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Identification of an alginate-based formulation of paraquat to reduce the exposure of the herbicide following oral ingestion

J.R. Heylings *, M.J. Farnworth, C.M. Swain, M.J. Clapp, B.M. Elliott

Syngenta Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ, United Kingdom

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Abstract

The herbicide paraquat has been widely used throughout the world for almost 50 years and is important in sustainable agriculture. When used correctly the chemical poses no known risk to human health. However, it is acutely toxic, and can be fatal, if the concentrated product is ingested orally. Despite many years of research there is no successful treatment for paraquat intoxication. In recent years we have turned our attention to understanding how we can make the product safer, if it is accidentally or intentionally consumed. We present in this paper a novel approach aimed at safening the paraquat product, Gramoxone. Following our previous research on the site and mechanism of paraquat absorption from the gastrointestinal tract we have identified a new formulation of paraquat, Gramoxone INTEON® that reduces the absorption of paraquat into the blood. This new formulation contains the polysaccharide, alginate, a natural product extracted from sea-weed. We have designed a preparation of paraquat and alginate with surfactants that is herbicidally active but has the unique property that it gels on contact with gastric acid in the stomach. The resulting mixture slows the dispersion and delivery of the toxic chemical to its site of absorption in the small intestine. Alginates also protect the mucosa against the damaging influence of topical gastric irritants, like paraquat. Our studies have shown that increasing the loading of alginate between 7 and 17 g/L causes a dose-related reduction in paraquat absorption *in vitro* in isolated rat ileum. This is also observed *in vivo*, as measured by paraquat plasma kinetics in the rabbit where the Area Under Curve (AUC 0–24 h) was reduced from 33.8 3 for Gramoxone to 12.5 6 (μg/mL) h for a formulation containing 17 g/L alginate. Such a reduction in systemic exposure to paraquat is expected to reduce the acute oral toxicity of the formulation. This should be particularly effective in a vomiting species such as man since we have shown in this investigation that alginates not only reduce the peak plasma paraquat values but also delay the time to peak levels. This provides the opportunity for a more effective emetic response since the highly viscous gelled material should remain in the stomach for longer than the liquid Gramoxone. Further research is required to understand and optimise the safening and herbicidal characteristics of these alginate acid-triggered gel formulations of paraquat. However, we anticipate that this alginate technology in Gramoxone INTEON® could have significant benefit in reducing human mortalities associated with the herbicide.

Keywords: Paraquat; *In vitro*; Isolated mucosa; Gastrointestinal tract; Absorption; Triggered gel; Alginate; Formulation; Gramoxone INTEON®

1. Introduction

Paraquat (1,1′-dimethyl-4,4′-bipyridylium) is widely used as a contact herbicide that offers unique benefits to millions of farmers throughout the world. It is fast-acting, offers excellent rainfastness, rapid deactivation

Abbreviations: CTL, Central Toxicology Laboratory

* Corresponding author. Tel.: +44 1625 514550; fax: +44 1625 590249.
E-mail address: jon.heylings@syngenta.com (J.R. Heylings).

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on contact with soil, no leaching and facilitates "no-till" farming. Detailed health surveys of occupationally exposed populations have confirmed the excellent safety record of paraquat in normal use (Hart, 1987; Senanayake et al., 1993). However, following the introduction of paraquat more than 40 years ago, there have been many documented cases of fatal human poisoning. Paraquat toxicity has been reviewed in a number of comprehensive publications (Human Toxicology, 1987; Bismuth and Hall, 1995; Lock and Wilks, 2001). Accidental paraquat poisonings are infrequent nowadays, particularly following the addition of alerting agents to the formulation, e.g. colour, stench and emetic as well as alteration in packaging. Suicidal ingestion of paraquat remains a problem in some countries and cases have been described extensively in the literature (Proudfoot et al., 1979; Vale et al., 1987; Houze et al., 1990; Bismuth et al., 1991).

Paraquat intoxication in animals and man results in renal failure and lung damage (Clark et al., 1966). The herbicide is rapidly but incompletely absorbed from the gastrointestinal tract (Smith et al., 1974). Paraquat is not metabolised to any significant extent in animals and man and is normally excreted as the parent compound. A reduced ability to excrete absorbed paraquat in urine due to nephrotoxicity (Haley, 1979; Lock, 1979; Proudfoot et al., 1979), coupled with lung accumulation often leads to lung fibrosis and, subsequently, death (Vale et al., 1987). The toxicokinetics of paraquat and the mechanism of toxicity have been the subjects of much research. Knowledge gained has provided clinicians with various treatment regimes following human poisoning. However, treatment has proved difficult and currently methods are aimed primarily at reducing the gastrointestinal tract absorption of the herbicide with oral adsorbents, coupled with osmotic purgatives to decontaminate the intestine, thereby preventing further absorption, or other methods aimed at removal of the herbicide from the blood, e.g. haemodialysis (Clark, 1971; Smith et al., 1974; Meredith and Vale, 1987). There is at present no specific "antidote" to paraquat poisoning which is safe to use clinically.

