible, and does not accept any liability, for the testing of pesticides for compliance with the specifications, nor for any methods recommended and/or used for testing compliance. As a result, FAO does not in any way warrant or represent that any pesticide claimed to comply with a FAO specification actually does so.

[1] This disclaimer applies to all specifications published by FAO.

INTRODUCTION

FAO establishes and publishes specifications* for technical material and related formulations of agricultural pesticides, with the objective that these specifications may be used to provide an international point of reference against which products can be judged either for regulatory purposes or in commercial dealings.

From 1999, the development of FAO specifications has followed the **New Procedure**, subsequently described in the 1st edition of "Manual for Development and Use of FAO and WHO Specifications for Pesticides" (2002) and amended with the supplement of this manual (2006), which is available only on the internet through the FAO and WHO web sites. This **New Procedure** follows a formal and transparent evaluation process. It describes the minimum data package, the procedure and evaluation applied by FAO and the Experts of the FAO/WHO Joint Meeting on Pesticide Specifications (JMPS). [Note: prior to 2002, the Experts were of the FAO Panel of Experts on Pesticide Specifications, Registration Requirements, Application Standards and Prior Informed Consent, which now forms part of the JMPS, rather than the JMPS.]

FAO Specifications now only apply to products for which the technical materials have been evaluated. Consequently from the year 2000 onwards the publication of FAO specifications under the **New Procedure** has changed. Every specification consists now of two parts, namely the specifications and the evaluation report(s):

Part One: **The Specification** of the technical material and the related formulations of the pesticide in accordance with chapters 4 to 9 of the "Manual on development and use of FAO and WHO specifications for pesticides".

Part Two: **The Evaluation Report(s)** of the pesticide, reflecting the evaluation of the data package carried out by FAO and the JMPS. The data are provided by the manufacturer(s) according to the requirements of chapter 3 of the "FAO/WHO Manual on Pesticide Specifications" and supported by other information sources. The Evaluation Report includes the name(s) of the manufacturer(s) whose technical material has been evaluated. Evaluation reports on specifications developed subsequently to the original set of specifications are added in a chronological order to this report.

FAO specifications developed under the **New Procedure** do not necessarily apply to nominally similar products of other manufacturer(s), nor to those where the active ingredient is produced by other routes of manufacture. FAO has the possibility to extend the scope of the specifications to similar products but only when the JMPS has been satisfied that the additional products are equivalent to that which formed the basis of the reference specification.

Specifications bear the date (month and year) of publication of the current version. Dates of publication of the earlier versions, if any, are identified in a footnote. Evaluations bear the date (year) of the meeting at which the recommendations were made by the JMPS.

* NOTE: publications are available on the internet at
http://www.fao.org/agriculture/crops/core-themes/theme/pests/jmps/en/

PART ONE

SPECIFICATIONS

PARAQUAT DICHLORIDE

PARAQUAT DICHLORIDE

INFORMATION

Common name (dication):

paraquat (E-ISO, (m)F-ISO, BSI, ANSI, WSSA, JMAF)

Synonyms:

methyl viologen

Chemical names:

dication -
IUPAC, 1,1'-dimethyl-4,4'-bipyridinium [1]
CA, 1,1'-dimethyl-4,4'-bipyridinium

dichloride -
IUPAC, 1,1'-dimethyl-4,4'-bipyridinium dichloride [1]
CA, 1,1'-dimethyl-4,4'-bipyridinium dichloride

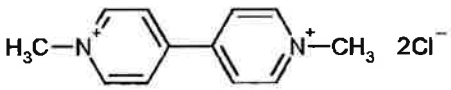
CAS No:

1910-42-5 (dichloride); 4685-14-7 (dication)

CIPAC No:

56 (dication); 56.302 (dichloride)

Structural formula (dichloride):



Molecular formula:

$C_{12}H_{14}Cl_2N_2$ (dichloride); $C_{12}H_{14}N_2$ (dication)

Relative molecular mass:

257.2 (dichloride); 186.3 (dication)

Identity tests (CIPAC G 56/SL/M-):

HPLC retention time; UV spectrum; addition of alkaline sodium dithionite to a dilute solution, where a blue colour indicates the presence of paraquat. The presence of the dichloride salt is tested with silver nitrate solution or, in the presence or absence of diquat dibromide, by capillary electrophoresis.

[1] The IUPAC name for the bipyridinium moiety is alternatively expressed as "bipyridinediium" or "bipyridilium".

PARAQUAT DICHLORIDE TECHNICAL CONCENTRATE (Note 1)

FAO Specification 56.302/TK (2003*)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose name is listed in the evaluation report (56.302/2003). It should be applicable to TK produced by this manufacturer but it is not an endorsement of those products, nor a guarantee that they comply with the specifications. The specification may not be appropriate for TK produced by other manufacturers. The evaluation report (56.302/2003), as PART TWO, forms an integral part of this publication.

1 Description

The material shall consist of paraquat dichloride, together with related manufacturing impurities, in the form of an aqueous solution , free from visible extraneous matter, and must contain an effective emetic (Note 2). The material may also include colorants and olefactory alerting agents.

2 Active ingredient

2.1 **Identity tests** (56/SL/M/2, CIPAC Handbook G, p.128, 1995)

The active ingredient (paraquat and chloride, Note 3) shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

2.2 **Paraquat dichloride content** (56/SL/M/3, CIPAC Handbook E, p.167, 1993)

The paraquat dichloride content (Note 4) shall be declared (not less than 500 g/l at 20 ± 2°C, Note 5) and, when determined, the average measured content shall not differ from that declared by more than ± 25 g/l.

3 Relevant impurities

3.1 **Free 4,4'-bipyridyl** (56/13/M/7.4, CIPAC Handbook 1A, p.1317, 1980)

Maximum: 1.0 g/kg (1000 ppm).

3.2 **Total terpyridines** (Note 6)

Maximum: 0.001 g/kg (1.0 ppm).

4 Physical properties

4.1 **pH range** (MT 75.3, CIPAC Handbook J, p. 131, 2000) (Note 1)

pH range: 2.0 to 6.0.

Note 1 The product must not be allowed to come into direct contact with metal. Containers may be manufactured from suitable polymeric materials or metal and must comply with pertinent national and international transport and safety regulations. If metal is used, containers must be lined with suitable polymeric material, or the internal surfaces treated to prevent corrosion of the container and/or deterioration of the contents.

Note 2 An effective emetic, having the following characteristics, must be incorporated into the TK.

- It must be rapidly absorbed (more rapidly than paraquat) and be quick acting. Emesis must occur in about half an hour in at least 50% of cases.
- It must be an effective (strong) stimulant of the emetic centre of the brain, to produce effective emesis. The emetic effect should have a limited 'action period', of about two to three hours, to allow effective treatment of poisoning.
- It must act centrally on the emetic centre in the brain.
- It must not be a gastric irritant because, as paraquat is itself an irritant, this could potentiate the toxicity of paraquat.
- It must be toxicologically acceptable. It must have a short half-life in the body (to comply with the need for a limited action period).
- It must be compatible with, and stable in, the paraquat formulation and not affect the herbicidal efficacy or occupational use of the product.

To date, the only compound found to meet these requirements is 2-amino-4,5-dihydro-6-methyl-4-propyl-*s*-triazole-(1,5*a*)pyrimidin-5-one (PP796). PP796 must be present in the TK at not less than 0.8 g/l.

The method for determination of PP796 content can be downloaded here:

Note 3 Chloride in paraquat dichloride TK may be identified by means of the white precipitate produced on reaction of a solution of the TK with silver nitrate solution. Alternatively or in addition, the method for identification of chloride in mixed formulations of diquat dibromide and paraquat dichloride may be used. This method can be downloaded here:

Note 4 To obtain the paraquat dichloride content, multiply the paraquat ion content (as determined by CIPAC method 56/SL/M/3) by 1.38.

Note 5 The lower limit of 500 g/l corresponds nominally to 442 g/kg and thus the tolerance of ± 25 g/l corresponds to ± 5% on a g/kg basis. If, in a particular case, the declared concentration exceeds 566 g/l (>500 g/kg), the tolerance shall be ± 25 g/kg, not ± 25 g/l (± 22 g/kg). If the buyer requires specification of both g/l at 20°C and g/kg, then in case of dispute the analytical results shall be calculated as g/kg.

Note 6 The method for determination of total terpyridines in technical and formulated paraquat dichloride is available from CIPAC at http://www.cipac.org/Inpub.htm.

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* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: http://www.fao.org/agriculture/crops/core-themes/theme/pests/jmps/en/ .

PARAQUAT DICHLORIDE SOLUBLE CONCENTRATE (Notes 1, 2 and 3)

FAO specification 56.302/SL (February 2008*)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose names is listed in the evaluation report (56.302/2003). It should be applicable to relevant products of this manufacturer, and those of any other formulators who use only TK from the evaluated source. The specification is not an endorsement of those products, nor a guarantee that they comply with the specification. The specification may not be appropriate for the products of other manufacturers who use TK from other sources. The evaluation report (56.302/2003), as PART TWO, forms an integral part of this publication.

1 Description

The material shall consist of technical paraquat dichloride, complying with the requirements of FAO specification 56.302/TK (2003), in the form of an aqueous solution (Notes 1 and 3), together with any other necessary formulants, and must contain an effective emetic (Note 2). The material may also include colorants, olefactory alerting agents and thickeners. It shall contain not more than a trace of suspended matter, immiscible solvents and sediment.

2 Active ingredient

2.1 Identity tests (56/SL/M/2, CIPAC Handbook G, p.128, 1995)

The active ingredient (paraquat and chloride components, Note 4) shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

2.2 Paraquat dichloride content (56/SL, CIPAC Handbook E, p.167, 1993, Note 2)

The paraquat dichloride content (Note 5) shall be declared (g/kg and/or g/l at 20 ± 2°C, Note 6) and, when determined, the average content measured shall not differ from that declared by more than the following tolerances.

Declared content, g/kg or g/l at 20 ± 2°C	Permitted tolerance
25 up to 100	± 10% of the declared content
Above 100 up to 250	± 6% of the declared content
Above 250 up to 500	± 5% of the declared content
Note: the upper limit is included in each range.	

3 Relevant impurities

3.1 Free 4,4'-bipyridyl (56/13/M/7.4, CIPAC 1A, p.1317, 1980)

Maximum: 1 g/kg (1000 ppm).

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* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: http://www.fao.org/agriculture/crops/core-themes/theme/pests/jmps/en/

3.2 **Total terpyridines** (Note 7)

Maximum: 0.001 g/kg (1.0 ppm).

4 Physical properties

4.1 **pH range** (MT 75.3, CIPAC Handbook J, p. 131, 2000)

pH range: 4.0 to 8.0.

4.2 **Solution stability** (MT 41, CIPAC Handbook F, p. 131, 1995)

The formulation, after the stability test at 54°C (see 5.2) and following dilution (Note 8) with CIPAC standard water D and standing at 30 ± 2°C for 18 h, shall give a clear or opalescent solution, free from more than a trace of sediment and visible solid particles. Any visible sediment or particles produced shall pass through a 45 µm test sieve (Note 9).

4.3 **Persistent foam** (MT 47.2, CIPAC Handbook F, p. 152, 1995) (Note 10)

Maximum: 60 ml after one minute.

5 Storage stability

5.1 **Stability at 0°C** (MT 39.3, CIPAC Handbook J, p. 126, 2000)

After storage at 0 ± 2°C for 7 days, the volume of solid and/or liquid which separates shall not be more than 0.3 ml.

5.2 **Stability at elevated temperature** (MT 46.3, CIPAC Handbook J, p.128, 2000)

After storage at 54 ± 2°C for 14 days, the determined average active ingredient content must not be lower than 97%, relative to the determined average content found before storage (Note 11), and the product shall continue to comply with the clause for:

- pH range (4.1).

Note 1 An effective emetic, having the following characteristics, must be incorporated into the SL.

- It must be rapidly absorbed (more rapidly than paraquat) and be quick acting. Emesis must occur in about half an hour in at least 50% of cases.
- It must be an effective (strong) stimulant of the emetic centre of the brain, to produce effective emesis. The emetic effect should have a limited 'action period', of about two to three hours, to allow effective treatment of poisoning.
- It must act centrally on the emetic centre in the brain.
- It must not be a gastric irritant because, as paraquat is itself an irritant, this could potentiate the toxicity of paraquat.
- It must be toxicologically acceptable. It must have a short half-life in the body (to comply with the need for a limited action period).
- It must be compatible with, and stable in, the paraquat formulation and not affect the herbicidal efficacy or occupational use of the product.

To date, the only compound found to meet these requirements is 2-amino-4,5-dihydro-6-methyl-4-propyl-*s*-triazole-(1,5*a*)pyrimidin-5-one (PP796). PP796 must be present in the SL at not less than 0.23% of the paraquat ion content.

The method for determination of PP796 content can be downloaded here:

Note 2 FAO specifications 55/SL and 56/SL are applied to mixed SL formulations, containing both paraquat and diquat. Emetic is added to all formulations containing paraquat and the extra precautions required for handling solutions of paraquat must be observed when handling the mixed formulation. If the SL contains both diquat and paraquat, CIPAC method 55+56/SL/M/3 (CIPAC Handbook E, p.75, 1993) should be used for determination of active ingredient content.

Note 3 The product must not be allowed to come into direct contact with metal. Containers may be manufactured from suitable polymeric materials or metal and must comply with pertinent national and international transport and safety regulations. If metal is used, containers must be lined with suitable polymeric material, or the internal surfaces treated to prevent corrosion of the container and/or deterioration of the contents.

Note 4 Chloride in paraquat dichloride SL may be identified by means of the white precipitate produced on reaction with silver nitrate solution. Alternatively or in addition, the method for identification of bromide and chloride in mixed formulations of diquat dibromide and paraquat dichloride may be used. This method can be downloaded here:

Note 5 To obtain the paraquat dichloride content, multiply the paraquat ion content (as determined by CIPAC method 55/SL/M/3) by 1.38.

Note 6 If the buyer requires specification of both g/l at 20°C and g/kg, then in case of dispute the analytical results shall be calculated as g/kg.

Note 7 The method for determination of total terpyridines in technical and formulated paraquat dichloride is available from CIPAC at http://www.cipac.org/lnpub.htm.

Note 8 The concentration for the test should not be higher than the highest concentration recommended for use.

Note 9 Some formulations containing additional wetter may show signs of layering and produce a trace of oily precipitate under the test conditions defined in MT 41. This is acceptable and does not affect biological efficacy or spray characteristics at normal spray dilution.

Note 10 The mass of sample used in the test should correspond to the highest concentration recommended for use.

Note 11 Samples of the product taken before and after the storage stability test should be analyzed concurrently after the test to reduce the analytical error.

PARAQUAT DICHLORIDE WATER SOLUBLE GRANULES (Note 1)

FAO Specification 56.302/SG (February 2008*)

This specification, which is PART ONE of this publication, is based on an evaluation of data submitted by the manufacturer whose names is listed in the evaluation report (56.302/2003). It should be applicable to relevant products of this manufacturer, and those of any other formulators who use only TK from the evaluated source. The specification is not an endorsement of those products, nor a guarantee that they comply with the specification. The specification may not be appropriate for the products of other manufacturers who use TK from other sources. The evaluation report (56.302/2003), as PART TWO, forms an integral part of this publication.

1 Description

The material shall consist of granules containing technical paraquat dichloride complying with the requirements of the FAO specification 56.302/TK (2003) and suitable carriers, if required, and it must contain an effective emetic (Note 2). The material may also contain colorants and olefactory alerting agents. It shall be homogeneous, free from visible extraneous matter and/or hard lumps, free flowing, and nearly dust-free. Insoluble carriers and formulants shall not interfere with compliance with clause 4.2.

2 Active ingredient

2.1 **Identity tests** (56/SL/M/2, CIPAC Handbook G, p.128, 1995)

The active ingredient (paraquat and chloride components, Note 3) shall comply with an identity test and, where the identity remains in doubt, shall comply with at least one additional test.

2.2 **Paraquat dichloride content** (55+56/SG/M/4, CIPAC Handbook E, p.78, 1993)

The paraquat dichloride content (Note 4) shall be declared (g/kg) and, when determined, the content measured shall not differ from that declared by more than the following:

Declared content, g/kg	Permitted tolerance
25 up to 100	± 10% of the declared content
Above 100 up to 250	± 6% of the declared content
Note: the upper limit is included in each range.	

3 Relevant impurities

3.1 **Free 4,4'-bipyridyl** (56/13/M/7.4, CIPAC Handbook 1A, p.1317, 1980)

Maximum: 1.0 g/kg (1000 ppm).

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* Specifications may be revised and/or additional evaluations may be undertaken. Ensure the use of current versions by checking at: http://www.fao.org/agriculture/crops/core-themes/theme/pests/jmps/en/ .

3.2 **Total terpyridines** (Note 5)

Maximum: 0.001 g/kg (1.0 ppm).

4 Physical properties

4.1 **pH range** (MT 75.3, CIPAC Handbook J, p. 131, 2000) (Note 1)

pH range of a 1% w/v dispersion: 6.0 to 8.0.

4.2 **Degree of dissolution and solution stability** (MT 179, CIPAC Handbook H, p.307, 1998)

Residue of formulation retained on a 75 µm test sieve after dissolution in CIPAC Standard Water D at 30 ± 2°C.

Maximum: 2% after 5 minutes.

Maximum: 2% after 18 hours.

4.3 **Persistent foam** (MT 47.2, CIPAC Handbook F, p. 152, 1995) (Note 6)

Maximum: 30 ml after 1 minute.

4.4 **Dustiness** (MT 171, CIPAC Handbook F, p.425, 1995) (Note 7)

Nearly dust-free, with a maximum of 1 mg (0.0033% by weight) dust collected by the gravimetric method.

4.5 **Flowability** (MT 172, CIPAC Handbook F, p.430, 1995)

At least 98% of the formulation shall pass through a 5 mm test sieve after 20 drops of the sieve.

4.6 **Attrition resistance** (MT 178.2, CIPAC Handbook K, p.140, 2003)

Minimum 99.5% attrition resistance.

5 Storage stability

5.1 **Stability at elevated temperatures** (MT 46.3, CIPAC Handbook J, p.128, 2000)

After storage at 54 ± 2°C for 14 days the determined average active ingredient content shall not be lower than 97% relative to the determined average content found before storage (Note 8) and the formulation shall continue to comply with the clauses for:

- pH range (4.1),
- degree of dissolution and solution stability (4.2),
- dustiness (4.4),
- flowability (4.5),
- attrition resistance (4.6).

Note 1 Containers may be manufactured from suitable polymeric materials or metal, and must comply with pertinent national and international transport and safety requirements. Where metal is used containers shall be lined with suitable polymeric material, or the internal surfaces treated to prevent corrosion of the container and/or deterioration of the contents. The product must not be allowed to come into direct contact with metal.

Note 2 An effective emetic, having the following characteristics, must be incorporated into the SG.

