

## Health Assessment Position

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### Development of a new safer formulation of paraquat, Gramoxone Inteon (A3879BU)

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 Inteon Technology (based on alginate gelling) represents a step change in setting new standards in safety.

#### 1. Introduction to Inteon 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. This specific project emerged out of a broader research programme and has led to the development of Gramoxone Inteon.

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) and wound healing (Agren, 1996).

The data presented in this document relate to Gramoxone Inteon (a soluble liquid (SL) formulation of paraquat that contains 200g/l paraquat ion) and is based on Inteon technology. This is the lead product for the Inteon technology development project, and is intended to replace Gramoxone (200g/l) the formulation 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 Gramoxone Inteon 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). This would reduce the amount of any ingested paraquat that would be released to the small intestine, the site of greatest absorption for paraquat (Heylings, 1991). Further the inclusion of magnesium sulphate, a known purgative Schiller LR (1999), should further reduce the absorption of any paraquat reaching the small intestine by stimulating purgation.

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The hypothesis is based on 3 processes (gelling, emesis and purgation), which in their own right should reduce the oral absorption of paraquat. Together they would act synergistically and should 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)
- 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. PP796 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 clears the small intestine.

### **3. Experimental data.**

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

It is concluded that the Gramoxone Inteon 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 lethal dose of Gramoxone, approximately 55mg formulation/kg. This demonstrates in the dog, a vomiting species, a substantial improvement in the safety of Gramoxone Inteon 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.

### **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 Gramoxone Inteon 200g/l SL 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

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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 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 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 44mg 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 the data obtained with the Gramoxone AWT formulation, A3879BU with that of Gramoxone. The emetic absorption profile was not measured in the Gramoxone study.

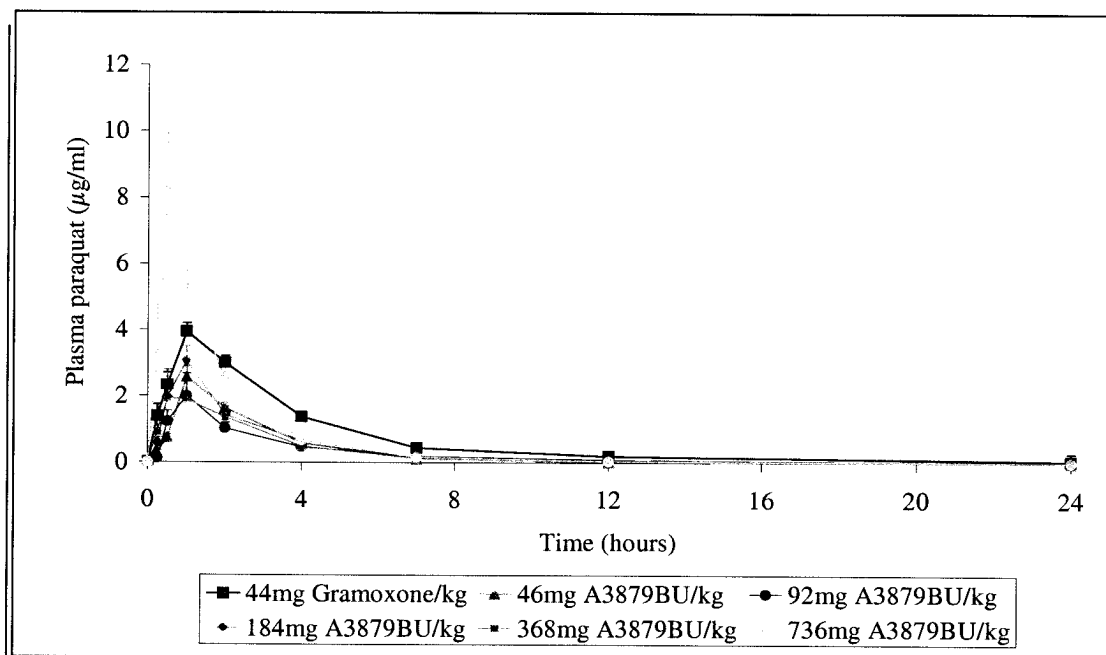
- All of the animals were clinically normal and remained in excellent clinical condition throughout the studies with both Gramoxone control and Gramoxone AWT (A3879BU). However following the highest dose of 736mg A3879BU formulation, clinical signs including prolonged retching, abdominal discomfort and decreased activity were observed for up to 3 hours after dosing. One animal, which had the highest peak plasma paraquat level, showed additional signs of inappetence, 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)