Research in our laboratory using *in vitro* rat isolated gastrointestinal mucosa has identified that paraquat enters the systemic compartment in part via an energy-dependent absorption process which is located on the brush border of epithelial cells lining the small intestine. This "active" uptake of paraquat predominates in the jejunum and obeys saturation kinetics (Heylings, 1991). It seems plausible that there is commonality between the paraquat uptake processes in different epithelial cell types, especially since it is known that paraquat accumulation in the epithelial cells of the lung is via a polyamine uptake process (Rose et al., 1974; Smith, 1982).

We have therefore utilised the isolated mucosa from the rat small intestine as an *in vitro* model to investigate the gastrointestinal absorption of paraquat, with a view to inhibiting this process *in vivo*. Recognising that the most effective way to reduce uptake of the bipyridyl into the blood is via inhibition of gastrointestinal uptake, we have focused on the identification of additives to the commercial formulation, Gramoxone, that are likely to interfere either directly with the absorption process or indirectly via effects on gastrointestinal physiology. Our previous work had identified that paraquat caused mucosal damage and increased passive flux across the mucosal barrier at high concentrations (Heylings, 1991). To reduce this effect it seemed appropriate to investigate ulceroprotective agents that could potentially be added to the herbicide product. The polysaccharide chemistry of alginate salts and alginic acid appeared attractive since alginates are not only effective at protecting the mucosa against topical damage in conditions such as gastric acid reflux, but they are also widely used in controlled drug delivery and release due to their gelling properties at different intraluminal acidities. Another key element of this research is the knowledge that peak blood levels following paraquat poisoning occur within about 1 h as the chemical rapidly reaches its absorptive site in the small intestine. Extending the retention time of the formulation in the stomach by exploiting the gelling and hence bulking properties of alginates at low pH would allow more time for treatment intervention and ultimate detoxification man. The extensive use, availability and excellent safety profile of alginates made the incorporation of these acid-triggered gelling agents into the commercial paraquat product, Gramoxone, an objective worth pursuing.

Due to the complexity of the interaction of alginates with the surface active adjuvants within the herbicide product the development of a "safer" formulation of paraquat naturally required a number of systematic investigations using an *in vitro* model as the primary screening tool. However, in order to validate our observations on paraquat uptake in the *in vitro* intestinal model, we have also examined the plasma paraquat kinetics *in vivo* in the rabbit, following a single oral dose of paraquat in alginate-based formulations. The overall aim was to build a new formulation of paraquat that could significantly reduce paraquat systemic exposure during the first few hours following oral ingestion but would retain all the key agricultural attributes of this important product.

2. Materials and methods

2.1. Formulations and reagents

Paraquat formulations were prepared and supplied by Syngenta Ltd., Yalding, Kent, UK. Each formulation was based on the commercial product Gramoxone and contained 200 g/L paraquat ion (w/v), surfactants, blue/green dye, pyridine bases, emetic agent (PP796) and other minor components. Alginate was supplied by ISP Alginates UK Ltd., and dissolved in the 200 g/L Gramoxone formulation at 7–17 g/L. *N*-methyl-2-pyrrolidone (NMP) and magnesium trisilicate were supplied by Sigma–Aldrich, UK.

Methyl [^{14}C]-labelled paraquat of specific activity of 110.8 mCi/mmol and a chemical purity of >98% was supplied by Amersham Pharmacia Biotech, Buckinghamshire, UK.

2.2. In vitro studies

Details of similar experimental models that utilise isolated gastrointestinal mucosa have been published previously (Heylings et al., 1984; Heylings, 1991). However, we have made some significant changes to the previous experimental model to incorporate a separate gastric incubation chamber and a re-cycling pump system to deliver the gastric digestate to the luminal side of six separate segments of small intestine. This gastrointestinal loop test (GILT), the primary screening tool for this investigation, has not been described previously.

Alderley Park strain male rats (Ap:Ak$_f$SD) were used for the *in vitro* studies. Animals were fasted overnight and humanely terminated by an overdose of halothane anaesthetic (Fluothane). Several 5 cm segments of ileum from adult male rats were removed immediately following termination. Prior to dissection any food debris was carefully extruded from the gut segments. Isolated mucosal tubes were prepared in an oxygenated physiological McIlvain's solution by blunt dissection and stripping of the outer mesentery and muscularis externae from each tube of intestine. The modified McIlvain's physiological solution used to prepare and bathe the isolated mucosa contained a number of electrolytes including Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, SO_4^{2-}, HPO_4^{2-}, glucose and mannitol, buffered at pH 7.2.

Each end of the mucosal segment (2 cm in length) was attached to external tubing via grommets using ligatures and checked for gross damage by syringing warmed saline through the segment. Six separate mucosal tubes were normally prepared from two to three rats per day. Each one was placed inside a water-jacketed outer glass vessel containing 50 mL of McIlvain's buffer at pH 7.2, gassed with 95% O_2 plus 5% CO_2 and maintained at 37 °C. This allowed the luminal side and blood side of the tissue to be bathed with separate solutions and to measure the paraquat flux across the isolated mucosa from lumen to blood side. Tissues can be maintained in a viable state, as qualified by measurement of electrogenic chloride transport, for up to 6 h under these bathing conditions, providing that they are continuously supplied with 95% O_2 plus 5% CO_2 and maintained at 37 °C (Heylings, 1991).