- It must be rapidly absorbed (more rapidly than paraquat) and be quick acting. Emesis must occur in about half an hour in at least 50% of cases.
- It must be an effective (strong) stimulant of the emetic centre of the brain, to produce effective emesis. The emetic effect should have a limited 'action period', of about two to three hours, to allow effective treatment of poisoning.
- It must act centrally on the emetic centre in the brain.
- It must not be a gastric irritant because, as paraquat is itself an irritant, this could potentiate the toxicity of paraquat.
- It must be toxicologically acceptable. It must have a short half-life in the body (to comply with the need for a limited action period).
- It must be compatible with, and stable in, the paraquat formulation and not affect the herbicidal efficacy or occupational use of the product.

To date, the only compound found to meet these requirements is 2-amino-4,5-dihydro-6-methyl-4-propyl-*s*-triazole-(1,5*a*)pyrimidin-5-one (PP796). PP796 must be present in the SG at not less than 0.23% of the paraquat ion content. The method for determination of PP796 content can be downloaded here:

Note 3 Chloride in paraquat dichloride SG may be identified by means of the white precipitate produced on reaction of a solution of the SG with silver nitrate solution. Alternatively or in addition, the method for identification of chloride in mixed formulations of diquat dibromide and paraquat dichloride may be used. This method can be downloaded here:

Note 4 To obtain the paraquat dichloride content, multiply the paraquat ion content (as determined by CIPAC method 55+56/SG/M/4) by 1.38.

Note 5 The method for determination of total terpyridines in technical and formulated paraquat dichloride is available from CIPAC at http://www.cipac.org/lnpub.htm.

Note 6 The mass of sample to be used in the test should correspond to the highest concentration recommended for use by the supplier. The test is to be conducted in CIPAC standard water D.

Note 7 The optical method, MT 171, would not give reliable values for dust at levels around the specified limit and should therefore not be used.

Note 8 Samples of the formulation taken before and after the storage stability test should be analyzed concurrently after the test in order to reduce the analytical error.

PART TWO

EVALUATION REPORTS

PARAQUAT

PARAQUAT

FAO EVALUATION REPORT 56.302/2003

Explanation

The data for paraquat dichloride were evaluated in support of a review of existing FAO specifications (AGP:CP/344, Rome,1996).

Paraquat dichloride is not under patent.

Paraquat was reviewed by the World Health Organization (WHO) and the United Nations Environment Programme (UNEP) in 1983, resulting in the publication of Environmental Health Criteria 39 (WHO, 1984), and by the International Programme on Chemical Safety (IPCS, 1991), resulting in IPCS Health & Safety Guide No 51. Paraquat was reviewed by the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) in 1986 and was scheduled for periodic re-evaluation in 2003. It has been evaluated by US EPA (USEPA, 1996) and is currently under evaluation by the European Commission.

The draft specification and the supporting data were provided by Syngenta Crop Protection AG, in 2002.

Uses

Paraquat dichloride is a non-selective contact herbicide, which is absorbed by foliage, with some translocation in the xylem. It is used in broad-spectrum control of broad-leaved weeds and grasses, in a wide range of agricultural applications, for general weed control on non-crop land and also for pasture restoration.

Identity

Common name (dication):

paraquat (E-ISO, (m)F-ISO, BSI, ANSI, WSSA, JMAF)

Synonyms:

methyl viologen

Chemical names:

dication -
IUPAC, 1,1'-dimethyl-4,4'-bipyridinium [1]
CA, 1,1'-dimethyl-4,4'-bipyridinium

dichloride -
IUPAC, 1,1'-dimethyl-4,4'-bipyridinium dichloride [1]
CA, 1,1'-dimethyl-4,4'-bipyridinium dichloride

CAS No:

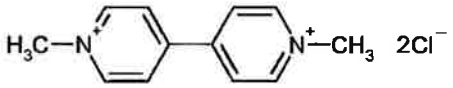
1910-42-5 (dichloride); 4685-14-7 (dication)

CIPAC No:

56 (dication); 56.302 (dichloride)

[1] The IUPAC name for the bipyridinium moiety is alternatively expressed as "bipyridinediium" or "bipyridilium".

Structural formula (dichloride):



Molecular formula:

$C_{12}H_{14}Cl_2N_2$ (dichloride); $C_{12}H_{14}N_2$ (dication)

Relative molecular mass:

257.2 (dichloride); 186.3 (dication)

Identity tests (CIPAC G 56/SL/M-):
HPLC retention time; UV spectrum; addition of alkaline sodium dithionite to a dilute solution, where a blue colour indicates the presence of paraquat. The presence of the dichloride salt is tested with silver nitrate solution or, in the presence or absence of diquat dibromide, by capillary electrophoresis.

Physicochemical properties

Table 1. Physicochemical properties of pure paraquat dichloride

Parameter	Value(s) and conditions	Purity %	Method reference
Vapour pressure	<<1x10-8kPa at 25°C (extrapolated)	99.5	OECD 104
Melting point, boiling point and/or temperature of decomposition	Melting point: >400°C Boiling point: not applicable Decomposition temperature: 340°C	99.5	OECD 102
Solubility in water	620g/l at 20 °C across pH range	99.5	OECD 105 (flask method)
Octanol/water partition coefficient	log P_{ow} = -4.5 at 20°C	99.5	OECD 107 (flask method)
Hydrolysis characteristics	Paraquat dichloride is hydrolytically stable under acidic, neutral and alkaline conditions, no significant decrease in concentration having been recorded at pH 5, 7 and 9 after 30days at 25°C and 40°C.	Not stated	Analysis of sterile aqueous buffer solutions containing known amounts of paraquat dichloride before and after storage.
Photolysis characteristics	The environmental half-life of paraquat dichloride in water under mid-European conditions was calculated to be between 2 and 820 years, depending upon seasonal sunlight and depth of water.	99.7	Measurement of molar extinction coefficients and quantum yield, then these data used in the Frank and Klöpffer model to obtain an estimate of half-life.
Dissociation characteristics	In aqueous solution the paraquat dichloride is completely dissociated.	Not applicable	-

Table 2. Chemical composition and properties of paraquat dichloride (TK)

Manufacturing process, maximum limits for impurities ≥ 1 g/kg, 5 batch analysis data	Confidential information supplied and held on file by FAO. Mass balances were 98.1-99.3% and percentages of unknowns were 1.9-0.7%.
Declared minimum paraquat dichloride content	500 g/l (442 g/kg).
Relevant impurities ≥ 1 g/kg and maximum limits for them	4,4 bipyridyl, 1 g/kg (1000 ppm).
Relevant impurities < 1 g/kg and maximum limits for them	Total terpyridines 0.001 g/kg (1.0 ppm)
Stabilisers or other additives and maximum limits for them	An effective emetic (reference to effective emetic criteria) – see below. PP796, 2-amino-4,5-dihydro-6-methyl-4-propyl-s-triazole-[1,5-a]pyrimidin-5-one is the only emetic known to meet these effective emetic criteria. If PP796 is the effective emetic employed, it must be present at a minimum level of 0.23% by weight of the paraquat ion content[0.17% on a paraquat dichloride basis]
Melting or boiling temperature range	340°C, at which decomposition occurs

Criteria for effective emesis.

- The emetic must be rapidly absorbed (more rapidly than paraquat) and be quick acting. Emesis must occur in about half an hour in at least 50% of cases.
- The emetic must be an effective (strong) stimulant of the emetic centre, to produce effective emesis. The emetic effect should have a limited "action period" of about two to three hours, to allow effective treatment of poisoning.
- The emetic must be act centrally on the emetic centre in the brain.
- The emetic must be not be a gastric irritant because, as paraquat is itself an irritant, this could potentiate the toxicity of paraquat.
- The emetic must be toxicologically acceptable. It must have a short half-life in the body (to comply with the need for a limited action period).
- The emetic must be compatible with, and stable in, the paraquat formulation and not affect the herbicidal efficacy or occupational use of the product.

Toxicological summaries

Notes. (i) The proposer confirmed that the toxicological and ecotoxicological data included in the summary below were derived from paraquat dichloride having impurity profiles similar to those referred to in the table above.
(ii) The conclusions expressed in the summary below are those of the proposer, unless otherwise specified.

Table 3. Toxicology profile of paraquat dichloride TK, based on acute toxicity, irritation and sensitization

Species	Test	Duration and conditions or guideline adopted	Result (paraquat dichloride technical / paraquat cation).
Rat, Alpk:ApfSD, male	oral	OECD 401, 14 day observation	MLD = 344 [246 – 457] mg paraquat dichloride technical/kg bw, equivalent to 113.5 mg/kg bw expressed as paraquat cation.
Rat, Alpk:ApfSD, female	oral	OECD 401, 14 day observation	MLD = 283 [182 – 469] mg paraquat dichloride technical/kg bw, equivalent to 93.4 mg/kg bw expressed as paraquat cation.

Table 3. Toxicology profile of paraquat dichloride TK, based on acute toxicity, irritation and sensitization

Species	Test	Duration and conditions or guideline adopted	Result (paraquat dichloride technical / paraquat cation).
Rat, Alpk:ApfSD, male and female	dermal	OECD 402, 24 hour, occluded, 14 day observation	MLD = >2000 mg paraquat dichloride technical/kg bw equivalent to >660 mg/kg bw expressed as paraquat cation.
Rat, Alpk:Ap, male and female	inhalation	OECD 403, 4 hour nose only*, 14 day observation	LC_{50} = 0.83 – 1.93 mg/m^3 expressed as paraquat cation.
Rabbit, New Zealand White, female	skin irritation	OECD 404, 4 hour, occluded, 34 day, observation	Slight but persistent skin irritant.
Rabbit, New Zealand White, female	eye irritation	OECD 405, 28 day observation	Persistent, moderate to severe irritant to the rabbit eye [Class 5 on a 1-8 scale].
Guinea pigs, Dunkin Hartley, female	skin sensitization	OECD 406, Magnusson and Kligman maximization test, 24 hour, occluded, 48 hour observation	Negative, not a skin sensitizer.

* Paraquat dichloride is non-volatile and formulations containing paraquat are not applied through equipment which will generate a significant proportion (>1% w/w) of spray droplets of diameter less than 50 µm. Therefore, respirable vapour or droplets of paraquat dichloride will not be produced in practice and these toxicity data are not relevant to assessment of human risks.

Table 4. Toxicology profile of paraquat TK, based on repeated administration (sub-acute to chronic)

Species	Test	Duration and conditions or guideline adopted	Result
Rabbit, New Zealand White, male and female	Short-term dermal toxicity	21-day dermal toxicity	NOEL = 1.57 mg paraquat dichloride/kg bw/day equivalent to 1.15 mg/kg bw/day, expressed as paraquat cation. LOEL = 3.61 mg paraquat dichloride /kg bw/day, equivalent to 2.6 mg/kg bw/day, expressed as paraquat ion.
Mouse, ICR-CRJ SPF, male and female	Short-term toxicity	13-week dietary	NOEL = 100 ppm, equivalent to approximately 12 and 14 mg/kg bw/day, expressed as paraquat ion in males and females, respectively. LOEL = 300 ppm, equivalent to approximately 36 and 42 mg/kg bw/day, expressed as paraquat ion in males and females, respectively.
Rat, Fischer CDF (F344), male and female	Short-term toxicity	13-week dietary	NOEL = 100 ppm, equivalent to approximately 6 and 7 mg/kg bw/day, expressed as paraquat ion in males and females, respectively. LOEL = 300 ppm, equivalent to approximately 20 and 21 mg/kg bw/day, expressed as paraquat ion in males and females, respectively.

Table 4. Toxicology profile of paraquat TK, based on repeated administration (sub-acute to chronic)

Species	Test	Duration and conditions or guideline adopted	Result
Dog, Beagle, male and female	Short-term toxicity	13-week dietary	NOEL = 20 ppm, equivalent to approximately 0.6 and 0.7 mg/kg bw/day, expressed as paraquat ion in males and females, respectively. LOEL = 60 ppm, equivalent to approximately 2 mg/kg bw/day, expressed as paraquat ion in males and females.
Dog, Beagle, male and female	Short-term toxicity	1-year dietary	NOEL = 15 ppm, equivalent to approximately 0.45 and 0.48 mg/kg bw/day, expressed as paraquat ion in males and females, respectively. LOEL = 30 ppm, equivalent to approximately 0.9 and 1.0 mg/kg bw/day, expressed as paraquat ion in males and females, respectively.
Mouse, Alpk Swiss-derived, male and female	Carcinogenicity	99-week dietary	Not tumorigenic. NOAEL = 12.5 ppm, equivalent to approximately 1.5 mg/kg bw/day, expressed as paraquat ion in males. NOEL = 37.5 ppm, equivalent to approximately 4.3 mg/kg bw/day, expressed as paraquat ion in females.
Rat, Fischer 344, male and female	Chronic toxicity / carcinogenicity	113-117 weeks for males and 122-124 weeks for females	Not carcinogenic. NOEL = 25 ppm, equivalent to approximately 1.25 mg/kg bw/day, expressed as paraquat ion. LOEL = 75 ppm, equivalent to approximately 3.75 mg/kg bw/day, expressed as paraquat ion.
Rat, Alpk:APfSD, male and female	Reproductive toxicity	3-generation, 2 litters per generation	No effect on reproductive parameters. NOEL for toxicity = 25 ppm, equivalent to approximately 2.3 mg/kg bw/day, expressed as paraquat ion. NOEL for reproductive effects = >150 ppm, equivalent to approximately 13 mg/kg bw/day, expressed as paraquat ion.
Mice, Crl:CD1 (ICR) BR, female	Developmental toxicity	Gavage	NOEL for both maternal and developmental toxicity = 15 mg/kg bw/day expressed as paraquat ion.
Mice, Alpk SPF, female	Developmental toxicity	Gavage	Not teratogenic. No significant influence on embryonic or foetal development. NOEL for developmental toxicity = >10 mg/kg bw/day expressed as paraquat ion.
Rat, Alpk:SPF, female	Developmental toxicity	Gavage	Not teratogenic. NOEL for maternal and developmental toxicity > 1mg/kg bw/day expressed as paraquat ion.
Rat, Alpk:APfSD	Developmental toxicity	Gavage	Not teratogenic. NOAEL for maternal and developmental toxicity = 3 mg/kg bw/day expressed as paraquat ion.

Table 5. Mutagenicity profile of paraquat dichloride TK, based on *in vitro* and *in vivo* tests

Species	Test	Conditions	Result
Mouse, lymphocytes (L5178Y)	OECD 476, L5178Y mouse lymphoma assay (*in vitro*)	Doses of 23 – 361 µg/ml	Negative
Human lymphocytes	OECD 473, Cytogenetic study (*in vitro*)	Dosed at 90, 903 and 1807 µg/ml	Positive
Chinese hamster lung fibroblasts	OECD 479, Sister chromatid exchange assay (*in vitro*)	Dosed at 0.9, 1.8, 9, 18, 90 and 177 µg/ml	Positive
Rat hepatocytes	OECD 482, DNA damage and repair/unscheduled DNA synthesis (*in vitro*)	Dosed at 0.19 ng/ml to 1.86 mg/ml	Negative
Rat somatic cells	Rat cytogenetic assay (*in vivo*)	Male and female Wistar rats given a single oral dose at 15, 75 and 150 mg/kg	Negative
Mouse somatic cells	OECD 474, Micronucleus test (*in vivo*)	Male and female C57BL/6J/Alpk mice given a single oral dose at 52 and 83 mg/kg	Negative
Rat somatic cells	UDS assay (*in vivo*)	Single oral dose at 42 to 120 mg/kg	Negative
Mouse germ cells	Dominant lethal (*in vivo*)	Male CD1 mice dosed orally at 0, 0.04, 0.4 and 4.0 mg/kg for 5 days.	Negative

Table 6. Ecotoxicology profile of paraquat dichloride TK.

Species	Test	Duration and conditions	Result
Daphnia magna, (water flea)	Acute toxicity	EEC Method C2, Static system, 20-21°C, 48-hour observation	24 and 48 hour EC_{50} = 11.8 and 4.4 mg/l, expressed as paraquat ion, respectively. 48 hour NOEC = 2.2 mg/l expressed as paraquat ion.
Daphnia magna, (water flea)	Chronic toxicity	21-day exposure, based on OECD Guideline 202, modified by individually separating the Daphnia static system, growth and reproduction monitored	NOEC = 0.12 mg/l expressed as paraquat ion.
Oncorhynchus mykiss, (rainbow trout)	Acute toxicity	EEC Method C1, static system at 15°C	24, 48, 72 and 96 hour LC_{50} = 33, 22, 22 and 19 mg/l, expressed as paraquat ion, respectively. 96 hour NOEC = <0.3 mg/l, expressed as paraquat ion
Cyprinus carpio, (mirror carp)	Acute toxicity	EEC Method C1, static system at 22°C	24, 48, 72 and 96 hour LC_{50} = >112, >112, >112 and 98 mg/l expressed as paraquat ion, respectively. 96 hour NOEC = 60 mg/l expressed as paraquat ion.

Table 6. Ecotoxicology profile of paraquat dichloride TK.

Species	Test	Duration and conditions	Result
Oncorhynchus mykiss, (rainbow trout)	Chronic toxicity	21-day fish juvenile growth test, based upon OECD Method 204, with the exposure period extended to 21 days. Broadly in agreement with the draft OECD guideline 'Fish, juvenile growth test - 28 days', except that the exposure was for 21 days. Flow through system at 15°C	NOEC = 8.5 mg/l expressed as paraquat ion.
Selenastrum capricornutum, (green alga)	Effect on growth	Based on OECD Guideline 201 but with an extension of the exposure period to 96 hours. Static system at 24°C, biomass and growth rate observed	EbC_{50} = 0.075 mg/l expressed as paraquat ion. ErC_{50} = 0.20 mg/l expressed as paraquat ion. NOEC = 0.016 mg/l expressed as paraquat ion.
Eisenia foetida, (earthworm)	Acute toxicity	Laboratory study in artificial soil	LC_{50} = >1000 mg/kg dry soil, expressed as paraquat ion
Apis mellifera (honey bee)	Acute oral toxicity	Based on UK data requirements for approval under the Control of Pesticides Regulations, Working Document D3 (revised 1979). Consistent with EPPO guideline 170. Controlled environment at 22°C	24, 48, 72, 96 and 120 hour LD_{50} = 154, 50.9, 26.3, 19.5 and 11.2 µg/bee, expressed as paraquat ion, respectively.
Apis mellifera (honey bee)	Acute contact toxicity	Based on UK data requirements for approval under the Control of Pesticides Regulations, Working Document D3 (revised 1979). Consistent with EPPO guideline 170. Controlled environment at 22°C	72, 96 and 120 hour LD_{50} = 108, 89.1 and 50.9 µg/bee, expressed as paraquat ion, respectively.
Colinus virginianus, (bobwhite quail)	Acute toxicity	Oral intubation in distilled water, 14 day observation	LD_{50} = 127 mg/kg bw expressed as paraquat ion. LLD = 115 mg/kg bw expressed as paraquat ion. NOEL = 72 mg/kg bw expressed as paraquat ion.
Anas platyrhynchos, (mallard duck)	Acute toxicity	Oral intubation in propylene glycol, 14 day observation	LD_{50} = 144 mg/kg bw expressed as paraquat ion.
Colinus virginianus, (bobwhite quail)	Short-term toxicity	5 days treatment, 3 days observation	LC_{50} = 711 mg/kg diet expressed as paraquat ion.
Anas platyrhynchos, (mallard duck)	Short-term toxicity	5 days treatment, 3 days observation	LC_{50} = 2932 mg/kg diet expressed as paraquat ion.
Coturnix japonica, (Japanese quail)	Short-term toxicity	5 days treatment, 3 days observation	LC_{50} = 703 mg/kg diet expressed as paraquat ion

Table 6. Ecotoxicology profile of paraquat dichloride TK.

