

Development of a new safer Novel formulation of paraquat, (Gramoxone AWT, (A3879BU)

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Syngenta propose to introduce a new formulation of paraquat that provides all the important agronomic and environmental benefits of paraquat in a reduced hazard formulation. - Syngenta's AWT (Alginate Wall Technology) formulation represents a step change in setting new standards in safety.

1. Introduction to AWT technology

Syngenta has for many years, undertaken research to improve the safety of existing products. Over the last five years considerable effort has been expended in striving to reduce the acute toxicity of paraquat through the use of a novel formulation technology based on alginate gelling agents derived from the *Ascophyllum* seaweed extract. This specific project emerged out of the broader research programme. This formulation technology development project has led to the launch of a new Gramoxone-based product, Gramoxone INTEON is named Alginate Wall Technology (AWT).

Alginates are carbohydrates of polymannuronic and polyguluronic acid. They are non-toxic and are commonly used in the food industry as gelling agents. They are also used in the pharmaceutical industry for their therapeutic properties, for example in treating dyspepsia (Mandel *et al.* 2000)(~~JH TO ADD REFERENCE~~) and wound healing (Agren, 1996)(~~JH TO ADD REFERENCE~~).

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The data presented in this document relate to a soluble liquid (SL) formulation of paraquat that contains 200g/l paraquat ion and is based on AWT technology. This is the lead product for this formulation technology development project, as it represents the formulation concentration most commonly used globally. This formulation also contains a blue/green dye, an olfactory alert and the effective centrally acting emetic (PP796), as stipulated in the FAO specification for paraquat products.

Field studies providing a comparison of herbicidal activity with existing paraquat formulations have demonstrated that this AWT formulation offers equivalent or superior efficacy performance. Although it forms a gel under acid conditions, the formulation did not give rise to blocked spray nozzles or other application difficulties.

2. The hypothesis

It was conceived that if alginates could be incorporated into a paraquat formulation and a pH-trigger used to ensure effective gelling in the stomach, this would slow the emptying of the formulation from the stomach, leading to more productive emesis (a greater amount of the formulation being emitted before entering the small intestine~~s~~). This would reduce the amount of any ingested paraquat that would be released to the

small intestines, the site of greatest absorption for paraquat (Heylings, 1991). Further the inclusion of magnesium sulphate, a known purgative Schiller LR (1999), ~~(JH TO ADD-REFERENCE)~~, should further reduce the absorption of any paraquat reaching the small intestines by stimulating purgation.

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The hypothesis is based ~~on each of the~~ 3 processes (gelling, emesis and purgation), which in their own right could reduce the oral absorption of paraquat. Together they would act synergistically and could potentially improve (reduce) the oral toxicity in a vomiting species following oral ingestion. The dog is the animal model chosen for this work since it has similar gastrointestinal physiology to man, including a vomiting reflex.

Key features of this hypothesis which make the technology viable:

- It has been demonstrated that paraquat is much more readily absorbed from the small intestine, most specifically the jejunum, than either the oesophagus or the stomach. (Heylings JR, 1991)
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- It is known that the stomach reacts to bulk by closing the pylorus and delaying emptying.
- The alginate is soluble in water at neutral pH. However in the acid environment of the stomach it rapidly forms a gel.
- PP796, a triazolopyrimidine emetic, acts in the vomit center of the brain via inhibition of phosphodiesterase
- The emetic PP796 is well absorbed from the gastrointestinal tract and is fast acting, with emesis typically occurring in about half an hour. This drug also inhibits gastric emptying by closure of the pylorus.
- Magnesium sulphate is an osmotic purgative agent that clears the bowel by stimulating the osmoreceptors in the duodenum. This causes a prompt influx of water into the bowel to equalise the osmotic pressure between blood and lumen. This stretch reflex closes the pylorus and raises the intraluminal pressure and effect clears the small intestine.

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3. Experimental data.

This hypothesis has been tested in a toxicokinetic study in the dog (Brammer, 2004) and the results, together with comparisons to earlier data on a non-toxic base line dose of Gramoxone (Heylings, Swain and Brammer 2004) are presented below.

It is concluded that the Gramoxone AWT formulation offers a significant reduction in the absorption of paraquat into the blood following oral ingestion. Doses of 46 – 736mg A3879BU formulation/kg, were well tolerated in the dog. The highest dose used represents more than 10 times the toxic dose of Gramoxone, approximately 55mg formulation/kg. This demonstrates in the dog, a vomiting species, a substantial improvement in the safety of A3879BU formulation, compared to the standard Gramoxone formulation. Syngenta consider that the available results indicate that the technology would be expected to provide a significant reduction in the amount of paraquat absorbed, and hence acute toxicity, in humans.