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- 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 level of paraquat dropped to below that of the 44mg 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 12 $\mu$ g/ml) and AUC (20.5  $\mu$ g/ml.h), following the 736mg A3879BU/kg and showed some inappetence, decreased activity and weight loss for several days following dosing. The other 2 dogs had no pathology of the lung.

Figure 1

**Plasma paraquat following an oral dose of A3879BU (46 - 736mg formulation/kg b wt.) in dogs (n = 3)**



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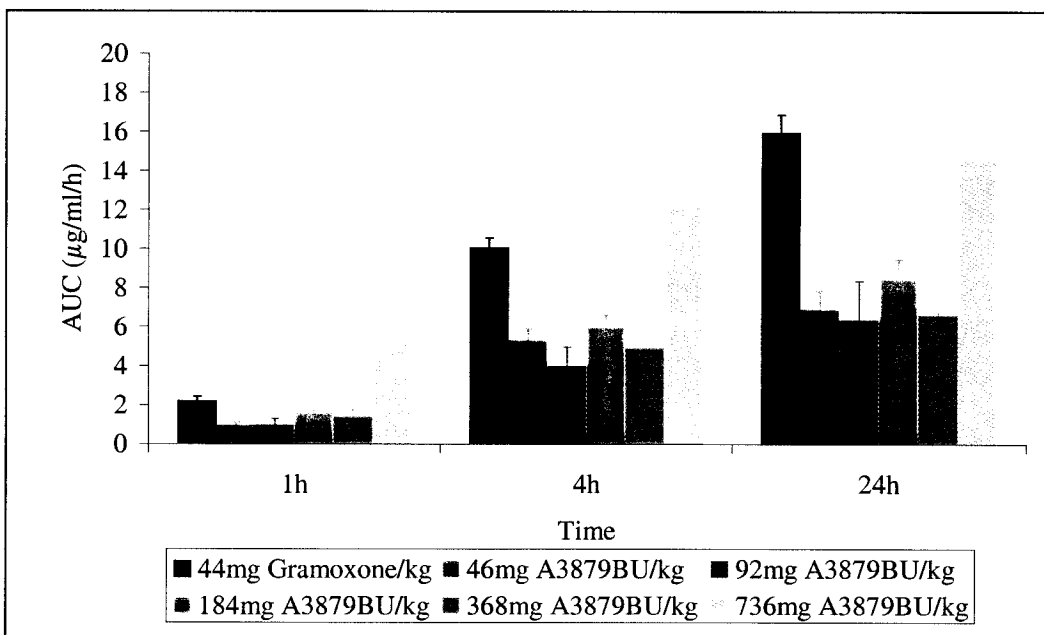
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- This is confirmed by the area under the curve (AUC) calculations, which show lower values for all AWT formulation doses at 24 hours compared to Gramoxone at 44mg formulation/kg. AUC's of 40µg/ml/h. or greater or a peak plasma paraquat well above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

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Figure 2.