Species	Test	Duration and conditions	Result
Colinus virginianus, (bobwhite quail)	Reproductive toxicity	18 week dietary treatment. Egg laying and collection started after 10 weeks on treated diet and lasted for 8 weeks.	NOEC for toxicity and reproduction = 100 mg/kg diet expressed as paraquat ion.
Anas platyrhynchos, (mallard duck)	Reproductive toxicity	18 week dietary treatment. Egg laying and collection started after 10 weeks on treated diet and lasted for 8 weeks.	NOEC for toxicity = 100 mg/kg diet expressed as paraquat ion. NOEC for reproduction = 30 mg/kg diet expressed as paraquat ion.

Paraquat dichloride was evaluated by WHO (WHO, 1984), by IPCS (IPCS, 1991) and by the FAO/WHO JMPR in 1986 (by which it is subject to a periodic re-evaluation in 2003). The IPCS (1991) review concluded that residue levels of paraquat in food and drinking-water, resulting from its normal use, are unlikely to pose a health hazard for the general population.

The WHO/PCS hazard classification (WHO 2002) of paraquat dichloride is: moderately hazardous, class II.

The US EPA concluded, from acute toxicity studies on laboratory animals, that paraquat is highly toxic by the inhalation route and was placed in Toxicity Category I (the highest of four levels) for acute inhalation effects. However, the EPA established that the large droplets arising in agricultural practice (400 to 800 µm) are well beyond the respirable range and therefore inhalation toxicity is not a toxicological endpoint of concern. Paraquat is moderately toxic (Category II) by the oral route and slightly toxic (Category III) by the dermal route. Paraquat will cause moderate to severe eye irritation and minimal dermal irritation and has been placed in Toxicity Categories II and IV for these effects (USEPA, 1997). Paraquat was classified as a "Group E" chemical, i.e. one showing evidence of non-carcinogenicity to humans. The no observed effect levels (NOEL) for maternal toxicity are equal to, or more conservative (protective) than, the NOEL based on developmental toxicity. There is no evidence that paraquat is associated with reproductive effects. Paraquat also shows no evidence of causing mutagenicity. The US EPA has determined that there is a reasonable certainty that no harm will result to infants and children or to the general population from aggregate exposure to paraquat dichloride residues. The EPA does not believe that the effects produced by paraquat would be cumulative with those of other, structurally related, compounds.

Formulations

The main formulation types available are SL and SG.

The SL formulations are registered and sold in many countries throughout the world. SG formulations are registered in Europe and sold mainly in the UK.

Methods of analysis and testing

Analytical methods for the active ingredient (including identity tests) were published in CIPAC Handbook E, pp. 75 and 167, and utilise a colorimetric procedure based on

the blue free-radical ion produced by paraquat. The method(s) for determination of impurities are based on GC-FID, GC-MS and CE.

Relevant impurity, 4,4'-bipyridyl, is determined by GC-FID (CIPAC 56/13) the group of relevant impurities, the terpyridines, are determined by GC-MS.

The methods for the terpyridines and the emetic have been peer evaluated for the TK but peer validation for the analysis of formulations is still to be finalized[1][2].

Test methods for determination of physico-chemical properties of the technical active ingredient were essentially OECD methods, with CIPAC procedures being used for formulation assessment (as indicated in the specifications).

Physical properties

The physical properties, the methods for testing them and the limits proposed for the SL and SG formulations, comply with the requirements of the FAO Manual (5th edition).

Containers and packaging

Detailed requirements for containers are given in the specifications, as a note, but it is important to prevent paraquat dichloride from coming into contact with metals.

Expression of the active ingredient

The active ingredient is expressed as paraquat dichloride.

Appraisal

Data submitted were in accordance with the FAO/WHO Manual (2002, 1st edition) and supported the proposed specifications.

Paraquat dichloride specifications were previously developed under the old FAO procedure in 1994 (TK and SL) and published by FAO. Revised FAO specifications (TK and SL) and an additional specification (SG) for paraquat dichloride were proposed under the new procedure by Syngenta Crop Protection AG.

Paraquat dichloride is no longer under patent.

Paraquat dichloride is a non-selective contact herbicide, highly soluble and stable in water (pH 5-9), only very slowly subject to photolysis and essentially non-volatile. It very readily, and essentially irreversibly, binds to soils and sediments.

The proposer provided the meeting with commercially confidential information on the two manufacturing processes (a third manufacturing process was no longer in use) for paraquat dichloride and concomitant impurities. Data for five batches from each of the two manufacturing processes were provided for the TK. Addition of water and an emetic (after reactions are complete) complete the TK manufacturing process. Other safening additives, such as warning colorants, stenching agents and

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[1] The method for determination of total terpyridines in technical and formulated paraquat dichloride was accepted by CIPAC in 2007 and is available at http://www.cipac.org/lnpub.htm.

[2] The method for determination of the emetic in technical and formulated paraquat was peer-validated in 2003 and is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here..

thickeners (for liquid formulations) are also incorporated. Mass balances were good: 99.0-99.3% characterized one manufacturing process, while 98.1-99.0% characterized the second process.

The proposer identified two relevant impurities of manufacturing (4,4'-bipyridyl and total terpyridines), both of which are normally below 0.5 g/kg. Minimum levels were specified for the emetic additive, and maximum levels for the two proposed relevant impurities, in the draft specifications for paraquat dichloride TK, SL and SG. Data submitted to FAO for TK purity, impurities and emetic content were similar to those submitted for registration of paraquat dichloride in the UK. A difference between the two sets of data was that terpyridines were not included in the UK data, because the concentrations are well below 1 g/kg. Both the terpyridines and 4, 4' bipyridyl were below 1 g/kg in batch analysis data submitted to FAO, regardless of which of the two current manufacturing processes was employed. The proposer noted that terpyridines are highly toxic, whilst, in some respects, 4,4'-bipyridyl is rather more toxic than paraquat dichloride. WHO/PCS opinion was to accept these views. The proposed new limit of 1 g/kg for 4,4'-bipyridyl is below the level of the previous FAO Specification (56/TK/S/F-1994). The Meeting agreed that the two impurities should be considered as relevant.

The method of analysis for paraquat dichloride is based on a colorimetric procedure, in which the blue paraquat radical, formed upon addition of alkaline sodium dithionite, is measured (CIPAC Handbook E, pages 75-78 and 167-168). The presence of paraquat as the dichloride salt may be identified by a check for chloride, using silver nitrate solution.

Methods for impurities are based on GC-FID (4,4' bipyridyl, CIPAC Handbook E, p.168 and CIPAC Handbook 1A, p. 1245) or GC-MS (terpyridines). Determination of the content of emetic, PP796, is based on capillary GC. The methods for the emetic and terpyridines have under gone satisfactory peer validation for the TK but further validation is underway for analysis of the formulations[12].

The proposer stated that physiochemical properties of paraquat dichloride were essentially determined using OECD methods, with CIPAC procedures used for assessment of formulation characteristics, as indicated in the specifications.

Paraquat dichloride was evaluated by WHO IPCS (1983 and 1991) with a classification of moderately hazardous assigned. The acceptable daily intake estimated by the FAO/WHO JMPR is 0-0.004 mg/kg. The US EPA has assigned a Category II acute toxicity to paraquat dichloride, which indicates it is moderately toxic. However, once paraquat is ingested and absorbed in sufficient amount, poisoning is essentially irreversible, with death as the probable end-point. Thus, all paraquat products must contain an effective emetic, to reduce the risk of accidental or deliberate ingestion and absorption. Paraquat is of low dermal toxicity but the US EPA classified paraquat dichloride in its highest toxicity class, Category I, for inhalation hazard. Nonetheless, the agency noted that, because the spray droplets produced in normal agricultural uses are too large to be respirable, the inhalation risk

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is actually very low. Paraquat dichloride is moderately toxic to aquatic invertebrates, slightly toxic to fish, moderately toxic to avian species and relatively non-toxic to bees.

As a result of evaluation of paraquat under Directive 91/414/EEC, the European Commission is proposing to make a colorant, an effective emetic and a stenching (or other olfactory alerting) agent, mandatory requirements for paraquat formulations. The proposer recommended the revised specifications be amended to reflect these same standards. The Meeting accepted the requirements for a stenching agent and emetic in paraquat product descriptions. The Meeting also agreed that a note to the specifications should identify the only emetic currently known to be satisfactory and provide both a minimum concentration and a suitable analytical method for it. The Meeting agreed that the note on emetic content should allow for a possible alternative compound, by describing the characteristics required for an effective emetic.

Paraquat dichloride is not mutagenic and EPA placed it in Group E for chemicals showing evidence of being non-carcinogenic to humans. Further, the evidence available indicates that paraquat dichloride has no effect on reproduction parameters and is non-teratogenic.

Certain amendments were made to the draft specifications, as agreed between the Meeting and the proposer. Apart from the exceptional requirements identified in the appraisal, the specifications were in accordance with the normal requirements of the FAO/WHO Manual.

Recommendations

The Meeting recommended that the specification for paraquat dichloride TK, as amended, should be adopted by FAO. The Meeting recommended that the specifications for SL and SG, as amended should be adopted by FAO, subject to satisfactory completion of peer validation of the analytical method for terpyridines[1] and the emetic[2].

References

Text reference	Publication details
FAO/WHO 2006	Section 2.9, p. 16. Manual on development and use of FAO and WHO specifications for pesticides. March 2006 revision of the first edition. Available only on the internet at http://www.fao.org/ag/agp/agpp/pesticid/ and http://www.who.int/whopes/quality.
IPCS, 1991	Health and Safety Guide No. 51. Paraquat Health and Safety Guide. World Health Organization, Geneva. 1991.
US EPA, 1996	Reregistration Eligibility Decision (RED), Paraquat dichloride. List A Case 0262. United States Environmental Protection Agency, 1996.
USEPA, 1997	R.E.D. Facts. Paraquat dichloride (EPA-738-F-96-018). United States Environmental Protection Agency, 1997.

[1] The method for determination of total terpyridines in technical and formulated paraquat dichloride was accepted by CIPAC in 2007 and is available at http://www.cipac.org/lnpub.htm.

[2] The method for determination of the emetic in technical and formulated paraquat was peer-validated in 2003 and is available from the Pesticide Management Group of the FAO Plant Protection Service or can be downloaded here..

Text reference	Publication details
WHO, 1984	Environmental Health Criteria 39: Paraquat and diquat. World Health Organization, Geneva, 1984.
WHO, 2002	The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification 2000-2002 (WHO/PCS/01.5). World Health Organisation, Geneva, 2002.

Human Toxicol. (1987), **6**, 49–55

Treatment of Paraquat Poisoning in Man: Methods to Prevent Absorption

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Theoretically, absorption of an ingested dose of paraquat may be reduced by (1) gastric lavage, (2) induced emesis, (3) whole-gut lavage or (4) by the oral administration of adsorbent substances.
1 Animal experiments suggest that paraquat is absorbed poorly from the stomach and absorbed incompletely (< 5%) from the small intestine over a 1–6-h period. Although gastric lavage would therefore seem a logical way to ameliorate the toxicity of an ingested dose of paraquat, peak plasma concentrations are attained rapidly and evidence for the efficacy of gastric lavage in man is poor.
2 In 1977, a potent emetic (PP796) was added to liquid and solid formulations of paraquat because experiments in primates had demonstrated a fivefold reduction in toxicity. In man, ingestion of formulations containing an emetic is more likely to cause spontaneous vomiting within 30 min than non-emetic preparations. However, definite evidence of benefit, as judged by improved patient prognosis, has yet to be established.
3 Gut lavage has been shown to remove only a small proportion of an ingested dose of paraquat. At the flow rates employed in man (75 ml/min), approximately 0.5–1.0 litres of lavage fluid/h may be absorbed across the intestinal wall. Since there is a theoretical risk of increasing paraquat absorption, the use of whole-gut lavage cannot be recommended.
4 Bipyridilium herbicides are adsorbed by soil and clay minerals, and montmorillonite in particular has been shown to be a strong binding agent *in vitro*. Accordingly, the use of Fuller's Earth (calcium montmorillonite) and Bentonite (sodium montmorillonite) for the treatment of poisoning has been investigated in animal models. Both agents reduce plasma paraquat concentrations and mortality in animals when administered after an oral dose of paraquat. Recently, other adsorbent materials have been found to have high maximum adsorption capacities for paraquat. In particular, activated charcoals and cation-exchange resins have attracted interest. Unfortunately, as yet, there is no evidence that the use of adsorbents in man is of therapeutic value.

Introduction

Paraquat (1,1-dimethyl-4,4-bipyridilium) is a potent contact herbicide that is potentially lethal to man if ingested. Death due to paraquat poisoning is usually characterized by pulmonary oedema and fibrosis but, if large amounts are ingested, multiple organ failure may develop (Vale *et al.*, 1987). The precise mechanism of toxicity is uncertain but, once a critical plasma concentration is exceeded, active accumulation of paraquat in the lung occurs, with formation of superoxide anion and depletion of NADPH (Smith, 1987). There is no effective antidote for paraquat poisoning (Bateman, 1987) and measures designed to enhance the elimination of paraquat from the body have not proven satisfactory (Bismuth *et al.*, 1987; Proudfoot, 1987). Attention has therefore been directed at the various means by which the absorption of an ingested dose of paraquat may be either prevented or reduced, namely gastric lavage, induced emesis, whole-gut lavage or the oral administration of adsorbent substances. The rationale for the use of each form of treatment is considered below and the evidence for their value in man is reviewed critically.

Gastric lavage

Paraquat is absorbed incompletely from the gut and, in man, it has been estimated that less than 5% of

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an ingested dose is absorbed over a 1–6-h period (Conning *et al.*, 1969). Animal experiments suggest that paraquat is absorbed poorly from the stomach but that facilitated absorption takes place in the small intestine. Thus, Smith *et al.* (1974) found that 10–40% of an orally administered dose remained in the rat stomach at 16 h. In the same study, a linear relation was noted between the paraquat content of the small intestine and the plasma concentration of paraquat. No such relation was observed between the paraquat content of the stomach and the plasma paraquat concentration. Bennett *et al.* (1976) demonstrated dose-dependent absorption in greyhound dogs. When propantheline, an anticholinergic drug which delays gastric emptying, was administered intravenously 15 min before an oral dose of paraquat, the time at which the peak plasma concentration of paraquat occurred was shifted from 75 min to 3–6 h.

Paraquat absorption from the gut may be incomplete but it is rapid, as evidenced by the time at which peak plasma concentrations are observed in different animal species. For example, peak concentrations occur in guinea pigs at 60 min (Conning *et al.*, 1969), in cats at 60 min (Clark, 1971) and in dogs at 60–75 min (Bennett *et al.*, 1976; Nakamura *et al.*, 1982). In man, the time at which the paraquat concentration in plasma peaks is not known with certainty. However, paraquat may be detected in the urine as early as 1 h after ingestion of an overdose and, to judge by the plasma concentration data published by Proudfoot *et al.* (1979), peak concentrations in man are certainly attained within 4 h. Active accumulation of paraquat by lung tissue and subsequent toxicity occurs once a threshold plasma concentration is exceeded. To be effective therefore gastric lavage, and other methods used to reduce absorption, must be employed sufficiently early to blunt or abolish the rapid rise in the plasma paraquat level so that the threshold concentration is not achieved.

Surprisingly, there is very little experimental information relating to the use of gastric lavage alone in the treatment of paraquat poisoning. As part of a study to determine the effect of single dose administration of oral adsorbents, Clark (1971) gave four cats 62.5 mg of paraquat/kg by stomach tube and then performed gastric lavage 60 min later. A 'marked reduction in the levels of paraquat in the blood' was reported in comparison with untreated control animals. However, scrutiny of the data suggests that the reduction in blood paraquat concentrations achieved was only from 16 to 12 mg/l at 5 h after dose administration.

The role of gastric lavage in the treatment of all forms of poisoning in man has been questioned recently since the evidence for its value is poor. Proudfoot (1984), in a review of the subject, considered seriously whether use of the procedure should be abandoned. Kulig *et al.* (1985) undertook a prospective study of 592 patients admitted over an 18-month period to Denver General Hospital following the ingestion of a drug overdose. Gastric lavage was not found to be helpful in the majority of patients, although it did appear to be of some value in 'obtunded' patients provided that it was undertaken within 1 h of ingestion of the overdose.

So far as the treatment of paraquat poisoning is concerned, there have been only two clinical studies published where the authors have made specific mention of the efficacy of gastric lavage. Bismuth *et al.* (1982), in a review of 28 patients, were not able to establish the value of gastric lavage. Bramley & Hart (1983), in a study of 262 cases of paraquat poisoning in the UK, were unable to demonstrate an improved prognosis resulting from the use of gastric lavage. There are further theoretical objections to a stomach washout following the ingestion of paraquat. Ulceration of the oropharyngeal and oesophagogastric mucosal surfaces by concentrated formulations of paraquat can make the procedure hazardous. Furthermore the use of gastric lavage may delay the deployment of alternative forms of treatment with greater theoretical value, for example, administration of oral adsorbents.

In conclusion therefore there is no definite evidence of the value of gastric lavage in the treatment of paraquat poisoning in man and any possible benefit is likely to be confined to use within 1 h of ingestion.

Induced emesis

In 1977, the manufacturers of paraquat (Imperial Chemical Industries PLC) added a potent emetic, PP796, a phosphodiesterase inhibitor, to liquid and solid formulations of paraquat because experiments in primates (T. B. Hart, personal communication) had demonstrated a fivefold reduction in toxicity.

There are a few published laboratory experiments relating to the use of emetic formulations of paraquat, and the principal source of data is a study, undertaken by Nakamura *et al.* (1982), designed originally to investigate the efficacy of gut lavage. Eleven mongrel dogs were given paraquat (250 mg/kg) by stomach tube. Five dogs were given an emetic preparation and all vomited within 15 min; six dogs received a non-emetic preparation of paraquat and vomited approximately 1 h later. The upper duodenum and rectum of each dog were ligated under general anaesthesia 4 h after the administration of paraquat; the gut was then lavaged through a duodenostomy and the lavage fluid collected through a sigmoidostomy. Plasma paraquat concentrations were not reduced significantly in the group of dogs that received the emetic formulation of paraquat (Table 1). Moreover, for reasons that were unclear, the percentage recovery of the administered

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Table 1 Plasma concentrations of paraquat (mg/l)[a] in dogs following the administration of emetic/non-emetic formulations [adapted from K. Nakamura, M. Yamashita & H. Naito (1982) *Vet. Hum. Toxicol.*, 24 (Suppl.), 157–158]

Group	*1 h*	*2 h*	*4 h*
Paraquat alone ($n = 6$)	122.7 ± 73.1	82.3 ± 41.6	52.9 ± 36.2
Paraquat + emetic ($n = 5$)	124.5 ± 43.9*	72.9 ± 40.8*	23.7 ± 6.7*

[a] Mean ± SD
* Not significant

dose of paraquat was strikingly small in both groups of dogs (paraquat alone 4.3 ± SD 4.5%; paraquat + emetic 2.5 ± 1.0%).