To study the absorption of paraquat across the gastrointestinal mucosa the formulation was spiked with radiolabelled paraquat, which was directly incorporated into the formulations of paraquat. Each formulation contained all the adjuvants and additional components present in the commercial product, including the alerting/safening chemicals that included a green/blue dye, pyridine bases stench and emetic agent (PP796). The homogeneity of the preparation was checked following thorough mixing to ensure even distribution of the radiolabel in the formulation prior to use.

In order to mimic the possible intragastric conditions that a paraquat product would encounter if ingested orally, 13 mL of each formulation concentrate (containing 200 g paraquat ion/L) was added to 130 mL of simulated gastric juice, based on McIlvain's but buffered at pH 2. The final luminal concentration of paraquat was 20 mg paraquat ion/mL. This represents a potential human intraluminal concentration and was selected as the optimum exposure dose in this test system previously (Heylings, 1991). Several alginate-based formulations were tested alongside the control, Gramoxone, and additional standards designed to increase or decrease paraquat absorption. Each formulation tested *in vitro* was spiked with methyl [^{14}C]-labelled paraquat to give approximately 0.13 μCi/mL, radioactive solution. The specific activity of the formulation was checked at the end of each experiment.

The 1:10 gastric mixture (formulation plus simulated gastric juice) was maintained at pH 2 in a water-jacketed vessel at 37 °C. To ensure good mixing of the formulation with the simulated gastric juice, a magnetic stirrer was placed underneath the gastric chamber mixing vessel and the contents were slowly stirred. This created significant turbulence and acted as a surrogate for gastric motility. The gas pressure and position of the outlet was maintained constant for each experiment. The inlet tubes to the mucosae were placed in the gastric chamber and the "gastric" contents were pumped from this reservoir through the luminal side of the tissues and re-cycled back to the gastric reservoir via outlet tubes. This closed system was circulated for 15 min with the buffer alone prior to each experiment and then for 4 h following addition of formulation. The peristaltic pump speed was set at 3.8 mL/min. This arrangement of re-cycling the luminal perfusate proved to be more useful than a single pass system, although we recognise that the small intestinal lumen would normally be exposed to a higher pH than the gastric mixture *in vivo*, due to bicarbonate secretion by the epithelia and the bulk neutralising effect of pancreatic secretion. The experimental rig for the GILT is shown diagrammatically in Fig. 1.

In order to measure paraquat absorption through the small intestine, 50 μL samples were taken from the blood side of each mucosal tube at 0, 0.25, 0.5, 1, 2, 3 and 4 h following addition of the formulation to the gastric chamber. These samples were analysed for ^{14}C paraquat by scintillation counting with a Packard Canberra 2700 TR scintillation counter. Results were expressed as amount of paraquat absorbed over the 4 h time course as well as rate or percentage of the applied dose absorbed over a specified time period. Mean values S.E.M.

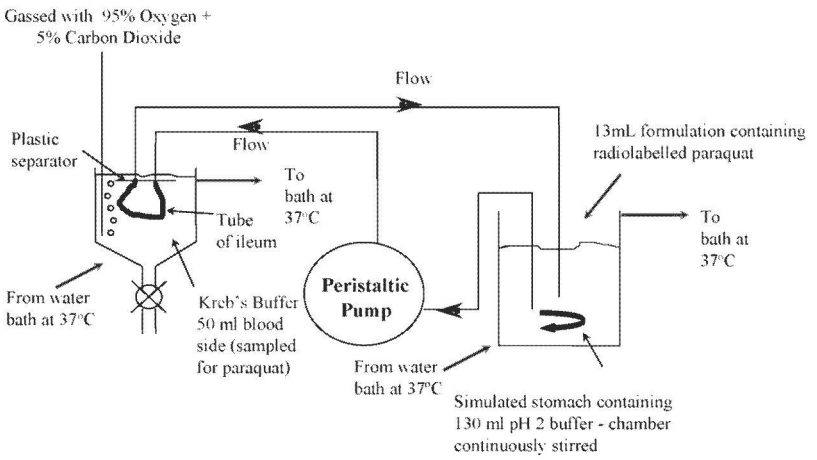

Fig. 1. Diagrammatic representation of the gastrointestinal loop test (GILT). Paraquat formulations were added to simulated gastric juice at pH 2 and 37 °C. The mixture was pumped through isolated rat ileum maintained under physiological conditions by gassing with 95% oxygen plus 5% carbon dioxide, pH 7.2. Paraquat absorption was measured by sampling the blood side of the tissue and analysis by scintillation counting.

are presented for six separate mucosae for each formulation studied. Experiments were designed to ensure that each formulation and contemporary controls were randomised as much as possible between each rig and the animals used for each gut segment.