Plasma paraquat AUC values following an oral dose of A3879BU (46 - 736mg formulation/kg b wt.) in dogs (n = 3) in comparison with Gramoxone

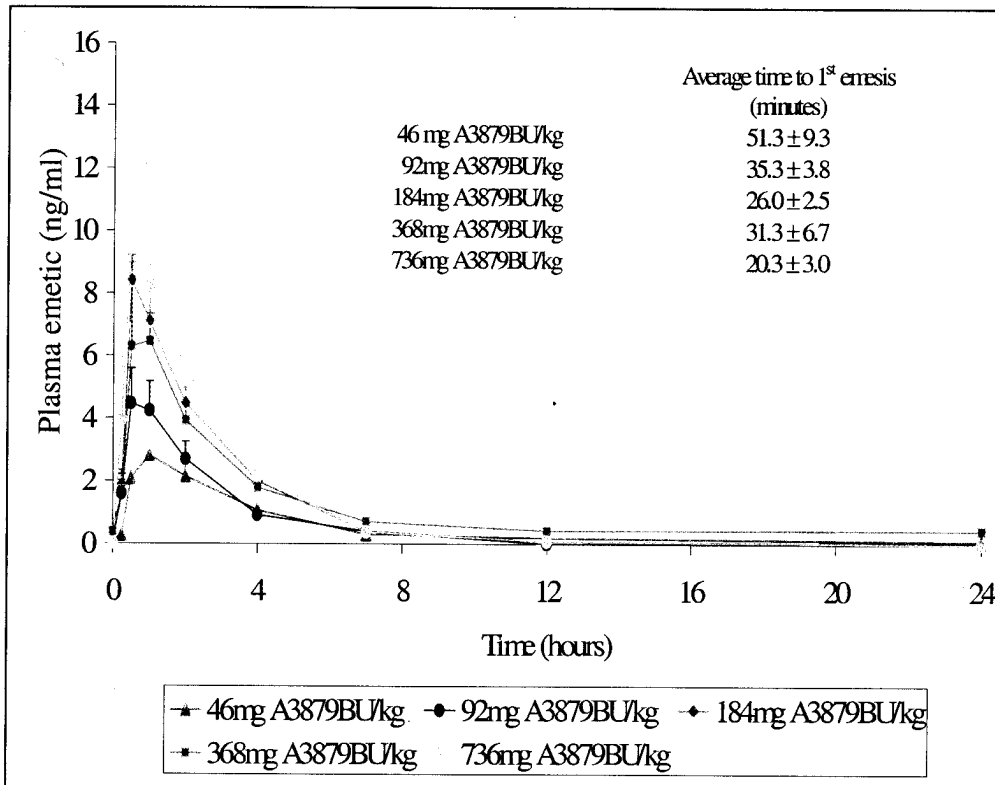


with the plasma emetic kinetics, which shows increased levels of absorbed emetic with increasing dose.

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Figure 3

Plasma emetic levels following an oral dose of A3879BU (46 - 736mg formulation/kg b wt.) in dogs (n = 3).



- 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 of the emetic, leading to significant reduction in paraquat absorption over the first 15 to 30 mins following

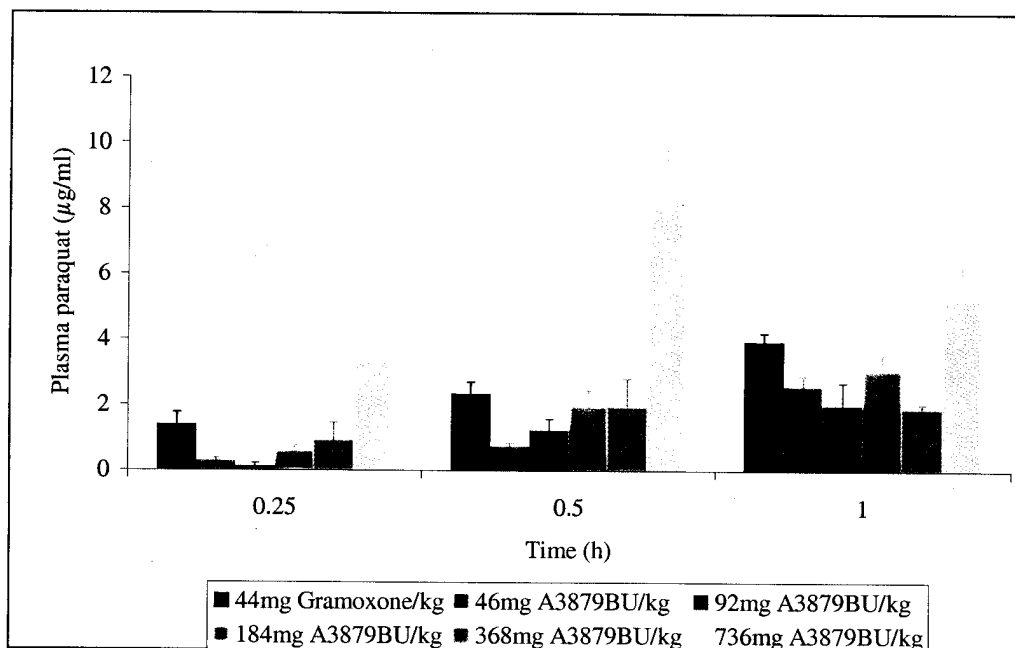
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dosing.

