BACKGROUND

Paraquat (1,1'- dimethyl 4,4'- bipyridilium) is a non-selective contact herbicide and is the active ingredient in the important Zeneca Agrochemicals product, GRAMOXONE.

Paraquat has been involved in a large number of human poisonings since its introduction more than 25 years ago. The vast majority of cases have involved purposeful ingestion and have occurred despite many different preventative measures. These have included changes in concentration, packaging and addition of alerting agents to the various paraquat products. Furthermore, the research into antidotes to paraquat has yet to yield a successful treatment.

The objective of a paraquat research programme at CTL, set up in 1987, was to identify the cellular process for paraquat absorption from the gastrointestinal (GI) tract and ultimately to identify agents which have specific pharmacological effects to reduce the absorption process.

This research programme involved the development of specific *in vitro* and *in vivo* methods to characterise the site and mechanism for paraquat absorption in experimental animals.

METHODS

All our studies have compared oral dosing of formulations based on the 200 g/l paraquat concentrate GRAMOXONE. These studies involved male rats for the optimisation of GRAMOXONE to reduce paraquat absorption. Male dogs were used for the estimation of a safety factor in a species with very similar GI tract physiology to man.

All studies were conducted under a Home Office project licence and were designed to minimise animal usage. In all dog studies toxicity was assessed by extrapolation of blood paraquat levels at sub lethal dose levels. Thus, the total amount of paraquat present in the blood over the first 24 hours after a single oral dose was calculated as the area-under-curve (A.U.C.). Specific formulations were given in incremental doses in the same animals until the AUC value predicted that systemic paraquat toxicity would be likely to occur at the next dose level.

A comparison of the tolerated paraquat dose for a new formulation in mg paraquat ion/kg was made with GRAMOXONE controls which gave equivalent AUC values at 10 mg/kg. This ratio is the safety factor and was achieved without signs of paraquat toxicity in the dog experiments.

IDENTIFICATION OF SAFENING COMPONENTS. 3.

MAGNESIUM ION

The search for a "safer formulation" affording at least a five-fold reduction in oral toxicity was commissioned using the idea of encapsulating the paraquat within a multiple emulsion which on dilution for spraying would release paraquat. In order to balance the osmotic pressure of the formulation, various chloride salts were added to the external water phase of the emulsion.

In toxicological testing both emulsion and aqueous paraquat formulations containing magnesium salts had low toxicity. Furthermore, calcium ions enhanced paraquat toxicity and a saturable calcium-dependent uptake process for paraquat which could be inhibited by magnesium was characterised *in vitro* and *in vivo*.

MAGNESIUM SULPHATE

Paraquat is absorbed by an energy-dependent process and selectively by the small intestine *in vitro* (1) and *in vivo* (2). In order to minimize the uptake and residence time of paraquat in this region of the GI tract, $MgSO_4$ was chosen as the magnesium additive to standard GRAMOXONE. In addition to blocking paraquat uptake, this salt, when present at levels that result in a higher osmotic pressure

than the blood, causes fluid secretion and purgation of the small intestine, rapidly clearing the paraquat from the gut.

MAGNESIUM TRISILICATE

Additional magnesium can be accommodated in a paraquat concentrate by suspending an insoluble magnesium salt. The choice of magnesium trisilicate (MgTS) as such a salt was based on several facts:

- This compound is a pharmaceutical antacid which reduces irritancy to the stomach lining.
- MgTS reacts with gastric HCl to release more soluble magnesium.
- The negatively charged trisilicate binds paraquat cation.
- The reaction of MgTS with gastric acid produces silicon dioxide gel.

The silicon dioxide gel adheres to the stomach lining providing a physical barrier of high viscosity. The MgTS therefore slows gastric emptying as well as binding the

paraquat electrostatically.

By using KELZAN as the MgTS suspending agent there is a further increase in viscosity with reduction in pH. By combining gels with $MgSO_4$, the paraquat either remains in the stomach or is cleared by purgation. Both actions keep the chemical away from its site of absorption in the small intestine.

EMETIC

Gramoxone contains the emetic agent PP796 at 0.5 g/l. Our studies in the dog suggest that a higher concentration of 1.5 g/l produces much more effective emesis and removes substantially more paraquat from the stomach. More importantly, the emesis occurs before the paraquat reaches the small intestine.

When higher emetic levels were combined with the purgative $MgSO_4$ and the gelling agent MgTS a synergistic safening effect was observed. Thus, we could not only demonstrate a reduction in absorption of paraquat but by removing the unabsorbed and trapped chemical more rapidly by emesis, the emetic agent now becomes an effective built in antidote.

PATENTS

Due to the importance of the different Magnesium salts as safening additives to GRAMOXONE and the experimental evidence that Mg ions reduce paraquat absorption and toxicity, CTL has applied for patents (3) covering Agrochemicals bipyridyl products. The patents cover a wide range of the various magnesium additives. The authors have named this formulation unofficially as 'MAGNOXONE'.

OPTIMISATION OF MAGNOXONE

Much optimisation of the individual levels of $MgSO_4$, MgTS and PP796 was carried out at CTL during 1991 and 1992. At this stage formulation experts at Yalding made a small scale version of 'MAGNOXONE' to the CTL specification (YF8004). This met the original criteria of storage stability, dilution and sprayability, and importantly >95% herbicidal efficacy in glasshouse and field trials when compared with GRAMOXONE.

'MAGNOXONE' YF8004 was found to be at least 20X less toxic to dogs compared to GRAMOXONE (4). It is estimated from existing human poisoning data that this will translate to an equivalent safety factor in man. Therefore, the current median lethal volume of GRAMOXONE to an adult human which is 15 ml becomes at least 300 ml with 'MAGNOXONE'.

Based on human poisoning surveys where the approximate volume of ingested product is known, a 300 ml volume would include more than 95% of attempted suicides as potential survivors. This is of course based on the fact that 'MAGNOXONE' will have the same effect in man as the dog. There is much data supporting this case since the concentrations of $MgSO_4$, MgTS and PP796 in

'MAGNOXONE' have proven pharmacological effects in man at the doses which would be achieved in an attempted paraquat poisoning.

SUMMARY

A new 200 g/l paraquat concentrate 'MAGNOXONE' has been developed at CTL. Animal studies have shown that a small scale version of this formulation, 'MAGNOXONE' YF8004, is at least 20X less toxic by volume compared to GRAMOXONE when given orally. The development of this formulation as a new paraquat product is dependent on a number of criteria which include successful formulation scale-up and the ultimate demonstration that toxicological data generated in experimental animals will translate to man.

REFERENCES

1. Heylings J.R. (1991). Toxicol. Appl. Pharmacol. 107, 482-493.

2. Heylings J.R. and Farnworth M.J. Toxicol. Appl. Pharmacol. (submitted).

3. Heylings J.R. (1990). UK Patent Application No. 9015134.1.

4. Heylings J.R. and Farnworth M.J. (1993). CTL report CTL/R/1115.