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3.1. Study design

3.1.1. Toxicokinetic dose response for AWT formulation A3879BU

A group of three male beagle dogs received oral doses (by capsule) of Paraquat 200g/l ~~AWTSL~~ formulation (A3879BU), on 5 occasions at monthly intervals. The nominal dose levels used were 8, 16, 32, 64 and 128mg paraquat ion/kg. Allowing for specific gravity and purity, these doses were equivalent to achieved dose levels of 46, 92, 184, 368 and 736mg A3879BU formulation/kg.

Following each dose, the dogs were observed continuously for 4 hours and then frequently during the remainder of the day. All incidences of emesis were recorded and vomit and faeces were removed immediately to prevent possible re-ingestion. Blood samples were taken at intervals following each dose to enable a plasma profile of paraquat and PP796 (the emetic) to be determined. Veterinary examinations (including cardiac and pulmonary auscultation) were made prior to each dose, during the observation period, and prior to termination. General clinical observations, bodyweights and food consumption were measured ~~frequently at weekly intervals~~ throughout the study. Clinical pathology parameters were measured from blood samples taken prior to and then 24 hours after each dose. At the end of the study period, the animals were killed and examined *post mortem*. Kidney and lung samples were taken for subsequent histopathological examination.

3.1.2. Base line data for Gramoxone

Data have been extracted from ~~a series of~~ research studies conducted at CTL between 1987 and 1991, which compared the absorption of paraquat from different paraquat formulations with that of the commercial product Gramoxone (CTL studies XD1236 and XD1328). The data extracted from these studies provide a toxicokinetic profile following an oral dose of Gramoxone (200g/L formulation) at a nominal concentration of 8mg paraquat ion/kg, equivalent to 46mg formulation/kg administered by gavage or gelatine capsule. This dose was chosen since it is just below a lethal dose (Widdop, 1977).

Plasma samples were collected from male dogs during the 24h period after dosing and the concentration of paraquat in these plasma samples was determined. The toxicokinetic parameters AUC_{0-1} , AUC_{0-4} and AUC_{0-24} (area under the curve between the time zero and 1h, 4h and 24h respectively) were calculated. Clinical observations, including time to onset of emesis, were made frequently during the 24-hour post dose period.

3.2. Results

The toxicokinetic profiles below have compared d the data obtained with AWT formulation, A3879BU with that of Gramoxone. The emetic absorption profile was not measured in the Gramoxone study.

- All of the animals were clinically normal and remained in excellent clinical condition throughout the studies with both Gramoxone control and Gramoxone AWT (A3879BU). However following the highest dose of 736mg A3879BU formulation, clinical signs including prolonged retching, abdominal discomfort

and decreased activity were observed for up to 3 hours after dosing. One animal Male 3, which had the highest peak plasma paraquat level, showed additional signs of inappetance, weight loss and decreased activity for several days following this dose.

- Kidney and liver function tests and veterinary examination have shown no adverse effects in any dog over this dose range (46 - 736mg A3879BU formulation/kg)
- The AWT formulation results in slower absorption and lower plasma paraquat levels compared to Gramoxone across a 16-fold increase in dose.
- Generally peak plasma levels were observed at 1 hour with significant elimination after 2 and 4 hours and almost complete elimination by 7 hours.
- In the dogs exposed to 736mg A3879BU formulation/kg (16 fold higher than the Gramoxone dose), the initial peak plasma paraquat levels were higher than those at other doses. This was only transient and plasma levels of paraquat dropped to below that of the 46mg Gramoxone formulation/kg by 2 hours.
- At termination one animal had some pathology of the lung (slight focal interstitial fibrosis, slight alveolar macrophage infiltration and slight focal pneumonocyte hypertrophy) consistent with signs of paraquat toxicity. This animal also had the highest peak plasma level (approximately 12µg/ml) and AUC (20.5 µg/ml.h), following the 736mg A3879BU/kg and showed some inappetance, decreased activity and weight loss for several days following dosing. The other 2 dogs had no pathology of the lung.

Figure 1

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- This is confirmed by the area under the curve (AUC) calculations, which show lower values for all AWT formulation doses at 24 hours compared to Gramoxone at 44mg formulation/kg. AUC's of 40µg/ml/h. or greater or a peak plasma paraquat well above of 10µg/ml, in combination with adverse clinical signs are indicators of paraquat toxicity and would lead to the animal being humanely removed from the study.

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Figure 2.

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- The reduction in time to first emesis with increasing dose of AWT formulation is consistent with the plasma emetic kinetics, which shows increased levels of absorbed emetic with increasing dose.

Figure 3.

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- AWT formulation provides more opportunity for productive emesis. The plasma paraquat kinetics are consistent with acid triggered gelling in the stomach, closure of the pylorus resulting from either bulking effect of the gel or the pharmacological action~~central activity~~ of the emetic, leading to~~and~~ significant reductions in paraquat absorption over the first 15 to 30 mins following dosing.