2.3. *In vivo studies*

To establish the usefulness of the *in vitro* GILT to predict *in vivo* paraquat absorption, we also examined a number of paraquat formulations in the rabbit. Our objective was to measure the paraquat systemic exposure over 24 h following an oral dose. These experiments were designed to look at kinetics and not an acute toxicity end point that may have ensued over the days following dosing. Thus, all animals were humanely terminated at the scheduled time point of 24 h, well ahead of the expected clinical signs of toxicity. The rabbit was chosen as the species to study the toxicokinetics of paraquat since the acute paraquat toxicity is quite well understood in this species (Butler and Kleinerman, 1971; Dikshith et al., 1979) and this larger animal allows serial samples of blood to be conveniently taken from an indwelling cannula. The rabbit develops acute renal failure similar to that observed in human poisoning but lung fibrosis is not a feature of paraquat intoxication in this species (Butler and Kleinerman, 1971). Another important point is the rabbit has no vomit reflex, so the intrinsic action of a formulation and its ability to affect gastrointestinal absorption can be assessed without the complication of emesis.

Adult male New Zealand white rabbits about 2.5 kg bodyweight were obtained from Charles River, UK Ltd. Following an acclimatisation period of at least 10 days, groups of four animals were dosed orally by gavage tube with undiluted formulation at 40 mg paraquat ion/kg. The concentration of each formulation was 200 g paraquat ion/L for the control Gramoxone and all test formulations and standards. The new formulations were based on the commercial product, Gramoxone, and contained the water soluble acid-triggered gel, alginate, at different concentrations. These were compared with the water insoluble gelling agent, magnesium trisilicate. Animals had access to food and water throughout. Blood samples were taken at 0, 0.25, 0.5, 1, 2, 4, 7, 12 and 24 h from a cannulated ear vein. Animals were humanely terminated at 24 h following the final blood sample. Analysis for paraquat in the blood was undertaken by radioimmunoassay, using ^{3}H-labelled paraquat and antibodies raised in rabbits, following preparation of plasma by centrifugation at 1000 × *g* for 15 min. This method has been described before and has been shown to be sensitive for analysing plasma, urine and other biological tissues (ICI, 1979). Paraquat (μg/mL) could be accurately detected over a range of 0.1–10 μg/mL in rabbit plasma by this method.

Mean values S.E.M. were calculated at each time point for the four replicates. Experiments were designed to intersperse the controls, receiving Gramoxone, with the alginate formulations as much as practical. Paraquat systemic exposure from 0 to 24 h was calculated by measuring the plasma area-under-curve (AUC) and is presented as mean S.E.M. for 0–1 h AUC, 0–4 h AUC and 0–24 h AUC, expressed in (μg paraquat/mL) h. The plasma exposure values for each test formulation were compared with control, Gramoxone, using Student's *t*-test, with $P < 0.5$ being considered statistically significant.

3. Results

3.1. *In vitro gastrointestinal mucosa*

Paraquat absorption across isolated rat ileum was linear over the 4 h exposure period and was very reproducible for all formulations tested. We therefore chose the mean 4 h absorption value for comparing formu-

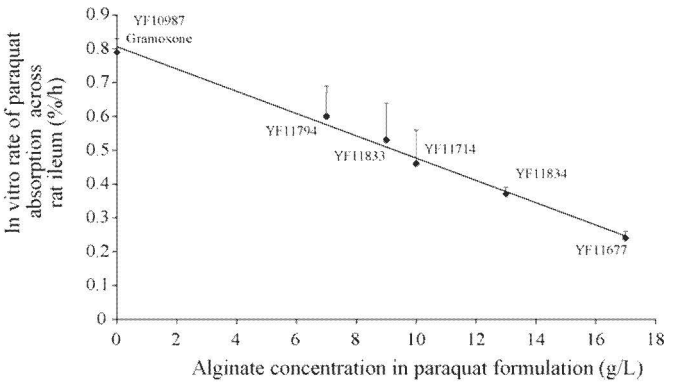

Fig. 2. Absorption of paraquat through rat isolated ileum in the GILT was compared for five different concentrations of alginate in a standard Gramoxone formulation. Addition of increasing concentrations of alginate in Gramoxone caused a dose-related reduction in paraquat absorption through the gastrointestinal mucosa compared with Gramoxone control (YF10987). Mean values S.E.M. are shown, $n = 6$ per formulation.

lations, expressed as % paraquat absorbed per hour. The alginate-containing paraquat formulations all visibly gelled on contact with our simulated gastric juice at pH 2. In contrast, the Gramoxone dispersed immediately with no evidence of gelling in the gastric chamber. The design of the GILT system is such that the resulting "mixture" is pumped on through the small intestine. Thus, the formulation has been exposed to the acidic conditions of the stomach before it comes in contact with its absorptive site in the small intestine. As shown in Fig. 2, paraquat absorption from the alginate-containing formulations was lower than from Gramoxone. In fact, there was a dose-related reduction in paraquat absorption between 7 and 17 g/L alginate. The 17 g/L alginate formulation resulted in 0.24 0.02% of the applied dose reaching the blood side of the isolated ileum per hour. This was significantly ($P < 0.01$) lower than Gramoxone controls where the absorption rate over the same time period was more than three times higher at 0.79 0.04% per hour. This clearly demonstrates the impact of alginate loading on absorption of paraquat in this *in vitro* model. The higher the alginate loading then the less paraquat appears on the blood side of isolated rat ileum. This relationship between alginate concentration and paraquat absorption was very significant with regression analysis giving an r^2 value of 0.99 across the 7–17 g/L range (Fig. 2).