Following the introduction of emetic preparations of paraquat, the London Centre of the National Poisons Information Service (NPIS) and ICI Plant Protection Division conducted jointly a survey of paraquat poisoning in the UK. The study commenced in 1980 and interim results for 262 patients were reported in 1983 (Bramley & Hart, 1983). The presence, or absence, of the emetic in the preparation of paraquat ingested was established in 103 of 262 cases, and the time at which spontaneous vomiting occurred was known in 61 of 103 patients (Table 2). There can be no doubt that ingestion of the emetic formulation induces earlier vomiting, and the difference between the number of patients in each group (emetic v. non-emetic) who vomit either before or after 30 min (or not at all) is highly statistically significant (χ^2 9.87 corrected for continuity; $P < 0.005$). Furthermore, with the preliminary reported results of the survey, it is possible to show that, in the manner of a dose-response curve, vomiting is more likely to occur the greater quantity of paraquat ion ingested (Table 3). Unfortunately, despite the occurrence of earlier vomiting, Bramley & Hart (1983) were unable to demonstrate an improved prognosis in patients who had ingested emetic, rather than non-emetic, formulations of paraquat. Subsequent reports (Denduyts-Whitehead *et al.*, 1985; Onyon & Volans, 1987) from the same study have suggested a small, but inconclusive, fall in mortality since the introduction of the emetic, PP796. A reduction in the mortality from paraquat poisoning as a result of the emetic preparation has not been noted by other workers (Bismuth *et al.*, 1982; Nakamura *et al.*, 1982; Naito & Yamashita, 1987).

Thus far, then, it has not been possible to prove that any clinical benefit has derived from the introduction of emetic formulations of paraquat. In some ways, though, this is not surprising for there is, increasingly, doubt about the value of induced emesis as a means of treating any other form of intoxication (Corby *et al.*, 1968; Boxer *et al.*, 1969; Neuvonen *et al.*, 1983; Curtis *et al.*, 1984; Kulig *et al.*, 1985).

Whole-gut lavage

Published laboratory data on whole-gut lavage are confined to the study, mentioned above, by Nakamura *et al.* (1982). Eleven mongrel dogs were given paraquat (250 mg/kg) by stomach tube. Gut lavage was performed 4 h later and only 2.5–4.3% of the administered dose of paraquat was recovered. To explain

Table 2 Time of spontaneous vomiting after ingestion of emetic/non-emetic formulations of paraquat [adapted from A. Bramley & T. B. Hart (unpublished data)]

	Vomiting		
Group	*< ½ h*	*> ½ h*	*No vomiting*
Non-emetic formulation ($n = 21$)	4(19)	4(19)	13(62)
Emetic formulation ($n = 40$)	26(65)	9(22)	5(13)

Percentages are given in parentheses
$P < 0.005$ (see the text for details)

Table 3 Incidence of spontaneous vomiting 30 min after the ingestion of emetic/non-emetic formulations of paraquat [adapted from A. Bramley & J. B. Hart (unpublished data)]

	Amount of paraquat ion ingested (g)		
Group	*< 2*	*2–5*	*> 5*
Non-emetic formulation ($n = 21$)	1/10 (10)	1/4 (25)	2/7 (29)
Emetic formulation ($n = 40$)	16/29 (55)	3/4 (75)	7/7 (100)

Percentages are given in parentheses

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the extremely low recovery of paraquat, it was hypothesized that either absorption must have occurred rapidly from the small intestine (peak plasma concentration ≤ 60 min; see Table 1), or that a substantial amount of paraquat must have remained in the stomach.

The only clinical report of whole-gut lavage where the procedure was used alone, without concomitant oral adsorbents, is that of Okonek *et al.* (1976). A 30-year-old male ingested an unknown quantity of Reglone (200 g of diquat/l) 30 h before admission. Whole-gut lavage was undertaken by using an electrolyte solution (6.14 g of NaCl/l, 0.75 g of KCl/l, 2.94 g of $NaHCO_3$/l) heated to body temperature which was fed into the patient by using a stomach tube and peristaltic pump. Approximately 27 mg of diquat was recovered in 6900 ml of lavage fluid. However, at the pumping rate employed (75 ml/min), it was found that 0.5–1.0 litres of lavage fluid were absorbed across the intestinal wall. Theoretically, this is likely to enhance absorption of diquat (or paraquat). Perhaps for this reason no subsequent studies have been reported using gut lavage alone. Certainly, there is no evidence to suggest that whole-gut lavage is of value in the treatment of paraquat poisoning in man.

Oral adsorbents

Bentonite and Fuller's Earth

In the period, 1965–1967, bipyridilium herbicides were found to bind strongly to soil and to clay minerals, in common with many other organic cations (Knight & Tomlinson, 1967). Study of the adsorption capacity and chemical composition of a variety of soils showed that montmorillonite in particular was a strong binding agent *in vitro* (Knight & Tomlinson, 1967).

Clark (1971) investigated the effect of single-dose administration of oral adsorbents on paraquat toxicity in animals. Preliminary experiments *in vitro* showed that the adsorption capacity of minerals varied, but that Bentonite (sodium montmorillonite) and Fuller's Earth (calcium montmorillonite) were particularly effective (Table 4). At the time that these experiments were undertaken and, for some years subsequently, emphasis was placed on the so-called strong adsorption capacity (SAC) of a substance. SAC is defined as the quantity of paraquat that can be adsorbed per unit weight of adsorbent before the adsorbent phase is in equilibrium with a detectable solution concentration (Knight & Tomlinson, 1967), in this instance 1 mg/l. In other words, there is a region of the adsorption isotherm in which paraquat cannot be detected in solution (this region has no physical significance but depends on the sensitivity of the analytical methods employed). The maximum adsorption capacity (MAC) of a substance (see below) is defined as the maximum quantity of paraquat that can be adsorbed per unit weight of adsorbent.

Clark (1971) went on to demonstrate that a single dose of adsorbent material administered to rats after a potentially lethal dose of paraquat could reduce mortality (Table 5). Bentonite and Fuller's Earth prevented some deaths even when administration was delayed for 3 h after dosing with paraquat. Further experiments in cats showed that some reduction in blood paraquat levels could be achieved following a single dose of either Fuller's Earth or Bentonite when compared with control animals (Clark, 1971).

Smith *et al.* (1974) investigated subsequently the effect of repeated doses of oral adsorbents on paraquat toxicity in animals. Rats were given four doses of a castor oil/magnesium sulphate/Bentonite mixture at 2–3-hourly intervals commencing 4–10 h after the oral administration of a lethal dose of paraquat (680 μmol/kg). Even when administration of the adsorbent/cathartic mixture was delayed for as long as 10 h, the mortality was considerably reduced.

Table 4 Strong adsorption capacities (SAC) of various minerals [adapted from D. G. Clark (1971) *Br. J. Indust. Med.*, **28**, 186–188]

Adsorbent	*SAC[a] (g of paraquat/100 g)*
Kaolin	0.5
Decalso[b]	1.4
Amberlite	1.7
Bentonite	5.0
Fuller's Earth	5.0

[a] Calculated on the basis of a 1 mg/l limit of detection
[b] Synthetic sodium aluminium silicate

Table 5 Mortality in rats due to paraquat following delayed administration of adsorbent materials [adapted from D. G. Clark (1971) *Br. J. Indust. Med.*, **28**, 186–188]

Adsorbent	*Time after dosing (h)*	*Paraquat dose and mortality[a] (mg/kg)*	
		200	*300*
None	–	6/6	6/6
Amberlite	0.5	6/6	6/6
Decalso	0.5	6/6	6/6
Bentonite	0.5	0/6	6/6
	1.0	0/6	6/6
	2.0	3/6	6/6
	3.0	5/6	6/6
Fuller's Earth	0.5	0/6	3/6
	1.0	1/6	6/6
	2.0	2/6	5/6
	3.0	4/6	6/6

[a] LD_{50} in rats 150 mg/kg

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Twenty-seven of 29 untreated control rats died, but not one of 10 rats died when treated at 4 h, and only two of 10 rats died when treated at 10 h after administration of the paraquat. Smith *et al.* (1974) were able to show that the reduction in mortality was associated with a concomitant reduction in the plasma concentration of paraquat and a reduction in the amount of paraquat accumulated in lung tissue.

Fuller's Earth is preferred in clinical practice because it can be used as a 30% (w/v) suspension, whereas Bentonite swells in water and can only be used as a 6 or 7% (w/v) suspension. Magnesium sulphate is usually administered at the same time as the adsorbent to increase the rate of elimination of the Fuller's Earth/Bentonite–adsorbed paraquat complex from the gut. Unfortunately, the use of these agents in poisoned patients has not met with the same success as in laboratory experiments. Thus, Park *et al.* (1975) gave 11 patients a 7% (w/v) Bentonite suspension, six of whom subsequently died; nine of 10 patients treated with 30% (w/v) Fuller's Earth by Vale *et al.* (1979) also died; 18 of 26 patients died in Belfast following the administration of Fuller's Earth (Coppel *et al.*, 1981); in Paris, 10 of 13 patients died despite being given a 15% (w/v) suspension of Fuller's Earth (Bismuth *et al.*, 1982). Finally, Bramley & Hart (1983), in a review of 262 cases of paraquat poisoning in the UK, were unable to demonstrate an improved prognosis associated with the use of Fuller's Earth. In this latter study, though, almost all patients received Fuller's Earth and the control group was too small.

Activated charcoal

At the time that Clark (1971) undertook his experiments with adsorbent substances in rodents, the assumption was made that activated charcoal would not bind paraquat. It is only recently that this assumption has been challenged and found to be false. Okonek *et al.* (1982) have shown *in vitro* that activated charcoal, despite having a low SAC, possesses a maximum binding capacity greater than that of either Fuller's Earth or Bentonite (Table 6). They also undertook experiments *in vivo*, using rats, similar to those of Clark (1971). A single 1-g dose of adsorbent was instilled by mouth at various times after the administration of a lethal dose of paraquat. Activated charcoal (Kohle-Compretten, Merck) effected a reduction in mortality greater than that achieved by either Fuller's Earth or Bentonite (Table 7).

Other workers have investigated the effect of single dose administration of activated charcoal in mice (Gaudreault *et al.*, 1985). Not only did activated charcoal appear to be effective, but the addition of a cathartic agent (magnesium citrate) increased the chances of survival in these experiments (Table 8).

Table 6 Maximum (MAC) and strong (SAC) adsorption capacities of various materials [adapted from S. Okonek, H. Setyadharma, A. Borchent & E. G. Krienke (1982) *Klin. Wochenschr.*, **60**, 207–210]

Adsorbent	*MAC (g of paraquat/ 100 g)*	*SAC[a] (g of paraquat/ 100 g)*
Fuller's Earth	6	5
Fullererde	2	<1.0
Bentonite	6	5
Bentonit APV	6	4–5
Bentonit SF	6	5
Activated charcoal (Kohle-Compretten, Merck)	>8	<1.0

[a] Calculated on the basis of a 0.5 mg/l limit of detection

Table 7 Mortality in rats due to paraquat following delayed administration of adsorbent materials [adapted from S. Okonek, H. Setyadharma, A. Borchent & E. G. Krienke (1982) *Klin. Wochenschr.*, **60**, 207–210.

Adsorbent	*Time after dosing (h)*	*Paraquat dose and mortality[a] (mg/kg)*	
		200	*300*
None	—	6/6	—
Fuller's Earth	0.5	0/6	6/6
	1.0	0/6	6/6
	2.0	1/6	6/6
	3.0	1/6	6/6
Bentonit APV	0.5	0/6	4/6
	1.0	2/6	5/6
	2.0	0/6	6/6
	3.0	0/6	6/6
Activated charcoal (Kohle-Compretten, Merck)	0.5	0/6	2/6
	1.0	0/6	4/6
	2.0	0/6	4/6
	3.0	2/6	5/6

[a] LD_{50} in rats 150 mg/kg

Table 8 Mortality in mice due to paraquat (200 mg/kg) followed by single dose treatment 30 min later [adapted from P. Gaudreault, P. A. Friedman & F. H. Lovejoy (1985) *Ann. Emerg. Med.*, **14**, 123–125]

Group	*Mortality*
No treatment	11/16
Magnesium citrate	5/16
Fuller's Earth	6/16
Activated charcoal	6/16
Activated charcoal + magnesium citrate	1/6[a]

[a] $P < 0.01$

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The type of activated charcoal employed was not stated.

It is important to recognize that not all forms of activated charcoal have the same capacity to adsorb paraquat (Table 9), a factor that may have some importance if poisoned patients are to be treated with this material rather than Fuller's Earth or Bentonite. However, results of multiple-dose administration of activated charcoal in the treatment of paraquat toxicity have not yet been reported for either animals or man.

Cation exchange resins

Recently, some interest has centred on cation exchange resins, normally used for the treatment of hypercalaemia, as an alternative means of binding paraquat in the gut to reduce systemic adsorption. Kayexalate (sodium polystyrene sulphate) and Kalimate (calcium polystyrene sulphate) have high MAC for paraquat (Table 9), and Nokata *et al.* (1984) have reported a reduction in morbidity in rats from paraquat toxicity following the delayed administration (up to 24 h) of these materials. Latterly, Yamashita *et al.* (1987) have reported the results of gastric and intestinal lavage with these materials in 22 patients. Six of 11 patients treated in this manner survived, but 11 patients who did not receive Kayexalate died. Unfortunately, it is not possible to judge whether the severity of poisoning was comparable in the two groups of patients because blood concentration data are not provided.

In conclusion then, so far as oral adsorbents are concerned, there is no definite evidence of their value in man for the treatment of paraquat poisoning. Nevertheless, the MAC of some activated charcoals are greater than those of either Fuller's Earth or Bentonite. As a means of reducing the absorption of drugs, though, activated charcoal has never been shown to reduce either the morbidity or mortality of any form of poisoning. In contrast repeated oral doses of activated charcoal may enhance the elimination of certain drugs, e.g. phenobarbitone (Berg *et al.*, 1982) whose toxic effects are then ameliorated as the blood concentration falls. Obviously, this situation is very different from that which obtains in paraquat poisoning.

Table 9 Maximum adsorption capacities (MAC) of activated charcoals and other materials [adapted from T. B. Hart, (personal communication)]

Adsorbent	*MAC (g of paraquat/100 g)*
Carbomix	9–10
Ultracarbon	8–9
Amoco AC	> 8
Medicoal	> 6
Norit AC	6
SK & F AC	< 1
Fuller's Earth	6
Kayexalate[a]	> 10
Kalimate[b]	> 10

[a] Sodium polystyrene sulphate
[b] Calcium polystyrene sulphate

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SYNG-PQ-00059888

June 1982

PARAQUAT POISONING IN THE UNITED KINGDOM

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SYNG-PQ-03720006_R

PARAQUAT POISONING IN THE UNITED KINGDOM

Introduction

Paraquat, a bipyridilium compound, was first put on the U.K. market as a contact herbicide by I.C.I. in 1962 (fig 1). By the late 1960's there were a significant number of fatal paraquat poisonings occurring each year (fig 2).

In the early 1970's a large amount of publicity was given by the press to deaths caused by paraquat poisonings, some of which involved very aggressive journalism (figs 3 & 4). This and the increased use of paraquat in the U.K. at this time, were probably significant factors in the number of deaths due to deliberate ingestion of paraquat (fig 5). The number of accidental deaths remained low, at about one or two a year.

Regulations following from the Poisons Act of 1972 stated that liquid formulations of paraquat (greater than 5% of paraquat ion, weight to volume) should only be used by professionals (that is, farmers, nursery gardeners and so on). This referred to the liquid concentrates such as Gramoxone and Dextrone. Granular formulations containing less than 5% of paraquat ion weight to volume, such as Weedol and Pathclear, were exempt from these regulations and could be used in domestic gardens.

In 1974, in response to the increasing number of poisoning incidents, I.C.I published a booklet entitled "The Treatment of Paraquat Poisoning" (fig 6). This outlined the toxic effects of the herbicide, and advocated the use of Fuller's Earth, followed by haemodialysis or charcoal haemoperfusion, for the treatment of paraquat poisoning(fig 7). The booklet distribution was followed by the dispatch of Fuller's Earth, the mainstay of treatment, to hospitals throughout the United Kingdom. One year later, a stenching

agent was added to liquid formulations of paraquat in an attempt to prevent the small number of accidental poisonings occurring each year. Subsequently in 1977 an emetic substance was added to paraquat formulations (solid and liquid) in an attempt to reduce the acute toxicity of those formulations, by inducing vomiting before a potentially lethal dose could be absorbed.

Present Study

a) Aims

In 1980 a survey of paraquat poisoning in the U.K. was initiated jointly by the National Poisons Information Service at Guy's Hospital, London, and I.C.I. Plant Protection Division. There were three main aims of this study (fig 8):

(i) To examine in detail the incidence of paraquat poisoning in the U.K.

(ii) To evaluate treatment methods, especially charcoal haemoperfusion and any new treatments being used for paraquat poisoning.

(iii) To evaluate the efficacy of the emetic added to paraquat formulations in reducing paraquat mortality.

b) Methods

Information about cases of paraquat poisoning was received from three sources (fig 9):

(i) The National Poisons Information Service, including the four regional centres at Belfast, Cardiff, Dublin and Edinburgh.

(ii) I.C.I. Plant Protection Division and Central Toxicology Laboratory.

(iii) Newspaper articles, via I.C.I. Publicity Departments.

I.C.I. and the NPIS were usually contacted in the first instance by doctors requesting advice on the management of poisoned patients or measurement of plasma paraquat levels. Requests to I.C.I. for replenishment of Fuller's Earth stocks also brought several patients to our attention. In each case a note was made of the caller, the hospital, name of the patient and any symptoms present, and this information was filed at the NPIS in London.

Further information on subsequent symptoms, treatment given, results of laboratory analyses, and outcome for each patient was obtained by contacting doctors by telephone, usually between two and seven days after the poisoning incident, if possible. In some cases, for example those brought to our attention by newspaper articles, several months had elapsed before we contacted the relevant doctors.

Finally, questionnaires were sent to doctors to obtain a complete case history for each patient, including name, age and sex of the patient, amount of formulation of paraquat ingested, whether the formulation contained emetic, symptoms, treatment given, laboratory analyses and outcome (figs 10, 11 & 12).

Presence or absence of the emetic in the paraquat formulation involved had to be confirmed in each case as there are still significant amounts of old formulations (not containing the emetic) in stock. This could be done by:

i) examination of the container (the presence of the emetic is indicated by a red chevron on the packets of Weedol and Pathclear, and by two black flashes on the Gramoxone label (fig 13).

ii) analysis of urine samples for the emetic metabolites.

iii) analysis of the original product for emetic parent compound.

Ideally, confirmation of the presence or absence of the emetic could be obtained by more than one of these methods.

c) Results and Discussion

i). Recovery of information

About 70% of the questionnaires sent out were returned with complete information. For a further 15% of patients, complete or almost complete information was obtained by telephone, leaving 13% about whom incomplete details were obtained, and 2% where hardly any information could be obtained at all (fig 14).