Figure 4.

**Plasma paraquat values following an oral dose of A3879BU (46 - 736mg formulation/kg b wt.) in dogs (n = 3) compared with Gramoxone (44mg formulation/kg b wt)**



### 3.3. Conclusion

It is concluded that the Gramoxone Inteon 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 lethal 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 Gramoxone Inteon formulation (A3879BU) would be expected to provide a significant reduction in the amount of paraquat absorbed in humans.

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#### 4.0. Irritation hazard

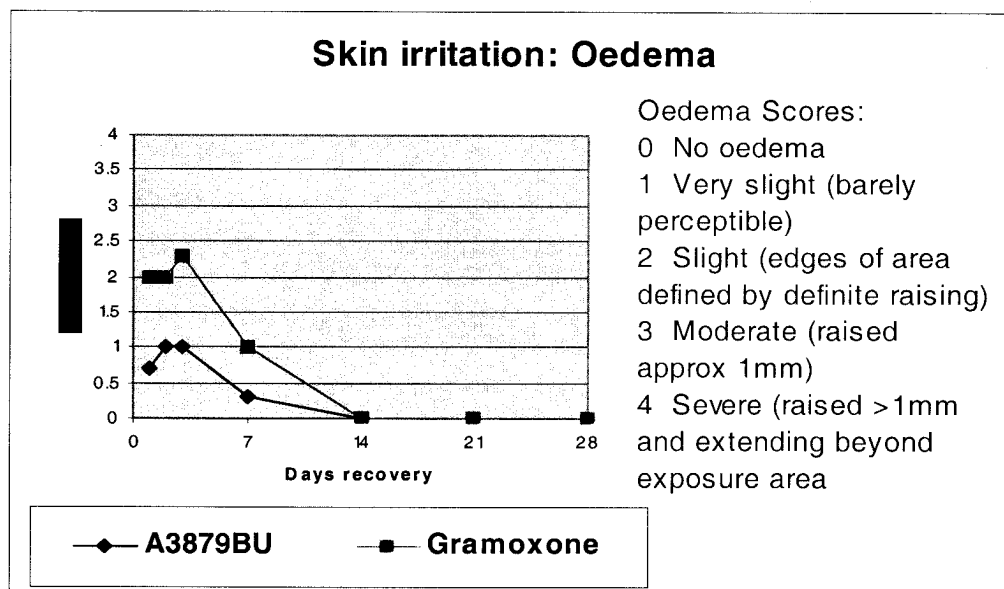
A further benefit of the Gramoxone Inteon formulation is a significant reduction in the irritancy of the concentrated formulation. Paraquat, being a polar chemical penetrates the skin extremely slowly (Wester et al 1984) <0.3% over 24 hours. The penetration, which does occur, is via hair follicles. The alginate gel precipitates on the skin surface following drying and forms a protective film. This reduces dermal penetration in rodent skin. In regulatory irritancy studies, although some topical damage 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:

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 signs of skin irritation. The comparative scores for erythema and oedema comparing current Gramoxone with Gramoxone Inteon are given in Figures 5 and 6.

Figure 5.