To put these observations into perspective we also examined positive and negative standard formulations to calibrate the *in vitro* test rig. For the positive absorption standard we added the powerful solvent, *N*-methyl-2-pyrrolidone (NMP) to the Gramoxone formulation. This additive acts as a membrane penetration enhancer and aids the transport of compounds across the epithelial cells of the intestinal mucosa. As shown in Table 1, in the GILT rig, the addition of 10 g/L NMP to Gramoxone significantly ($P < 0.01$) increased the absorption of paraquat (dosed as Gramoxone) from 0.79 0.04% to 1.58 0.03% of the applied dose per hour.

In contrast, addition of 100 mg/mL of the ulceroprotective antacid magnesium trisilicate to Gramoxone had the reverse effect and significantly reduced the absorption of paraquat to 0.29 0.03% per hour. The absorption values for the control Gramoxone, these positive and negative standards and different alginate formulations are summarised in Table 1.

Table 1
Absorption of paraquat *in vitro* in the GILT and *in vivo* kinetics in the rabbit

Formulation	Paraquat ion conc. (g/L)	Additive	*In vitro* paraquat absorption: rat ileum (% per h), mean S.E.M., $n = 6$	*In vivo* paraquat absorption: rabbit 0–24 h AUC (μg/mL)h, mean S.E.M., $n = 4$
YF8004	200	Magnesium trisilicate	0.29 0.03	7 6
YF11677	200	Alginate 17 g/L	0.24 0.02	13 6
YF11834	200	Alginate 13 g/L	0.37 0.02	29 3
YF11714	200	Alginate 10 g/L	0.46 0.10	29 4
Gramoxone	200	None	0.79 0.04	34 3
Gramoxone	200	*N*-Methyl-2-pyrrolidone	1.58 0.03	54 5

Various alginate-containing formulations of paraquat and standards have been directly compared in the two models.

3.2. In vivo rabbit kinetics

It was important at this stage of the investigation to understand how predictive these *in vitro* observations were in the context of *in vivo* paraquat absorption and hence paraquat toxicity for a selected number of formulations. There have been several previous studies demonstrating the prognostic value of plasma paraquat and survival following poisoning (Proudfoot et al., 1979; Houze et al., 1990). However, we needed evidence that reduced paraquat uptake in the isolated ileum translated into reduced paraquat absorption in an animal model. We therefore examined a selected range of alginate formulations in the rabbit alongside our Gramoxone control and standards, using plasma paraquat profile as the surrogate marker for expected acute toxicity. Previous studies had estimated the lethal acute oral dose of paraquat in the rabbit at 30–40 mg paraquat ion/kg (Lock and Wilks, 2001). By dosing formulations at 40 mg paraquat ion/kg and intervening (at 24 h) with a scheduled humane termination ahead of toxic signs, we were able to use plasma kinetics to determine the performance of a new formulation instead of clinical signs of toxicity. The Gramoxone control formulation was orally dosed to different groups of rabbits at 40 mg paraquat ion/kg on four separate occasions ($n = 4$, each). This gave a reproducible plasma paraquat profile with peak plasma values between 3.5 and 4.0 μg/mL between 30 min and 1 h following dosing. The 24 h AUC values were 33.4, 33.0, 35.6 and 46.2 (μg/mL) h for the groups of four rabbits. The C_{max} (approx 3.5 μg/mL) and T_{max} values (approx 1 h) were also consistent across separate experiments with the Gramoxone control. This acceptable reproducibility allowed us to examine the performance of new alginate-based formulations at the same oral dose under identical conditions. An additional Gramoxone control group was run alongside the alginate formulations as a contemporary control. This group had a mean 0–24 h AUC of 33.7 3.3 (μg/mL) h.

All formulations contained 200 g/L paraquat and were given orally via gavage at an achieved dose of 40 mg paraquat ion/kg. The more promising alginate mixtures identified *in vitro* that spanned a range that had reduced paraquat absorption (10 and 17 g/L) *in vitro* were examined *in vivo*. These alginate-based formulations of paraquat reduced both the peak plasma and 0–24 h AUC paraquat values when compared with a contemporary Gramoxone control group. As shown in Fig. 3, the intermediate alginate concentration of 10 g/L reduced the peak plasma paraquat from 3.8 0.2 in Gramoxone controls to 2.5 0.2 μg/mL. The AUC (0–24 h) was only marginally reduced from 33.8 3 to

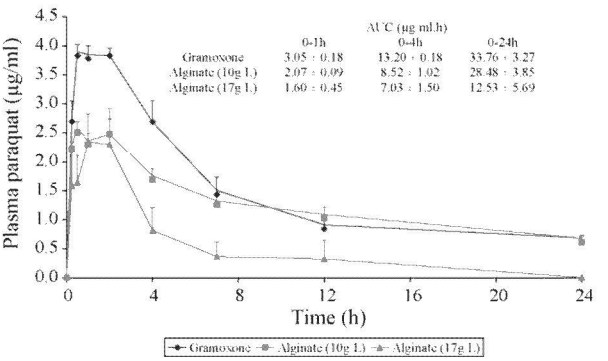

Fig. 3. Plasma paraquat concentrations in the rabbit following a single oral dose of formulation concentrate containing 200 g/L paraquat. Addition of 10 and 17 g/L alginate to Gramoxone reduced the plasma paraquat peak and AUC values observed with Gramoxone control. The highest alginate loading delayed the peak plasma paraquat and reduced the systemic exposure to paraquat at all time points. Mean values S.E.M., $n = 4$ are shown for each group.