There were two main problem areas in the survey. The first was in estimating the amounts of paraquat taken: doctors could only report what they had been told by patients, and symptoms and laboratory analyses did not always confirm their report.

The second, and major difficulty of the study has been in confirming the presence or absence of the emetic in paraquat formulations. There are several reasons for this. Often the containers are not available for doctors to examine, and so there can be no positive identification of emetic formulations from the label or from analysis of the original product. For a urine analysis to detect the emetic metabolites a sample needs to be taken within 48 hours of ingestion of paraquat; a number of cases were notified after this time period. When urine samples were requested from hospitals they were not always sent, and, if sent, did not always arrive. We were able to confirm either presence or absence of emetic in only 39% of the cases in the survey.

ii) Mortality Statistics

Between the beginning of January 1980 and the end of February 1982, 262 cases of paraquat poisoning were reported. The two main formulations involved were Weedol (47% of cases) and Gramoxone (32%) (fig 15). The majority of patients were adults (94%) (fig 16), and male (76%) (fig 17). 83% of the poisonings were deliberate, 11% were accidental, and for 6% no intent was specified (although for most of the latter deliberate ingestion was implied at the time of the original call) (fig 18). 94 patients died, 143 survived, and for 25 the outcome was unknown (fig 19).

The commonest symptoms reported were spontaneous vomiting (in 55% of patients whose symptoms were specified) - in half of these patients vomiting occurred within half an hour of paraquat ingestion; irritation or ulceration of the fauces (47%): nausea (42%); renal damage (32%) and pulmonary damage (32%) (fig 20).

As would be expected, mortality increased as the reported amount of paraquat ingested increased. The mortality of patients who had ingested 2g to 5g of paraquat ion as Weedol or Pathclear was lower than that of patients who had taken equivalent amounts of the concentrates Gramoxone or Dextrone (figs 21 & 22). The reason for this apparent difference in relative mortalities is unclear. It may be that it is harder for patients to estimate the dose ingested of liquid formulations than for the sacheted solid products. The overall mortality from taking Weedol or Pathclear was 16%, while that from taking Gramoxone or Dextrone was 78%.

When the cases were analysed according to intent (that is, deliberate or accidental ingestion of paraquat) it was found that out of 208 patients about whom these details were known, there were five deaths reported as being accidental in origin (fig 23). All of these patients were adults.

No deaths of children under 12 were reported, either accidental or deliberate.

Monthly variation of paraquat poisonings was also studied (fig 24). It was thought that there may be a seasonal pattern to poisonings with Gramoxone and Dextrone, with peak numbers during the months when these products are most used, that is late August to October. However, no such pattern could be found during the two years of the study. Weedol and Pathclear are used by amateur gardeners most of the year, and no seasonal pattern of poisonings was expected or found with these.

Towards the end of 1981 when it became apparent that there were a large number of poisonings occurring involving Gramoxone, which legally should only be sold to professional users, an effort was made to determine the occupation of patients. The majority of patients taking Gramoxone seemed to be, or to have some connection with legitimate users, such as farmers, farm labourers or garden nursery workers.

iii) Treatment

Early treatment of paraquat poisoning is considered essential, because plasma paraquat concentration may reach a peak relatively quickly from the time of ingestion (certainly within six hours). In this study, this concept appears to be true for those cases involving 'Weedol' or 'Pathclear', but not for those involving 'Gramoxone' or 'Dextrone' (fig 25). As the solid formulations tend to be associated with relatively low doses of paraquat, this observation supports the one made by Dr Keir Howard in a previous meeting of this association, in which he concluded that early treatment is of benefit in cases swallowing between 1g and 6g of paraquat ion.

For several years now, the mainstay of treatment of paraquat poisoning has been the use of gastric lavage, followed by oral administration of Fuller's Earth and a suitable purgative. It is reassuring to see that 69% of the patients considered received Fuller's Earth as a treatment and 51% of patients received gastric lavage (fig 26). Unfortunately, due to the small number of patients not treated with Fuller's Earth and the large number of variables present, such as the time lapse between ingestion and treatment, the amount of paraquat taken and the amount of Fuller's Earth given, it is not possible to determine whether or not either of these methods influence the outcome.

Haemoperfusion through a charcoal column has been used for some time now for the treatment of paraquat poisoning, but has been received with a very much mixed response. In this study, 15% of the patients were haemoperfused. Most cases involved the use of haemoperfusion on one occasion only and for a period of up to 22 hours. The time lapse between ingestion of paraquat and the start of haemoperfusion varied greatly, from about four hours to over sixty hours. All cases were confirmed, by urine and plasma analysis, as involving paraquat. Although the number of patients haemoperfused was relatively small, the figures shown seem to indicate that this method is not associated with lower mortality, and may, in fact, have an adverse effect (fig 27).

During the period of this study only one significant new treatment emerged – the use of ethacrynic acid. This treatment was used by intravenous injection at Ninewells Hospital, Dundee. Although initial success was claimed, further use of the drug in other patients did not succeed . Interest in this form of treatment has now largely subsided.

iv) Emetic

Despite the introduction of an emetic to paraquat formulations on the U.K. market in 1977, old stock not containing the emetic is still being involved in poisonings. Of the 103 cases in the study where emetic was identified as being present or absent, it was present in 62% and absent in 38% (fig 28). Of the 39 of these cases which involved Gramoxone, 20 (51%) were not emetic formulations. Weedol, which has a higher rate of stock turnover, was involved in 45 cases, only 13 (29%) of which were not emetic formulations (fig 29).

Although it is not possible to reach definite conclusions about the effectiveness of the emetic addition in reducing toxicity of paraquat formulations, the evidence clearly shows that this addition has increased the incidence of early spontaneous vomiting following ingestion of a paraquat formulation (fig 30).

Summary

Between January 1980 and January 1982, the number of fatal paraquat poisonings has been between 42 and 46 per annum, and has therefore remained fairly constant over the past six years (fig 2). Also over the last six years the majority of fatal poisonings have been associated with suicidal intent (approximately 95% in the last two years).

Statistics published by the Office of Population Censuses and Surveys show that the total number of deaths from suicide has remained fairly constant over the last decade, as have the number of deaths from suicide

associated with chemical poisonings. The latest figures, published for 1980, show that there were 4,321 deaths from suicide by any method and 1572 deaths from suicide associated with chemical poisoning. Suicidal deaths involving paraquat, therefore account for approximately 1% of all suicidal deaths and 2.5% of suicide deaths involving chemical poisoning. Fatal accidental poisoning with paraquat accounts for about 0.3% of all accidental fatalities involving chemicals.

The majority of patients involved with paraquat poisoning were male and adult. No children were involved in any fatal paraquat incidents. There appears to be no set monthly variation in the number of paraquat poisonings involving either liquid or solid formulations and most of the patients involved with 'Gramoxone' poisoning were reported to have connection with legitimate use of the product.

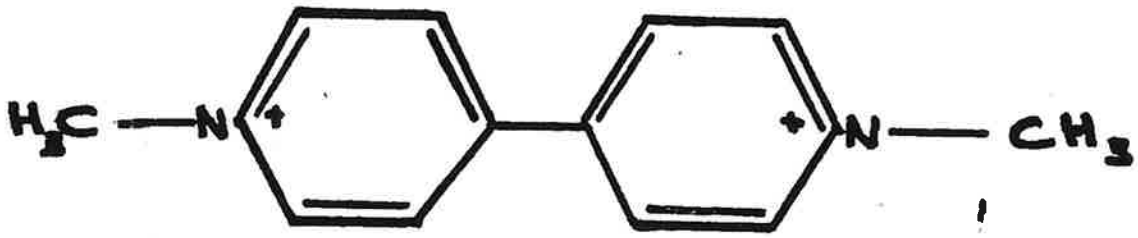
Early treatment of paraquat poisoning (up to 12 to 24 hours) appears to have some benefit when the dose of paraquat ingested is relatively low. We would recommend that although we cannot demonstrate an improvement in mortality with the use of Fuller's Earth or gastric lavage, these measures should be employed at the earliest opportunity, and are unlikely to be effective 24 hours or more after the time of ingestion. The results of using haemoperfusion through a charcoal column do not appear to be encouraging and it is unlikely that this method will be effective if used for single short periods of time. We would recommend that, if this method is to be used, it should be done within 24 hours of ingestion and should involve a different modus operandi.

We have not yet been able to evaluate fully the effectiveness of an emetic formulation in reducing mortality, but addition of the emetic significantly increases the incidence of early spontaneous vomiting. We are planning to continue to follow up paraquat poisoning cases, particularly those involving emeticised formulations. This continued follow-up will also attempt to study more cases involving early treatment with Fuller's Earth, and to evaluate any new treatment methods which may arise.

Finally, it is recommended that measures to prevent accidental paraquat poisoning are maintained and, if possible, improved upon. Widespread publicity of paraquat poisonings should be discouraged, because of its possible stimulus of suicide attempts with the chemical.

Fig. 1

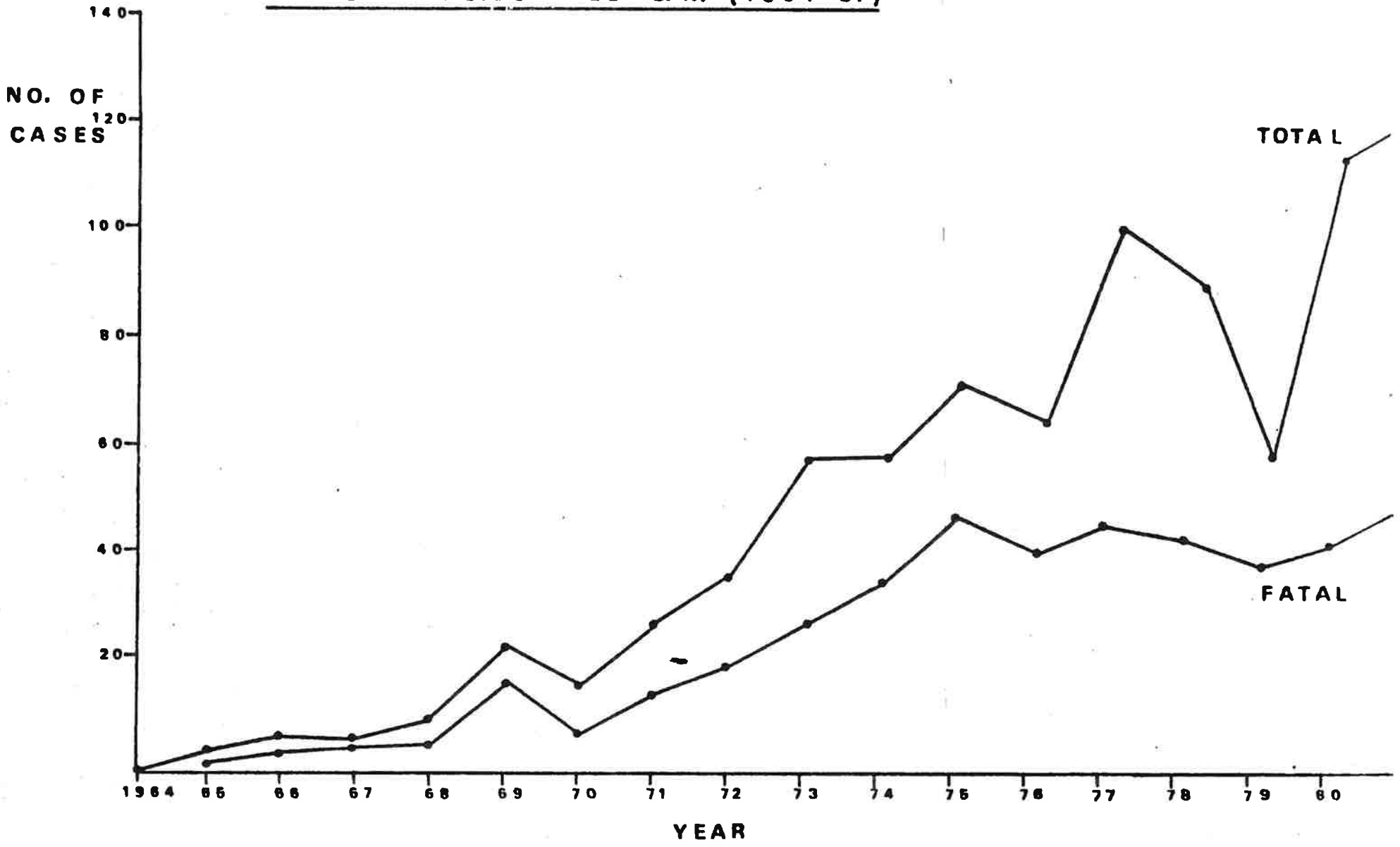
PARAQUAT DICHLORIDE



permanent

fig. 2

PARAQUAT POISONINGS - U. K. (1964-81)



19 FEB 75

BH45/74

Not suicide, says jury

SHOCK VERDICT IN PARAQUAT CASE

THE death of paraquat victim Redacted - EU PII Redacted - EU PII **was still a mystery last night.**

For all the fatal accident inquiry jury decided was that Redacted - EU PII did **NOT** commit suicide.

That was the unanimous verdict of the four women and three men.

At the end of the inquiry Mr Redacted - EU PII and Redacted - EU PII, solicitors for Redacted - EU PII and her family, made a formal statement.

They said: "We point out that the clear implication of the verdict was that while the jury did not believe the death to be suicidal, it could well be accidental.

Bowed

"Even if it were neither suicide nor accidental, it was not proved who might have been responsible for his death."

There was no stir at Kirkcudbright Sheriff Court as the foreman of the jury read out the verdict after two hours and 50 minutes deliberation.

The foreman said Redacted - EU PII died in Redacted - EU PII

Redacted - EU PII

"a terrible year."

Infirmary on the Redacted - EU PII as a result of poisoning by paraquat which was swallowed by him at Redacted - EU PII Redacted - EU PII on or about the 12th of July.

"We believe that Redacted - EU PII did not commit suicide.

"And we believe

Redacted - EU PII

... sat impassively.

the manufacturers are taking all reasonable precautions against the misuse of Gramoxone."

In the front row of the public benches, Redacted - EU PII widow, Redacted - EU PII sat impassively with her son Redacted - EU PII and her married daughter Redacted - EU PII

CONT/....

fq : Pwls

Irish Independent -

September 17, 1974 7

Paraquat: Most deadly killer since atom bomb'

A COUNTY physician last night called on the Government to put strict controls on "the most deadly killer since the invention of the atomic bomb"—paraquat.

Earlier, Redacted - EU PII Monaghan County Physician, had tried to raise the subject at the North Eastern Health Board, but the chairman, Senator D. Farrelly, asked him to put the item on the agenda for next month's meeting.

But Redacted - EU PII said that more people could die from paraquat poisoning and he wanted to warn the general public about the effects of using it. He suggested that the manufacturers should insert "a foul-smelling substance' 'into paraquat so that people would not mistake it for soft drinks.

He said: "I had the sad duty recently in Monaghan of sitting at the bedside of a perfectly healthy man who had taken paraquat. He asked when he was going home and I knew that he was going to his permanent home in about four days and that there was nothing I could do about it."

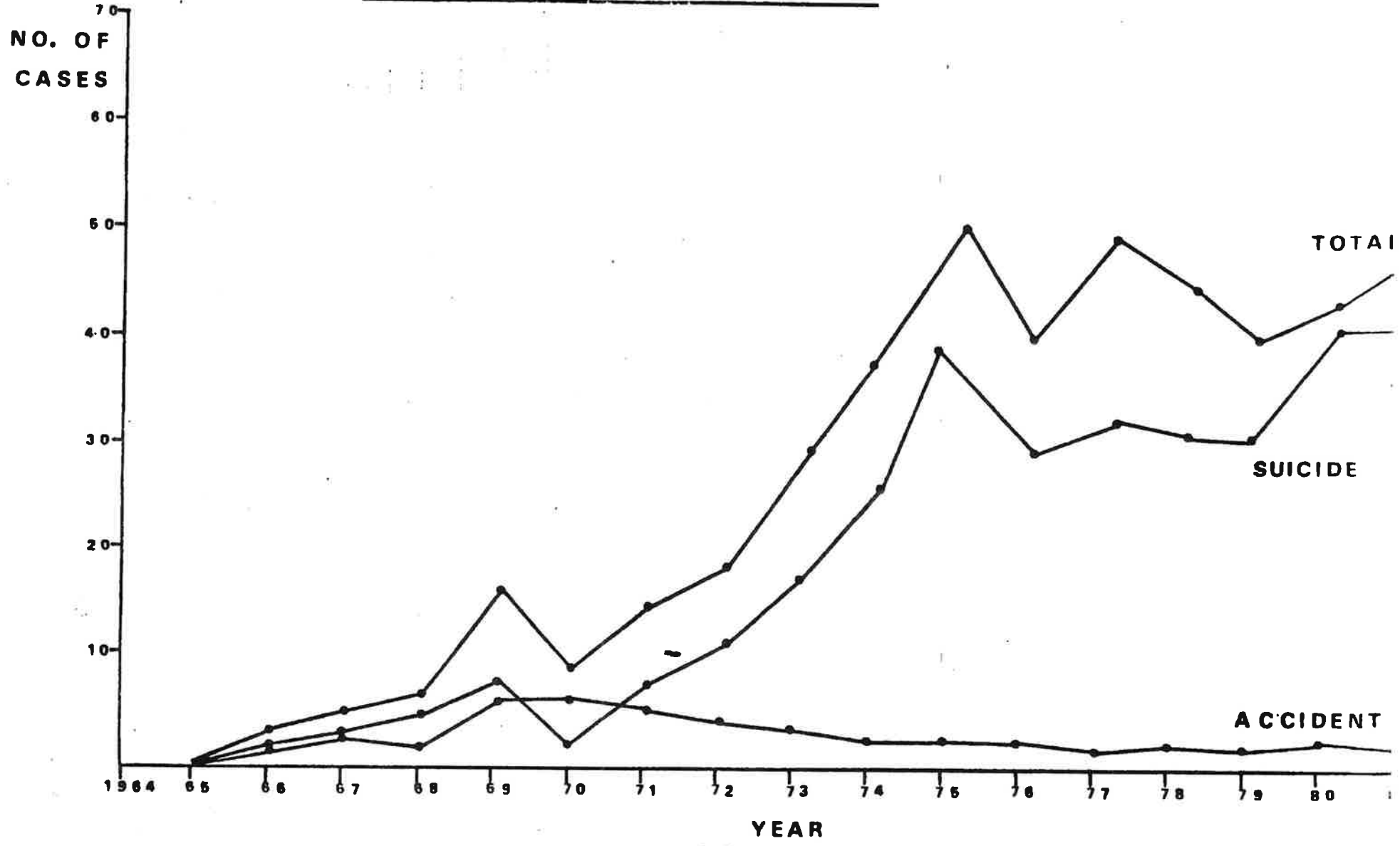
cc Mr J. S. L. Baber
.. K. D. Hughes
Legal Dept
Pub Relats.
Mrs J. A. Whitaker (CTL)
Dr E. G. Schumacher
Mr J. Swabey

permanent store for Dr Hart

fig. 5

U.K. PARAQUAT POISONINGS - FATAL (1964-81)