Figure 4.

Note y axis has paraquat spelled wrong and bracket missing –see other figs also

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3.3. Conclusion

It is concluded that the Gramoxone AWT formulation offers a significant reduction in the gastrointestinal absorption of paraquat following oral ingestion. Doses of 46 – 736mg A3879BU formulation/kg, were well tolerated in the dog. The highest dose used represents more than 10 times the toxic dose of Gramoxone, approximately 55mg formulation/kg. This demonstrates in the dog, a vomiting species, a substantial improvement in the safety of A3879BU formulation, compared to the standard Gramoxone formulation. Syngenta consider that the available results indicate that the technology would be expected to provide a significant reduction in the amount of paraquat absorbed in humans.

4.0. Irritation hazard

A further benefit of the alginate-containing AWT formulation is a~~the~~ significant reduction in the irritancy of the concentrated formulation. Paraquat, being a polar chemical~~has been shown to penetrate~~ penetrates the skin via hair follicles. The alginate gel precipitates on the skin surface following drying and forms a protective film, on the skin surface and together with the magnesium sulphate in the formulation This reduces dermal penetration in rodent skin. In regulatory~~rodent~~ irritancy studies, although some topical damage~~irritancy~~ is still observed, this is much reduced compared with that seen with current commercial paraquat formulations. A comparison of the scores observed is given in the attached graphs.

4.1. Skin irritation:

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Studies were conducted according to OECD 404 protocol. Three female New Zealand White albino rabbits each received a single four-hour application of 0.5ml of formulation to the shorn flank. The animals were assessed for up to 34 days for ~~any~~ signs of skin irritation.

Figure 5.

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Figure 6.

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According to EU Commission Directive 2001/59/EC, paraquat 200 g/l SL formulation (A3879BU) does not require classification for skin irritation. Based on the irritation scores at 72 hours only, A3879BU is assigned to EPA Toxicity Category IV. (Johnson IR, 2003)

4.2. Eye irritation:

Studies were conducted according to OECD 405 protocol. A volume of 0.1 ml of formulation was instilled into one eye of each of three female New Zealand White albino rabbits and an assessment of initial pain was made. The eyes were examined for 17 days to assess the grade of ocular reaction.

Figure 7.

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Figure 8.

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According to a modified form of the Kay and Calandra system, under the conditions of this study, paraquat 200 g/l SL formulation (A3879BU) is considered to be moderately irritating to the unrinsed eye. However, according to Commission Directive 2001/59/EC, the formulation does not require classification for eye irritation. Positive effects cleared within 10 days placing the formulation in EPA Category II for acute eye irritation. (Johnson IR, 2003)

5.0. Conclusion.

~~The new AWT formulation has been shown to be significantly less toxic significant~~
~~safening a~~

It is concluded that the Gramoxone AWT formulation (A3879BU) offers a significant reduction in both oral and dermal absorption of paraquat leading to a reduction in ~~acute oral toxicity and skin~~ irritancy. The highest ~~oral dose~~ tested in the dog ~~toxicokinetic study~~, 736mg A3879BU formulation/kg, was well tolerated ~~and~~ represents more than 10 times the toxic dose of Gramoxone, approximately 55mg formulation/kg. This demonstrates in the dog, a vomiting species, a substantial

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improvement in the safety of A3879BU formulation, compared to the standard Gramoxone formulation. Syngenta consider that the available results indicate that the technology would be expected to provide a significant reduction in the amount of paraquat absorbed in humans. Further this formulation is less irritant to both skin and eye and therefore has the potential to set a new standard for paraquat products in the market.

5.1. The potential contribution of AWT to the outcome of accidental incidents:

As a consequence of the measures taken previously, such as the inclusion of an alerting dye, a stench and a ~~centrall actingn-effective~~ emetic, mistaken ingestion of paraquat is now extremely rare. The AWT formulation contains an alerting blue/green dye, an olfactory alert and the effective emetic as stipulated in the FAO specification for paraquat products. The AWT technology provides a significant further safening. The available experimental data demonstrate a reduction in the gastrointestinal absorption of paraquat from the AWT formulation compared with Gramoxone at toxic doses. We therefore anticipate that the AWT formulation will eliminate fatalities following mistaken ingestion.

5.2. The potential contribution of AWT to the outcome of deliberate ingestion incidents:

Syngenta believes that the AWT formulation offers a positive contribution to addressing suicide as a public health issue, ~~in Taiwan~~. The available experimental data demonstrates a reduction in the gastrointestinal absorption of paraquat from the AWT formulation compared with Gramoxone at toxic doses. We therefore anticipate that the AWT formulation will significantly increase the survival rate following intentional ingestion ~~in man~~.

MJLC 19/02/04
~~JRH 24/02/04~~

6.0. References:

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