28 4 (μg/mL) h. However, the most noticeable impact on systemic exposure was over the first few hours following dosing where the AUC (0–4 h) was reduced from 13.2 0.2 to 8.5 1.0 (μg/mL) h.

The high alginate concentration formulation (17 g/L) gave an even better performance in the rabbit. Although the peak plasma level was only slightly lower than the 10 g/L alginate formulation at 2.3 0.5 μg/mL, it was delayed until 1 h following dosing. The other formulations reached peak plasma values by 30 min. In addition to this delayed peak with the high alginate loading the plasma paraquat levels were much lower than both Gramoxone and the low alginate loading formulation from 2 to 24 h (Fig. 3). The 0–24 h AUC for the 17 g/L alginate formulation was 12.5 6 (μg/mL) h compared to the 33.8 3 (μg/mL) h in Gramoxone controls, indicating that only about one third of the paraquat administered to the animals had been absorbed. Indeed, the systemic exposure of paraquat at the 0–1 and 0–4 h periods was significantly ($P < 0.05$) lower than Gramoxone.

The predictivity of the *in vitro* screen was further established with standards that were prepared with additives designed to either accelerate paraquat absorption or to inhibit it. Gramoxone containing 10 g/L of the membrane penetration enhancer and solvent, *N*-methyl-2-pyrrolidone (NMP) gave some of the highest *in vitro* and *in vivo* absorption values we have recorded in these models. As mentioned earlier, Gramoxone NMP doubled the absorption of Gramoxone *in vitro* in the GILT, over the 4 h exposure period. *In vivo*, the 24 h AUC increased from 34 3 to 54 5 (μg/mL) h *in vivo*. In addition, when Gramoxone contained the ulcero-

protective gelling antacid agent, magnesium trisilicate (100 g/L), this resulted in a very low 0–24 h AUC value of 6.9 5.5 (μg/mL) h *in vivo*, confirming the prediction from the *in vitro* GILT. We have therefore calibrated the responses for alginate formulations to our own internal positive and negative standards in addition to the Gramoxone control. A direct comparison of the *in vitro* GILT and the *in vivo* paraquat systemic exposure in the rabbit for the alginate formulations and our controls and standards is shown in Table 1.

4. Discussion

Our investigations using isolated mucosa from the rat and kinetic data generated in the rabbit have demonstrated that alginates added to the commercial product, Gramoxone, can reduce the gastrointestinal absorption of paraquat. We believe this is a novel finding and could have benefits in human paraquat poisoning, since these soluble polysaccharide polymers can be added directly to the herbicide formulation concentrate. We have also demonstrated that the systemic exposure to paraquat following oral administration of formulated products *in vivo* can be predicted by *in vitro* methods using isolated small intestine from the rat. This gastrointestinal loop test (GILT) therefore provides a screening opportunity to identify and optimise formulations that have low paraquat absorption characteristics with minimal animal testing.

The GILT was developed from our original isolated mucosa models (Heylings et al., 1984; Heylings, 1991) to incorporate the step of acidification in the stomach prior to pumping the mixture through the lumen of the small intestine. This is particularly important since the alginate was selected for its acid-triggered gelling properties with our rationale being that this increase in viscosity on contact with gastric acid would slow the dissolution or dispersion of the formulation in the gastric contents and minimise the contact of paraquat with the surface epithelial cells. These principles have been established before in pharmaceutical preparations used to treat gastric acid reflux and other upper gastrointestinal tract disorders, where alginate salts have been shown to have a range of therapeutic benefits (Brownlee et al., 2005). By slowing the dissolution of the ingested material, and consequently the delivery of the gelled mixture into the absorptive small intestine, it was envisaged that less of the toxic chemical would be absorbed giving more opportunity for the emetic agent in the formulation to work effectively. Although it must be recognised that an effective dose of the emetic must still be absorbed from the formulation. An additional property of alginate-containing pharmaceutical preparations is their ability to coat the gastric mucosal surface. Thus, there is the possibility that alginate present in a paraquat formulation may also enhance the barrier properties of the gastrointestinal mucosa, thereby reducing the opportunity for the topically irritant paraquat to come in contact with and damaging the surface epithelial cells.