ANALYSIS ACCORDING TO INTENT



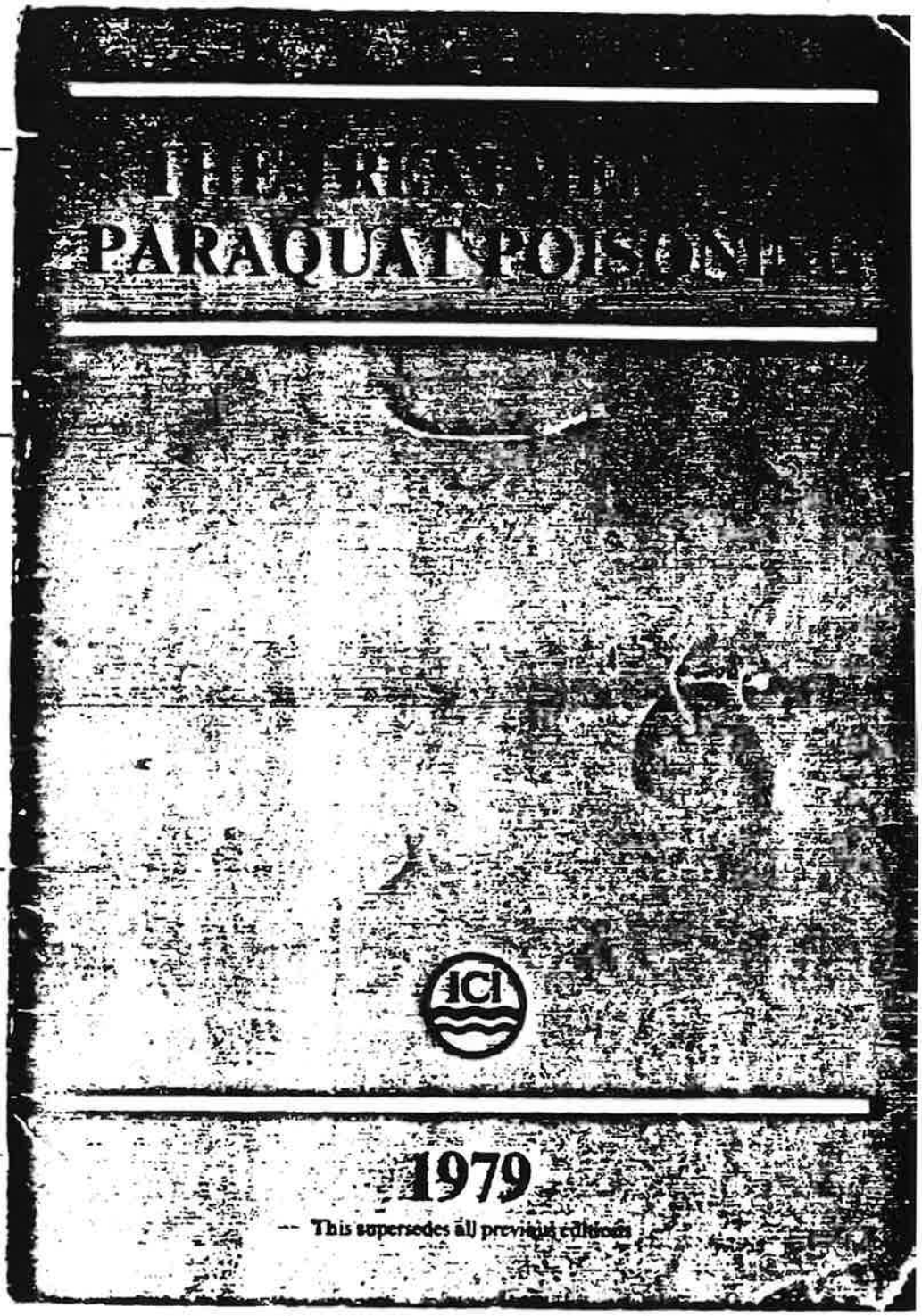

PARAQUAT POISONING
ICI
1979
— This supersedes all previous editions

Fig. 1

TREATMENT OF PARAQUAT POISONING FOLLOWING INGESTION

First Aid

Induce vomiting if not already occurring and send patient to nearest hospital immediately.

Hospital Treatment

1 Give stomach washout and at the same time test both urine and gastric aspirate for the presence of paraquat (see Appendix 1).

2 It is important to purge the gastro-intestinal tract immediately; within four hours if possible. Give up to one litre of 15% Fuller's Earth (Surrey Finest Grade), including 200 ml 20% mannitol in water. Alternatively, sodium or magnesium sulphate can be used as the purgative. Administration should normally be orally but, if this is not tolerated, stomach or duodenal intubation can be used. Continue purgation until the stools are seen to contain adsorbent.

3 CONTACT NEAREST POISONS INFORMATION CENTRE FOR FURTHER ADVICE ON TREATMENT.

4 Maintain and monitor fluid and electrolyte status on a daily basis.

5 Carry out haemodialysis or haemoperfusion (using a charcoal column) to remove paraquat from the plasma (Refs 2, 3). This will only be of use if carried out within 48 hours of ingestion. In some cases renal failure may necessitate the use of haemodialysis at a later stage.

6 In the event of respiratory difficulties, delay the use of oxygen as long as possible as it enhances the toxicity of paraquat.

7 In severe cases, particularly where shock has supervened, consider additional supportive therapy such as the use of steroids.

AIMS OF U.K. PARAQUAT POISONING SURVEY (fig 8)

1 To examine in detail the incidence of paraquat poisoning in the U.K.

2 To evaluate treatment methods.

3 To evaluate the efficacy of the emetic in reducing paraquat mortality.

Sources of information about paraquat poisonings (fig 9.)

1 National Poisons Information Service.

London

Belfast

Cardiff

Dublin

Edinburgh

2 I.C.I. Plant Protection Division

Central Toxicology Laboratory

3 Newspaper articles via I.C.I. Publicity Departments.

Fig 10

PARAQUAT QUESTIONNAIRE

A. PATIENT DETAILS

1. Name .....
2. Age .....
3. Sex .....
4. Hospital No. .....
5. Name of Doctor .....
6. Hospital .....

B. PRODUCT INGESTED

1. Formulation

Liquid: Gramoxone .....
Dextrone .....
Other (please state) .....

Solid: Weedol .....
Patholear .....
Other (please state) .....

2. Amount ingested .....
3. Time and date of ingestion .....
4. Time and date of admission .....
5. Ingestion

Accidental ☐
Suicidal ☐
Other ☐

6. Did product contain emetic? YES/NO
7. If yes, how was this ascertained
   a) from original container .....
   b) from gastric aspirate analysis .....
   c) from urine analysis .....

(Paraquat) Fig 11

C. SIGNS AND SYMPTOMS

	YES	NO	TIME AFTER INGESTION
Irritation/ulceration of fauces	☐	☐	. . . . .
Epigastric pain	☐	☐	. . . . .
Nausea	☐	☐	. . . . .
Vomiting - spontaneous	☐	☐	. . . . .
" - after treatment	☐	☐	. . . . .
Diarrhoea	☐	☐	. . . . .

Disturbance in:

Time after ingestion & duration

Renal function . . . . . .

Hepatic function . . . . . .

Pulmonary function . . . . . .

D. TREATMENT

	YES	NO	TIME AFTER INGESTION
Gastric lavage	☐	☐	. . . . .
Emetic	☐	☐	. . . . .
Fuller's earth	☐	☐	. . . . .
Haemodialysis	☐	☐	. . . . .
Haemoperfusion	☐	☐	. . . . .
Forced diuresis	☐	☐	. . . . .
Steroids	☐	☐	. . . . .
Other	☐	☐	. . . . .

E. OUTCOME

1. Survival (date of discharge). . . . . .
2. Fatal (date of death) . . . . . .
3. If fatal - cause of death . . . . . .
4. Is (3) based on clinical judgement? . . . post-mortem result? . . . . . .

Fig. 12

F. TOXICOLOGICAL ANALYSIS

	Date & Time	Result
Urine	.....	.....
Gastric aspirate	.....	.....
Serum/plasma	.....	.....

G. COMMENTS

.....

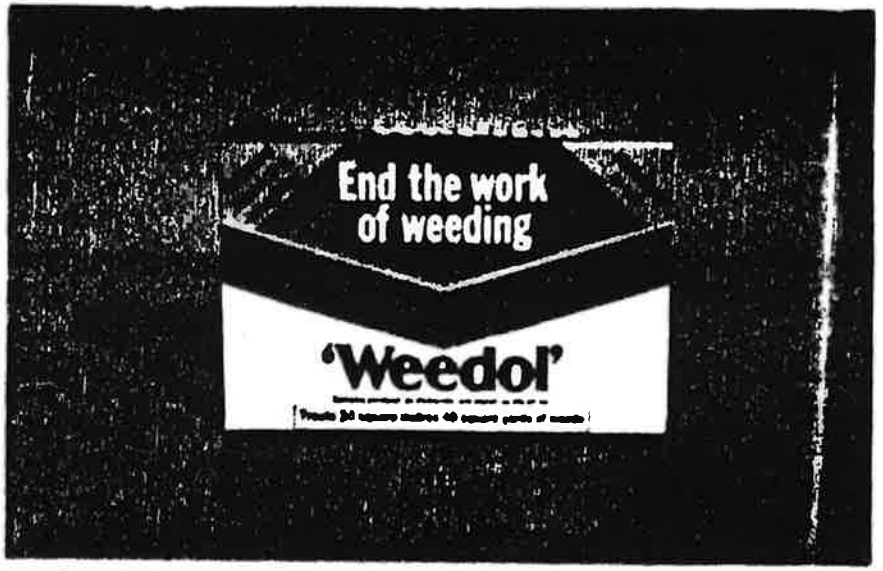
.....

.....

.....

Fig. 13

Light brown granules in 56g sachets.
(Paraquat 2.5% w/w: Diquat 2.5% w/w:
Simazine 5% w/w.)



Light brown granules in 56g sachets.
(Paraquat 2.5% w/w: Diquat 2.5% w/w.)

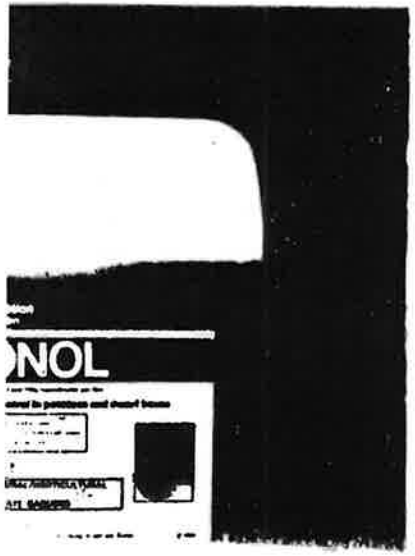
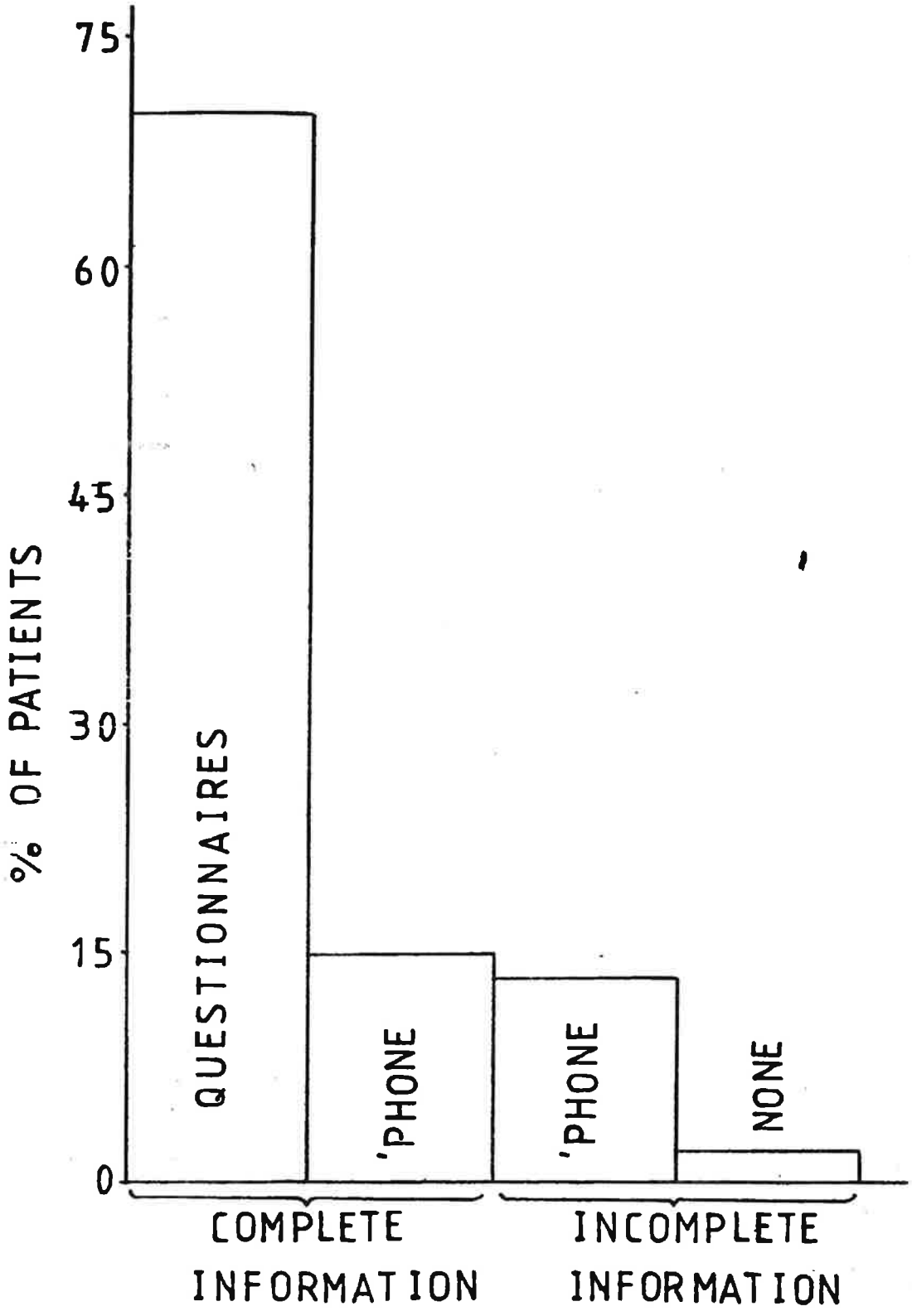


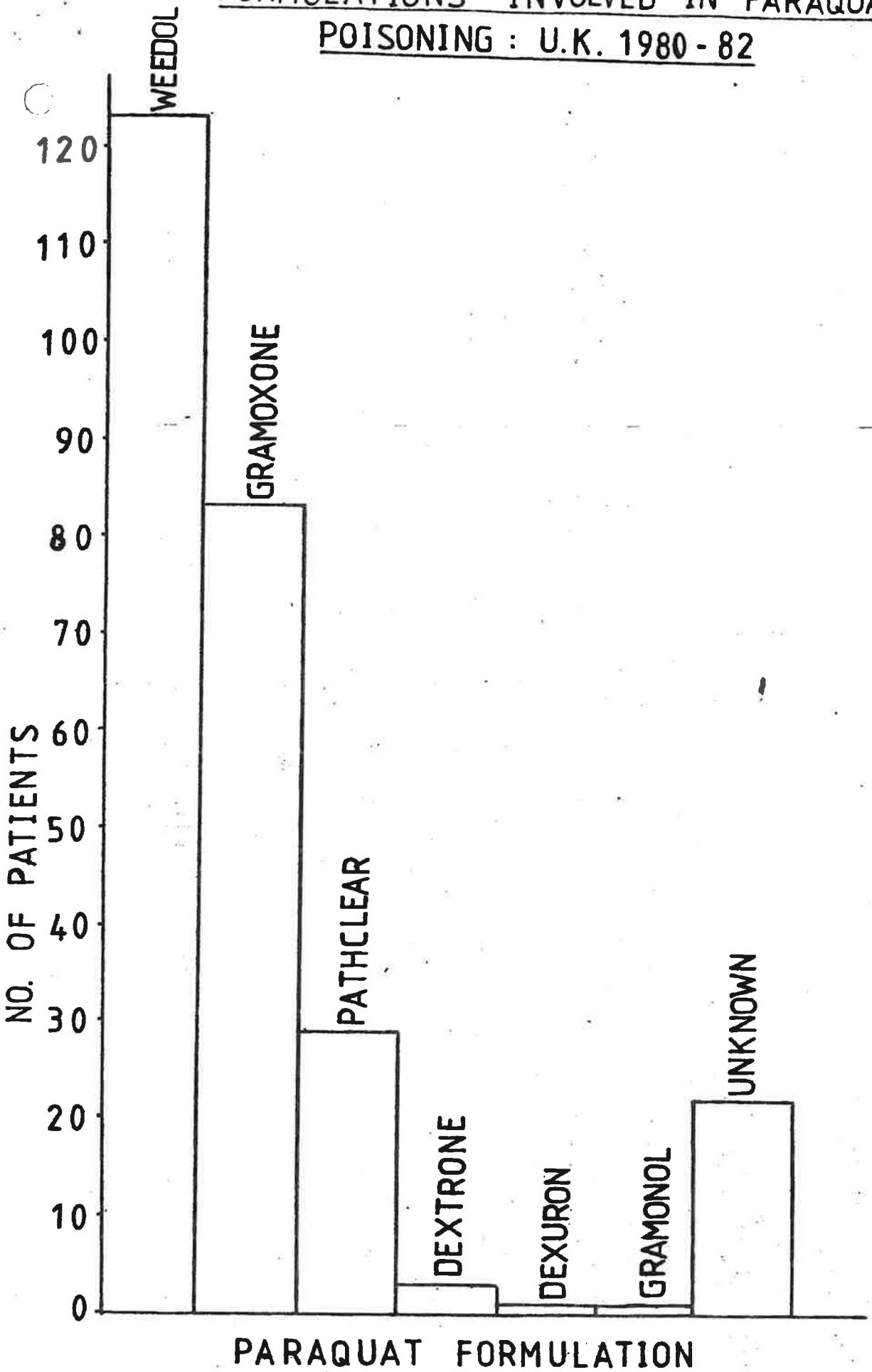
fig.14

RECOVERY OF INFORMATION



FORMULATIONS INVOLVED IN PARAQUAT POISONING : U.K. 1980 - 82

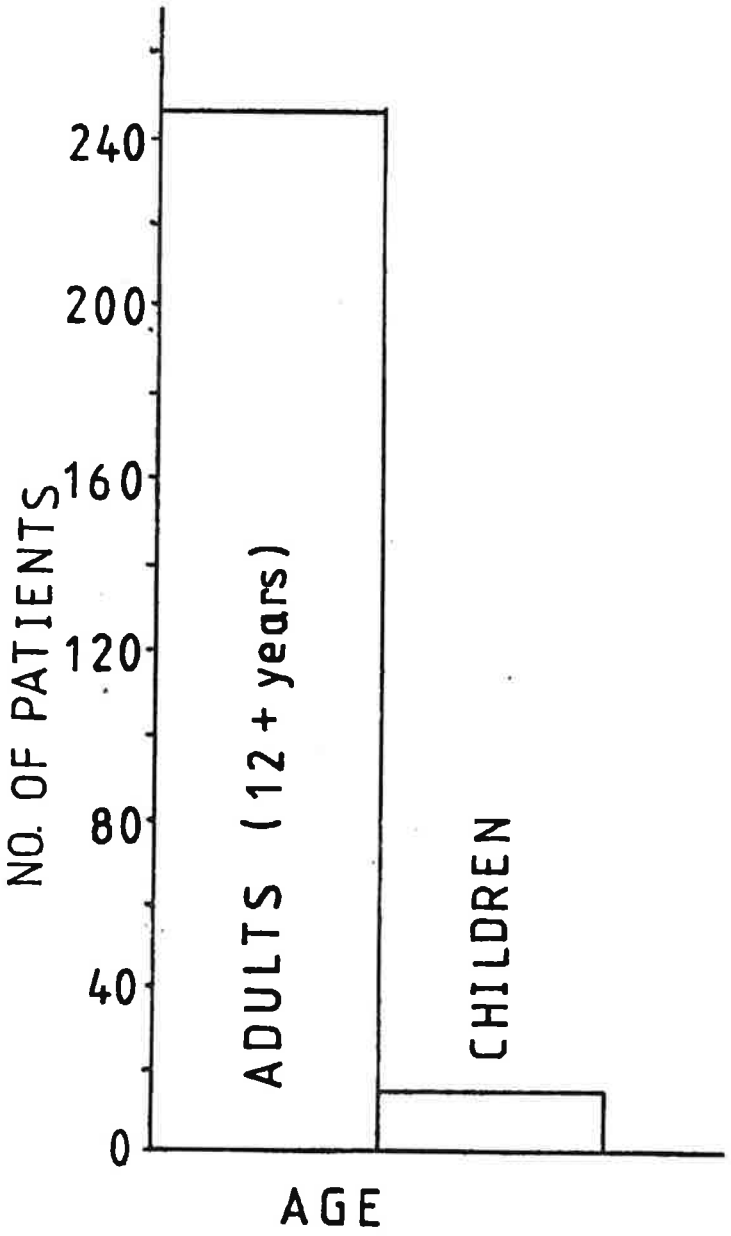
fig.1



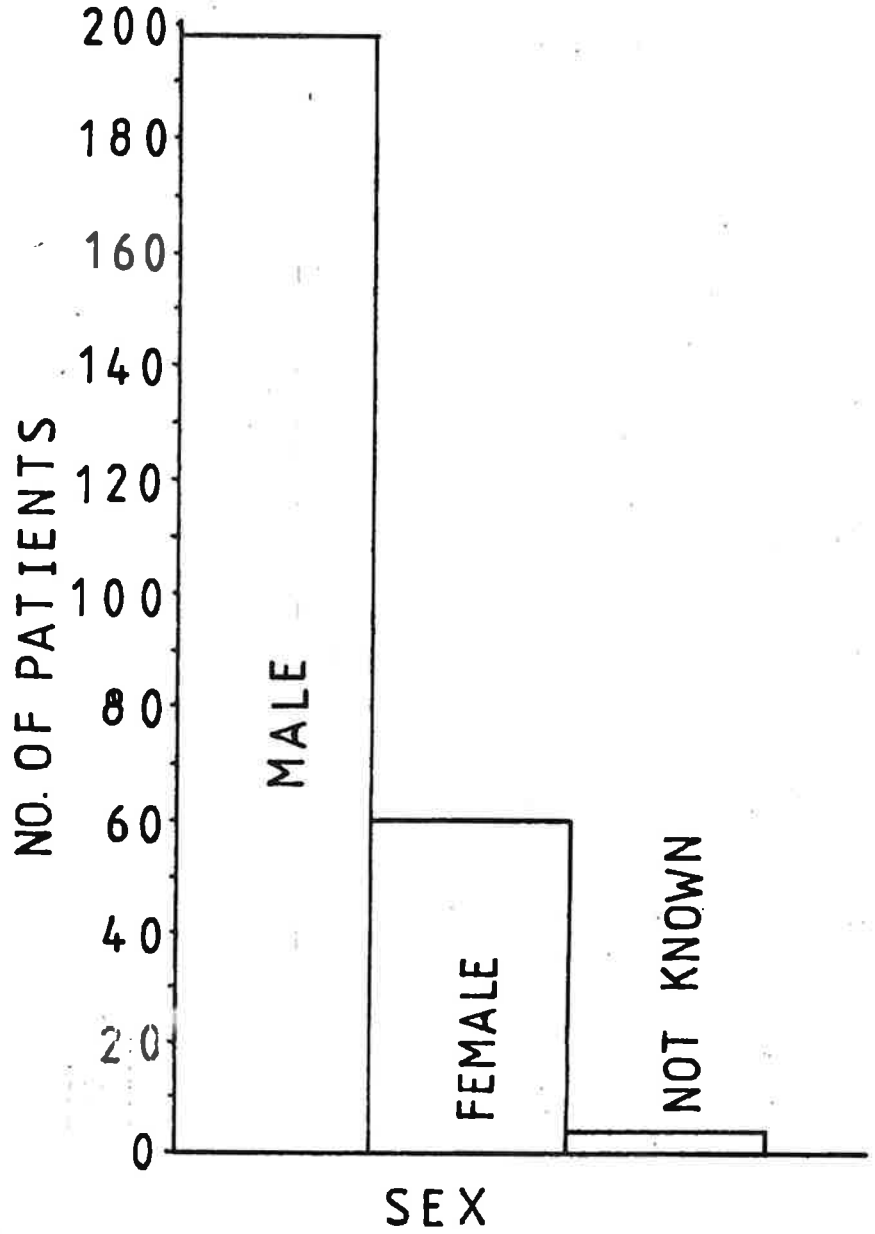
Heylings Dec Exhibit 32

SYNG-PQ-03720030_R

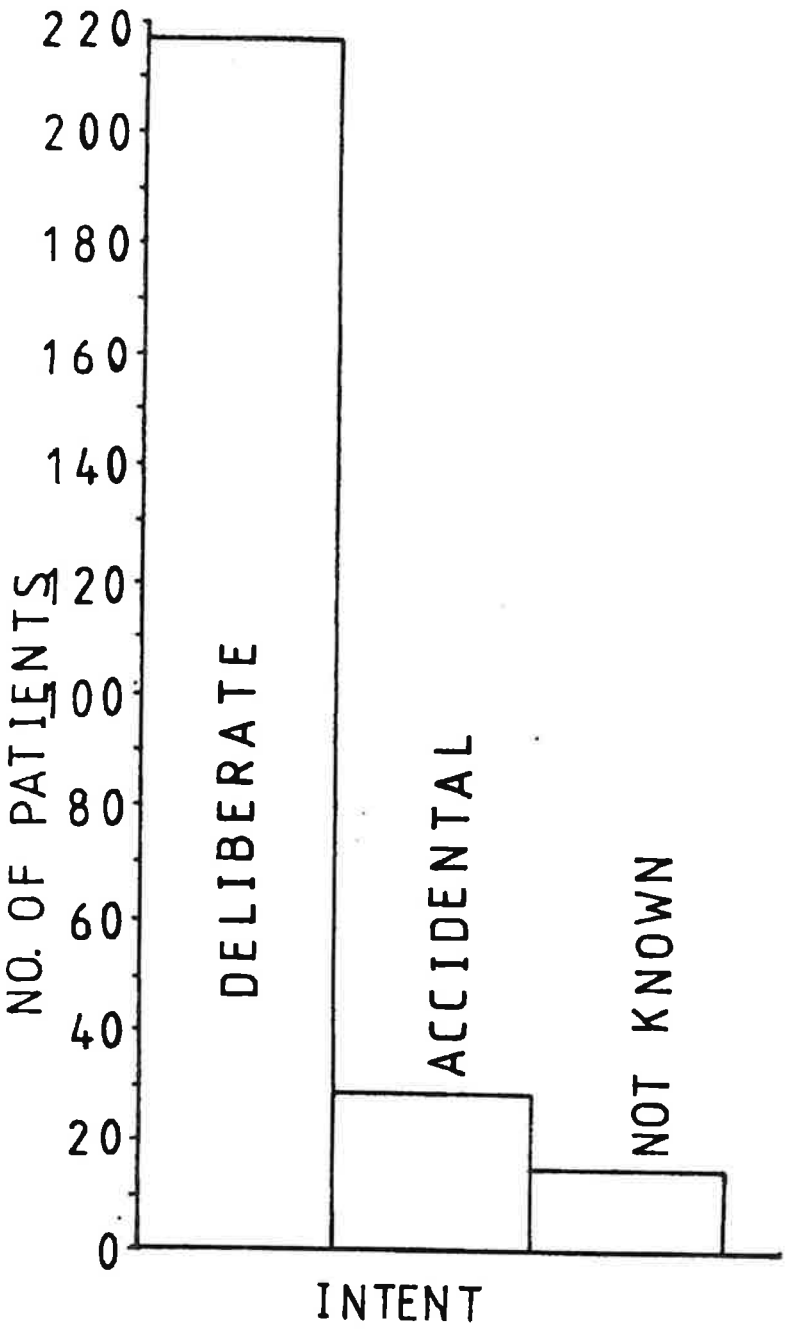
AGE OF PATIENTS fig.16



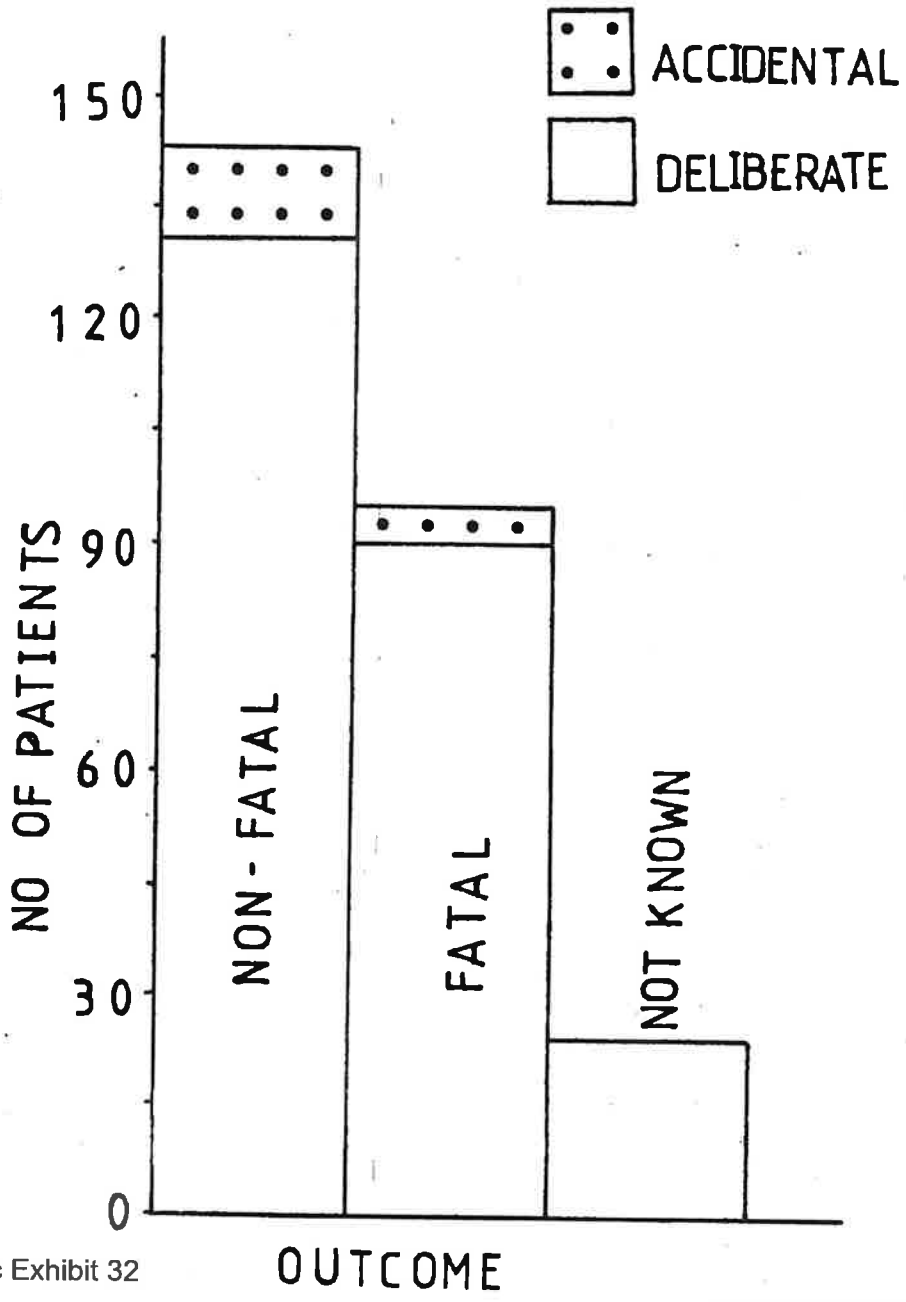
SEX OF PATIENTS fig.17



INTENT fig.18



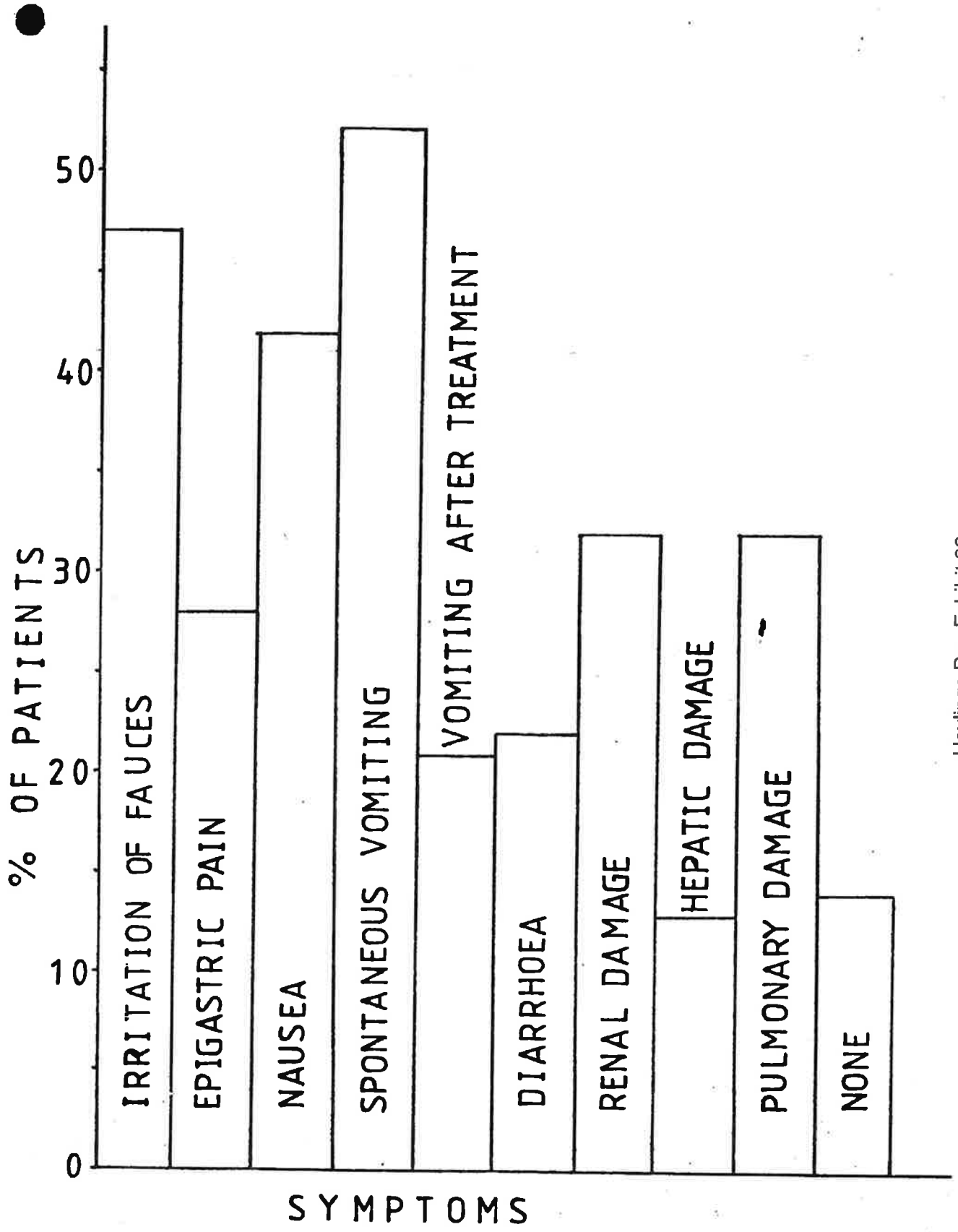
OUTCOME fig.19



Heylings Dec Exhibit 32

SYMPTOMS OF PATIENTS

fig. 20



Heylings Dec Exhibit 32

AMOUNT OF PARAQUAT TAKEN V. MORTALITY

[redacted]EEDOL/PATHCLEAR (Pg. 21.)