Our studies have shown that you can dissolve alginate in the standard commercial 200 g/L Gramoxone product and it does indeed form a viscous gel on contact with simulated gastric fluid at pH 2. We have demonstrated that the more alginate present *in vitro* and *in vivo* then the less paraquat reaches the blood side of the GILT *in vitro* or plasma *in vivo*. The 17 g/L alginate formulation was the most effective in both models, but this concentration makes the formulation more difficult to disperse in water. The 10 g/L alginate formulation concentrate has similar viscosity properties to the current commercial product, Gramoxone, at neutral pH. Of course, following ingestion the viscosity of the formulation concentrate increases substantially in the prevailing acidic environment of the human stomach. The 10 g/L alginate loading is therefore a more viable option for a new product development. It has also been shown to be effective as a herbicide where, of course, it is diluted and sprayed at neutral pH. A key consideration here is the compatibility of the 200 g/L paraquat:alginate mixture and its stability in a commercial product. To be an effective herbicide the product must contain surfactants to aid the spreading and delivery of the polar paraquat through plant cell membranes. Therefore the acid-triggered alginate gel mixture must be compatible with these adjuvants and remain in solution at an acceptable viscosity at neutral pH. Alginates are ideal compounds for this since they have good water solubility and, at the correct loading and selection of an optimum mannuronic:guluronic acid ratio, will only gel at quite an acidic pH and not during manufacture or in their normal field use. Paraquat products containing alginate below a 17 g/L alginate loading are visibly very similar to Gramoxone at neutral pH. They have all the physical characteristics of a typical Gramoxone product in terms of pourability, dissolution in water and spray properties. Importantly, the new alginate-containing product, Gramoxone INTEON®, has been shown to have equivalent herbicidal properties across a wide range of plant species and conditions as standard Gramoxone (unpublished observations).

Other antacid gelling agents that have also been shown to be therapeutically active, such as magnesium silicate and trisilicate are insoluble in water and consequently more difficult to formulate with the Gramoxone

components. Nevertheless, magnesium trisilicate proved to be a very useful positive standard to validate the *in vitro* model of paraquat absorption with the rabbit kinetics. Similarly, additives that are known to damage membranes and have powerful solvent actions, such as NMP, were shown in our studies to enhance paraquat absorption *in vitro* and *in vivo*, presumably by acting as a penetration enhancer of the polar paraquat across the mucosa.

An important aspect of the paraquat systemic exposure (in most cases of poisoning) is the plasma profile of the chemical over a 24 h period following ingestion. Paraquat plasma kinetics has mostly been studied in rats (Daniel and Gage, 1966; Rose et al., 1975). However, plasma profiles are very similar across all species (Lock and Wilks, 2001), including man at a minimally lethal dose. Blood profiles following oral poisoning with paraquat are characterised by a rapid absorptive phase which is, in part, active transcellular uptake by the gastrointestinal tract predominantly in the jejunum (Heylings, 1991; Nagao et al., 1993). The plasma profile is a good predictor of the prognosis following a single oral dose of paraquat and can be used to estimate the probability of survival if the blood sample time is known (Proudfoot et al., 1979). Significant reduction in the peak plasma and 0–24 h AUC paraquat levels is consistent with improved survival. This is the basis for our prediction that alginate formulations should improve oral toxicity.

In our investigations, the absorption of paraquat in the rabbit from the control formulation, Gramoxone, was reproducible when repeated on four separate occasions and showed a typical plasma profile seen in other species. The peak plasma values occurred about 30 min following dosing. This was transient with a steady decline in plasma paraquat from 2–7 h as the chemical was eliminated into urine. Paraquat is rapidly but incompletely absorbed from the gastrointestinal tract (Daniel and Gage, 1966). Being very polar and very stable to metabolism this rapid uptake into the blood is quite surprising. However, it is known that there is an energy-dependent uptake across the mucosa of the small intestine (Heylings, 1991) and this may be similar to the polyamine pathway demonstrated in the lung (Smith, 1982), kidney (Grabie et al., 1993) and gastrointestinal tract (Kumagai and Johnson, 1988). We also know that the transport of paraquat across the oesophagus, gastric mucosa and colonic mucosa is much lower than the small bowel where the chemical is preferentially taken up in the jejunum and ileum (Heylings, 1991). Thus, we believe that the early appearance of paraquat in the blood following ingestion of Gramoxone is due to this aqueous low viscosity mixture rapidly exiting the stomach through the pylorus and reaching the absorptive epithelia of the small intestine (and its site of active uptake) soon after ingestion. The surfactants present in the formulation then aid the transport of the chemical across the mucosa via their surface action on the mucous membrane. This process is likely to be exacerbated under conditions where there is minimal food in the stomach to slow gastric emptying. Being fully ionized at all pH values the presence or absence of gastric acid is not likely to affect paraquat absorption from Gramoxone.

The key aspect of this new paraquat:alginate technology in Gramoxone INTEON® relates to the unique conditions of human oral exposure. As soon as the dissolved alginate comes in contact with gastric acid, it is protonated and is transformed into a thick gelatinous mixture. This property is already used widely in the pharmaceutical industry to treat heartburn and acid reflux (Mandel et al., 2000) and also to cause satiety in the treatment of obesity, by virtue of its intragastric bulking properties (Hoad et al., 2004). This gelling of alginate at low pH would never be seen during the manufacture or normal use of the product as a herbicide. The alginate composition is unaffected at pH above about 4 units but mixture with pH 3 or less causes the 200 g/L paraquat concentrate to transform into a thick gel. In our *in vitro* studies we selected a gastric chamber pH of 2 to mimic likely human stomach conditions. However, we recognise that in reality the pH and, more importantly, the gastric output in terms of volume and acidity and other secreted or refluxed components present in the human stomach may have an impact on the gelling process and ultimately the absorption of paraquat. Nevertheless, under our simulated conditions we could clearly see a stepwise reduction in paraquat absorption with increasing alginate concentration.