Amount (g. p - ion)	Total	Fatal	Non-Fatal	Mortality
< 2	82	12	70	15%
2 - 5	12	1	11	8%
5 - 10	1	1	-	—
10+	1	1	-	-
TOTAL	96	15	81	16%

GRAMOXONE/DEXTRONE (Pg. 22)

Amount (g. paraquat ion)	Total	Fatal	Non-Fatal	Mortality
<.2	5	-	5	-
2 - 5	11	7	4	64%
5 - 10	14	12	2	86%
10 +	28	26	2	93%
TOTAL	58	45	13	78%

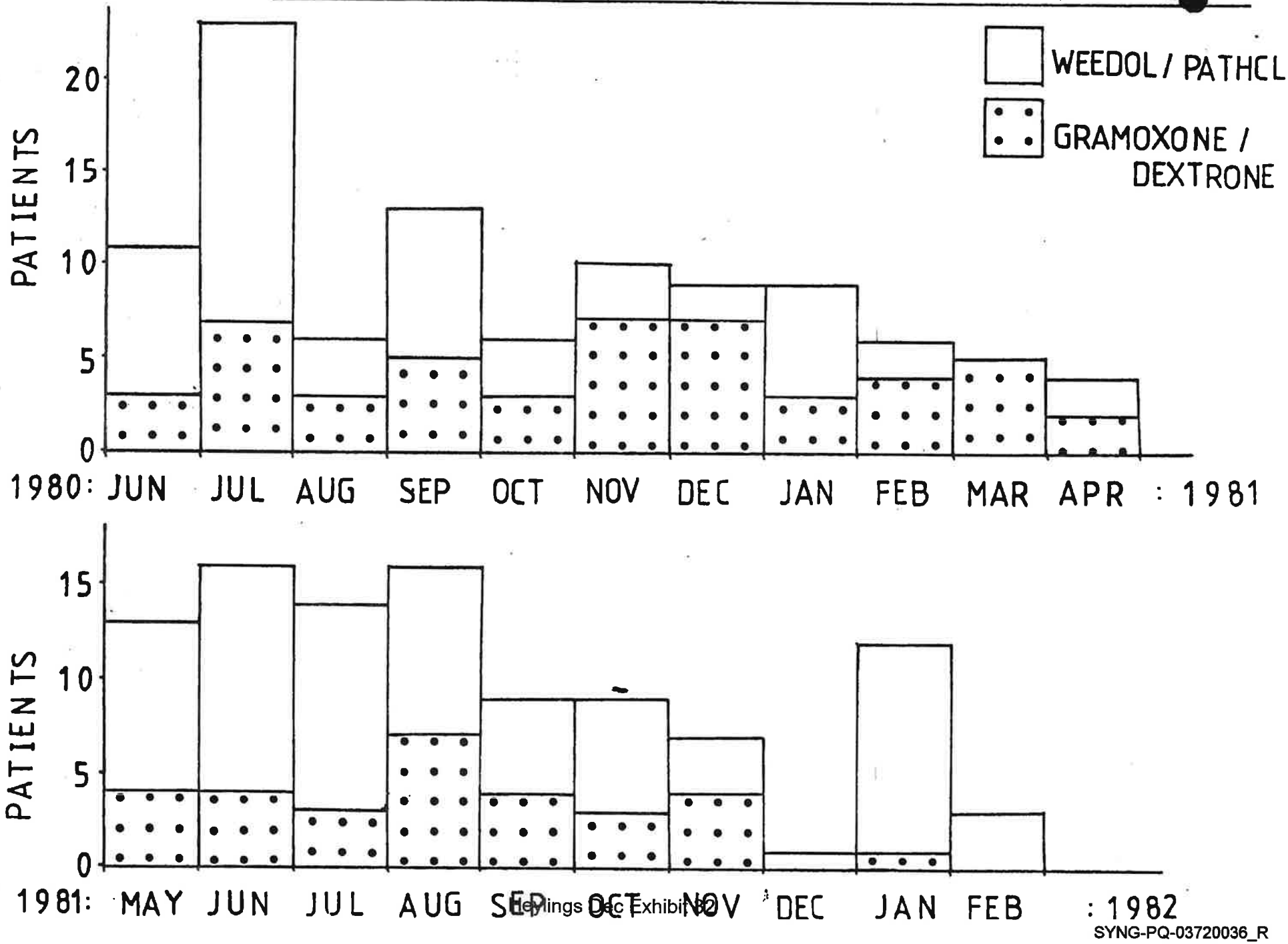
fig. 23

OUTCOME VERSUS INTENT AND AGE

OUTCOME	TOTAL	SUICIDE	ACCIDENTS	ADULTS	CHILDREN
FATAL	82	77	5	82	—
NON-FATAL	119	105	14	109	10
NOT KNOWN	7	7	—	7	—
TOTAL	208	189	19	198	10

fig.24

MONTHLY VARIATION OF PARAQUAT POISONINGS



Heylings Dec Exhibit 32
SYNG-PQ-03720036_R

TIME UNTIL TREATMENT VERSUS MORTALITY

Weedol/Pathclear

Time until treatment (hrs)	Fatal	Non-Fatal	Mortality
0 - 6	6	32	16%
6 - 12	2	13	13%
12 - 24	1	3	25%
24 - 48	3	-	100%

Gramoxone/Dextrone

Time until treatment (hrs)	Fatal	Non-Fatal	Mortality
0 - 6	23	8	74%
6 - 12	4	3	57%
12 - 24	2	2	50%
24 - 48	2	1	67%

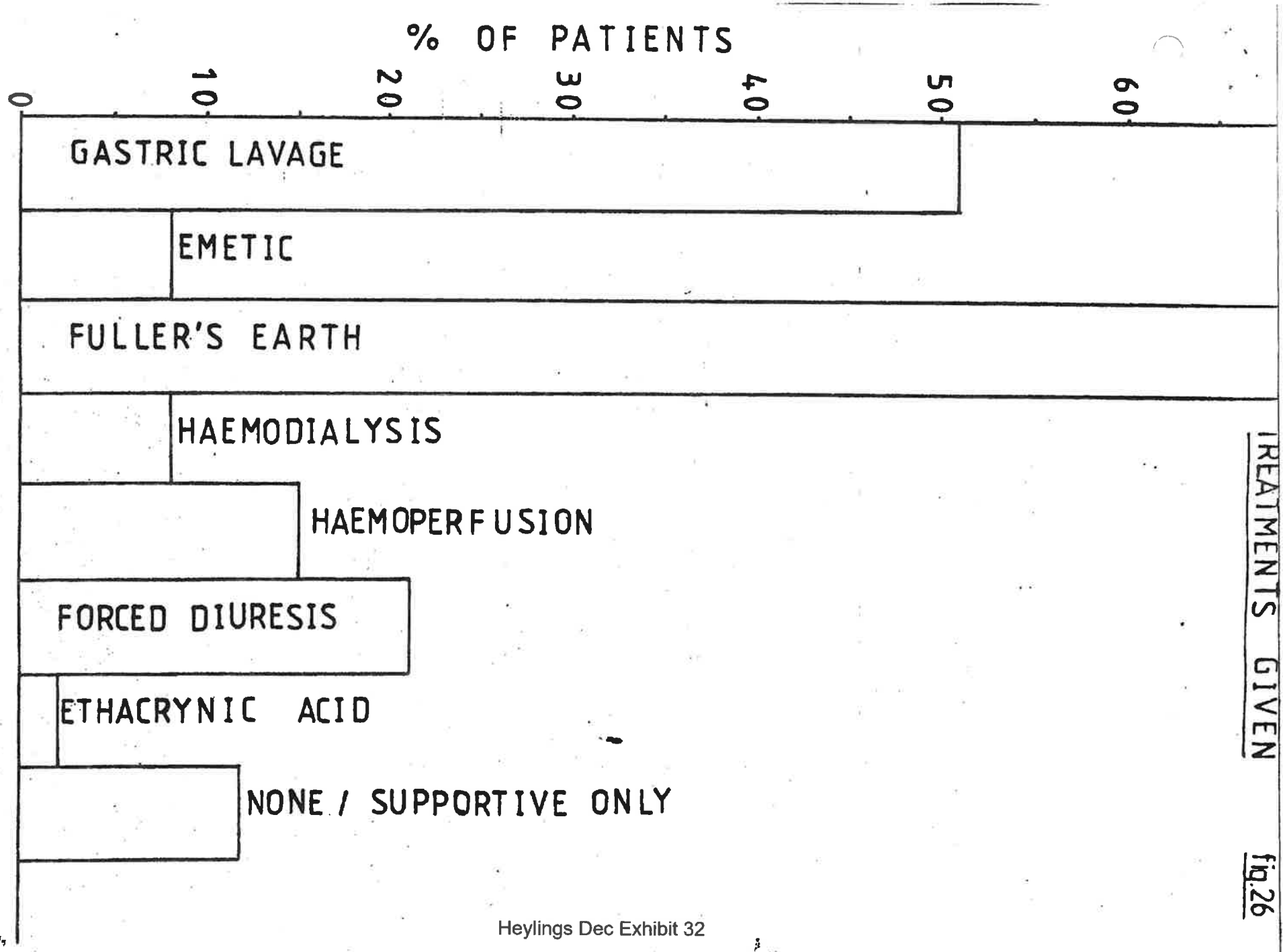


fig.26

SYNG-PQ-03720038_R

Heylings Dec Exhibit 32

HAEMOPERFUSION / MORTALITY (fig 27)

Patients haemoperfused

Amount (g. paraquat ion)	Total	Fatal	Non-Fatal	Mortality
< 2	11	3	8	73%
2 – 5	5	2	3	60%
5 – 10	6	–	6	100%
10 +	6	1	5	83%
TOTAL	28	6	22	79%

Patients not haemoperfused

Amount (g. paraquat ion)	Total	Fatal	Non-Fatal	Mortality
< 2	67	64	3	5%
2 – 5	15	11	4	27%
5 – 10	9	2	7	78%
10 +	19	2(?)	17	89%
TOTAL	110	79	31	28%

EMETIC PRESENT / ABSENT IN PARAQUAT FORMULATIONS TAKEN

fig.28

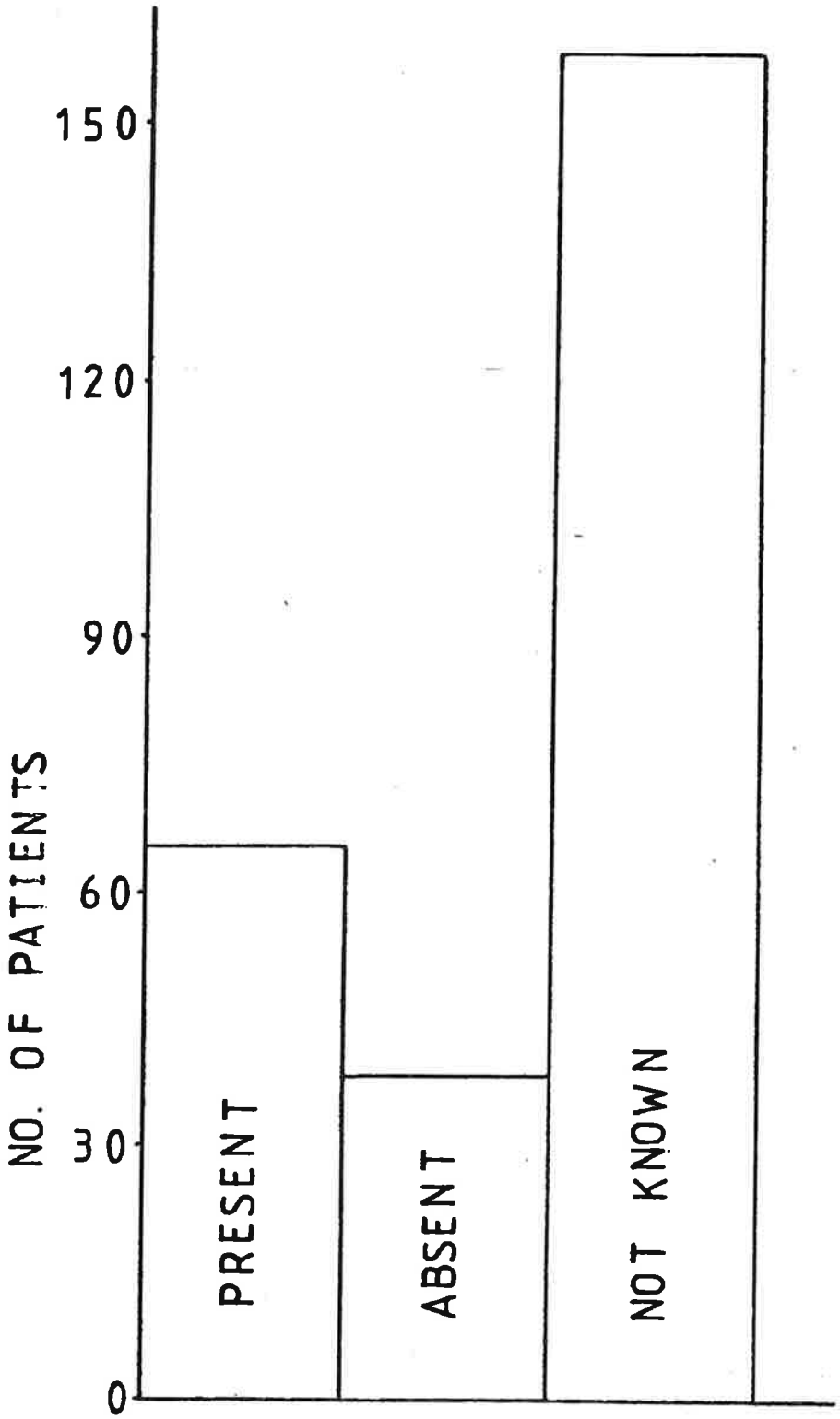


Fig. 29

Emetic - Present or Absent in Formulations

	Total	Weedol	Pathclear	Gramoxone	Dextrone	Dexuron	Gramonol	Not Known
Present +	64	32	12	19	1	-	-	-
Absent -	39	13	2	20	1	1	1	1
TOTAL	103	45	14	39	2	1	1	1

SPONTANEOUS VOMITING AFTER INGESTION OF EMETIC/NON-EMETIC FORMULATIONS OF PARAQUAT

Emetic present

Amount (g. paraquat ion)	Early Vomiting (< ½ hr p.i.)*	Late Vomiting	No Vomiting
< 2	16 (55%)	8	5
2 – 5	3 (75%)	1	–
5 – 10	1 (100%)	–	–
10 +	6 (100%)	–	–
TOTAL	26 (65%)	9	5

* p.i. = post ingestion

Emetic absent

Amount (g. paraquat ion)	Early Vomiting (< ½hr p.i.)*	Late Vomiting	No Vomiting
< 2	1 (10%)	2	7
2 – 5	1 (25%)	1	2
5 – 10	–	1	1
10 +	2 (40%)	–	3
TOTAL	4 (19%)	4	13

EXHIBIT 33
TO DECLARATION OF JON R. HEYLINGS

CHEV-SJ0022488	CHEV-SJ0081808	CHEV-SJ0109152 at 9154
CUSA-00044451	CUSA-00046646 at 6656-6657	CUSA-00075153
CUSA-00087955 at 8056-8057	CUSA-00087955 at 8090	CUSA-00087955 at 8096
CUSA-00087955 at 8114	CUSA-00087955 at 8119-8122	CUSA-00087955 at 8157
CUSA-00087955 at 8219-8220	CUSA-00088288 at 8290-8291	CUSA-00088288 at 8398
CUSA-00088288 at 8432	CUSA-00088288 at 8433	CUSA-00088288 at 8442-8451
CUSA-00088288 at 8470-8475	CUSA-00088288 at 8523	CUSA-00089087 at 9142
CUSA-00090216 at 0489-0490	CUSA-00090216 at 0538-0539	CUSA-00102373
CUSA-00108296	CUSA-00153678	CUSA-00186125 at 6583-6584
CUSA-00200666 at 0890-0891	CUSA-00232857	CUSA-00256176 at 6363-6364
CUSA-00265212	CUSA-00289880	CUSA-00289999
CUSA-00290556	CUSA-00292309	CUSA-00292312
CUSA-00292312 at 2314	CUSA-00292312 at 2315	CUSA-00292464
CUSA-00305732	CUSA-00305753	CUSA-00305755 at 5755-5762
CUSA-00305755 at 5765-5766	CUSA-00319174	CUSA-00324553
CUSA-00340569	CUSA-00384203	CUSA-00419109
CUSA-00420099	CUSA-00430884	SYNG-PQ-00059882
SYNG-PQ-00069432	SYNG-PQ-00524793	SYNG-PQ-00527245
SYNG-PQ-01765631	SYNG-PQ-01796364	SYNG-PQ-01829185
SYNG-PQ-01832461	SYNG-PQ-01843764	SYNG-PQ-01857812_R
SYNG-PQ-01858013_R	SYNG-PQ-02147610	SYNG-PQ-02432265_R
SYNG-PQ-02449462_R	SYNG-PQ-02450023_R	SYNG-PQ-02450030_R
SYNG-PQ-02450046_R	SYNG-PQ-02450068_R	SYNG-PQ-02450073_R
SYNG-PQ-02450103_R	SYNG-PQ-02450112_R	SYNG-PQ-02450184_R
SYNG-PQ-02450185	SYNG-PQ-02450186_R	SYNG-PQ-02450187_R
SYNG-PQ-02450188_R	SYNG-PQ-02450670_R	SYNG-PQ-02450673_R
SYNG-PQ-02450688_R	SYNG-PQ-02450689_R	SYNG-PQ-02450714_R
SYNG-PQ-02450720	SYNG-PQ-02450812_R	SYNG-PQ-02450823_R
SYNG-PQ-02450914_R	SYNG-PQ-02450931_R	SYNG-PQ-02450949_R
SYNG-PQ-02450951_R	SYNG-PQ-02450970_R	SYNG-PQ-02451010_R
SYNG-PQ-02451028_R	SYNG-PQ-02451086	SYNG-PQ-02451088_R
SYNG-PQ-02451102_R	SYNG-PQ-02451229_R	SYNG-PQ-02451257_R
SYNG-PQ-02451291_R	SYNG-PQ-02451399_R	SYNG-PQ-02451859_R
SYNG-PQ-02453690_R	SYNG-PQ-02469717	SYNG-PQ-02470031
SYNG-PQ-02470057	SYNG-PQ-02484950	SYNG-PQ-02491713_R
SYNG-PQ-02493940_R	SYNG-PQ-02494068_R	SYNG-PQ-02494081_R
SYNG-PQ-02494203	SYNG-PQ-02494291	SYNG-PQ-02506363
SYNG-PQ-02506882	SYNG-PQ-02507029_R	SYNG-PQ-02507056_R
SYNG-PQ-02508147_R	SYNG-PQ-02510856_R	SYNG-PQ-02510873_R
SYNG-PQ-02514408_R	SYNG-PQ-02514781	SYNG-PQ-02515147_R
SYNG-PQ-02515504	SYNG-PQ-02515536	SYNG-PQ-02515610_R

EXHIBIT 33
TO DECLARATION OF JON R. HEYLINGS

SYNG-PQ-02517085_R	SYNG-PQ-02518325_R	SYNG-PQ-02519034_R
SYNG-PQ-03705768	SYNG-PQ-03714546 at 4671-4689	SYNG-PQ-03719623_R
SYNG-PQ-03719624_R	SYNG-PQ-03719627	SYNG-PQ-03719628_R
SYNG-PQ-03719793_R	SYNG-PQ-03719794_R	SYNG-PQ-03719805
SYNG-PQ-03719807_R	SYNG-PQ-03719840_R	SYNG-PQ-03719841_R
SYNG-PQ-03719844_R	SYNG-PQ-03719845_R	SYNG-PQ-03719846_R
SYNG-PQ-03719847_R	SYNG-PQ-03719852_R	SYNG-PQ-03719874_R
SYNG-PQ-03719877_R	SYNG-PQ-03719883_R	SYNG-PQ-03719905_R
SYNG-PQ-03719953_R	SYNG-PQ-03719995_R	SYNG-PQ-03720006_R
SYNG-PQ-04087247	SYNG-PQ-04262278_R at 2370-2379	SYNG-PQ-04262278_R at 2668-2694
SYNG-PQ-04262278_R at 400-412	SYNG-PQ-04263349_R	SYNG-PQ-04267616_R
SYNG-PQ-04267671_R	SYNG-PQ-06550433	SYNG-PQ-13098668_R
SYNG-PQ-13098673_R	SYNG-PQ-13098675_R	SYNG-PQ-13113722_R
SYNG-PQ-13113942	SYNG-PQ-13113967	SYNG-PQ-13113976
SYNG-PQ-13113977	SYNG-PQ-13114571_R	SYNG-PQ-13119851
SYNG-PQ-14420786_R	SYNG-PQ-23666466_R	SYNG-PQ-30807695
SYNG-PQ-30827790	SYNG-PQ-30835261	SYNG-PQ-30880010
SYNG-PQ-33957765	SYNG-PQ-33960000	SYNG-PQT-ATR-13276729
SYNG-PQT-ATR-14192407		