The concept of incorporating specific formulation components to achieve different plasma exposure is fundamental to modern pharmaceutical research in the area of controlled drug release to optimise efficacy or reduce toxicity. In the pesticide field there are a number of publications where the absorption of the active compound has been modulated by the vehicle in which it has been dosed. For example, the absorption (and hence toxicity profile) of the pyrethroid insecticide deltamethrin in rats can be attributed to the vehicle it was dosed in (Crofton et al., 1995; Kim et al., 2007). However, in the present study we have designed the formulation so it is entirely compatible with the commercial needs of the product and the activation of the safening effect with alginate is via acid-triggered gelling in the stomach.

The design of the experiments in this study does not identify the actual mechanism by which alginates reduce absorption of paraquat. Our working hypotheses include the possibility that the alginate physically entraps the paraquat making it less available for absorption. There is no chemical affinity between paraquat and the alginate. Indeed, this would adversely affect its herbicidal properties. Our definition of entrapment relates to the slow release of paraquat from the gel bolus formed *in situ* in the stomach. Alternatively, the alginate could be acting independently of the paraquat becoming bound to the surface mucus layer to reduce the flux of paraquat from lumen to mucosa. The first concept is attractive since we can envisage a slowing of gastric empting *in vivo* caused by the bulking reflex closing the pylorus. This is consistent with our experiments with the high alginate concentration that delayed the peak blood levels following dosing. If the gastric mucosa is simultaneously protected by alginate any passive uptake of paraquat resulting from mucosal damage would also be minimised. Indeed, it has been shown that alginate can increase the thickness of the mucus layer and has the ability to form hybrid gels with the mucopolysaccharides already present (Taylor et al., 2005). In reality, as depicted in Fig. 4, a combination of events is probably the case, since the delivery of any ingested substrate from the gut lumen into the blood is the product of a multitude of physiological processes and chemical interactions and is affected by many factors including motility, volume, acid output, food, viscosity, mucosal defence, etc.

Our experiments do exclude one important event, however. The rabbit has no vomiting reflex and was chosen for this very reason. Our objective was to identify a safer formulation of paraquat that would have benefit even in the absence of emesis. If emesis could further improve this benefit then there are real possibilities to safen paraquat products that may be accidentally or intentionally ingested. All paraquat products contain an emetic drug (PP796). This is a phosphodiesterase inhibitor and acts centrally in the vomit centre of the brain where elevation of cAMP triggers closure of the pylorus and action on the musculature to expel the gastric contents up the oesophagus. Higher mammals, including humans have a vomit response that can be triggered locally or centrally. If the alginate-based paraquat formulations reduce absorption by slowing the dissolution of the formulation, by delaying gastric emptying or by reducing topical damage to the gastric mucosa, the protective effect of such acid-triggered gel formulations in a vomiting species may be even better than observed here in the rabbit. This may be particularly important in human paraquat poisoning where it is often too late to treat the patient using gastric lavage following ingestion. The liquid Gramoxone reaches the absorptive small intestine rapidly and early high blood levels have delivered a lethal dose of paraquat to the lungs. Therefore, any delay and/or reduction in gastrointestinal absorption of paraquat by alginate may make gastric decontamination in humans more effective and ultimately improve the chances of survival. The new alginate-containing paraquat product, Gramoxone INTEON® has now received registration in several countries, including the USA. It is anticipated that this novel formulation will improve survival following accidental or intentional ingestion of paraquat-containing agricultural products.

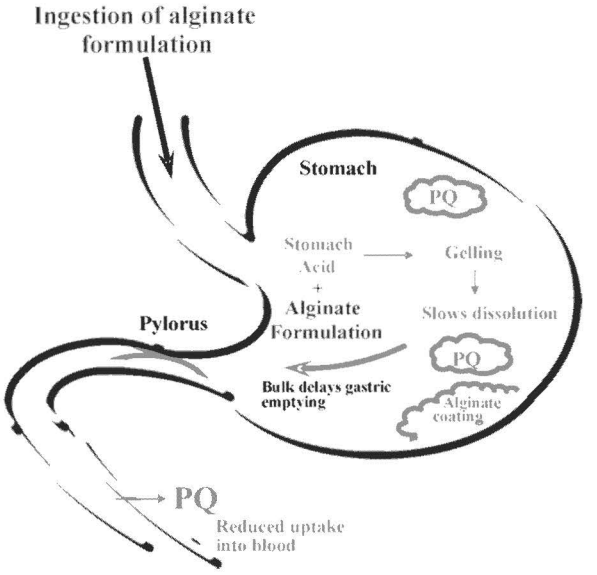

Fig. 4. Diagrammatic representation of the human stomach. On ingestion of a paraquat concentrate containing alginate the formulation will gel on contact with gastric acid producing a highly viscous mixture that should slow the dissolution of paraquat into the gastric contents. The bulking effect of the porous gel delays gastric emptying and delivery of the herbicide to its absorptive site in the small intestine. In addition, alginates are known to coat the surface of the mucosa and will improve the resistance of the mucosal barrier to the irritant effects of the paraquat formulation.

Conflict of interest

None.

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