**Comparative skin irritancy – Gramoxone Inteon formulation and Gramoxone formulation**



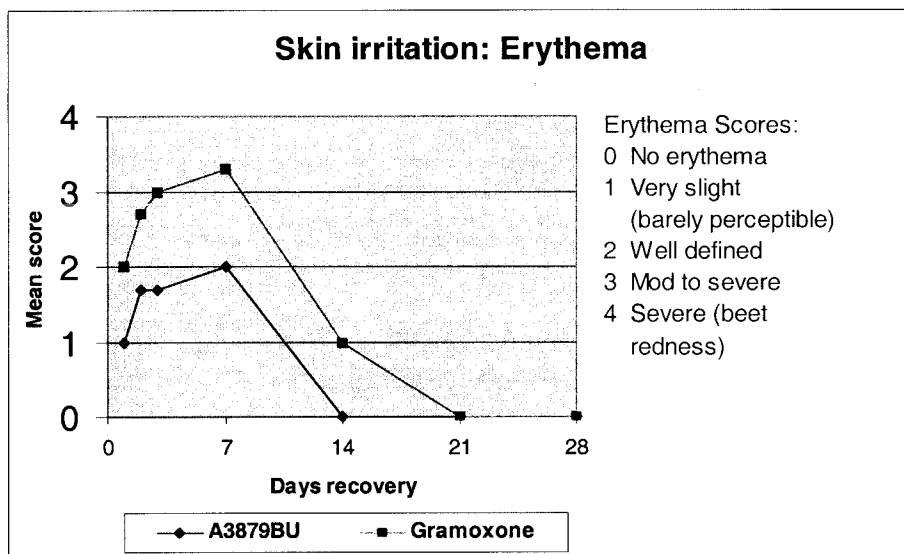
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Figure 6

**Comparative skin irritancy – Gramoxone Inteon formulation and Gramoxone formulation**



Gramoxone Inteon shows a reduction in the severity of both oedema and erythema and time to recovery compared with the standard Gramoxone (220g/l) formulation.

#### **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 shows a comparison on the total Kay and Calandra scores for the days after instillation for a current Gramoxone formulation compared with Gramoxone Inteon formulation (A3879BU). Figure 8 shows the scores for the different regions of the eye; cornea, iris and conjunctiva.

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Figure 7.

Comparison on the total Kay and Calandra scores following instillation into the eye for a current Gramoxone formulation compared with Gramoxone Inteon formulation (A3879BU)

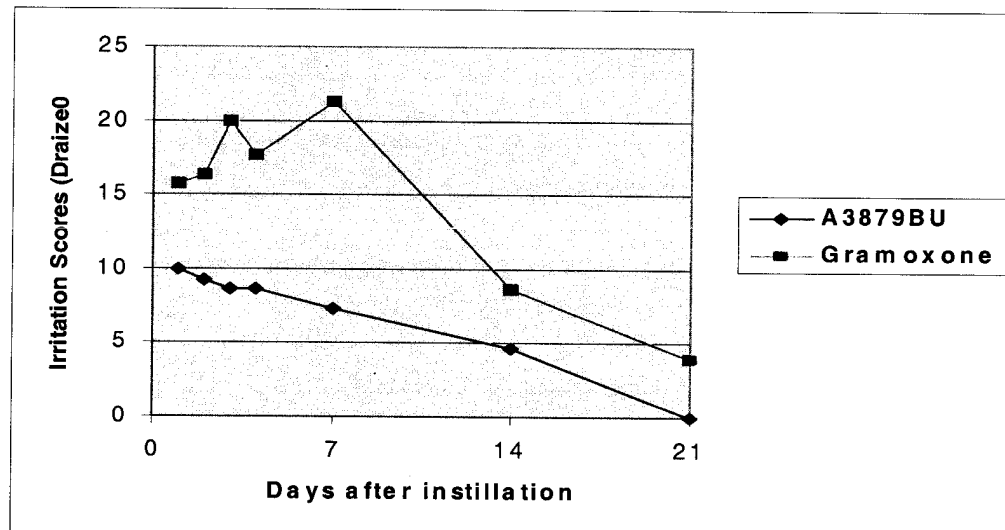
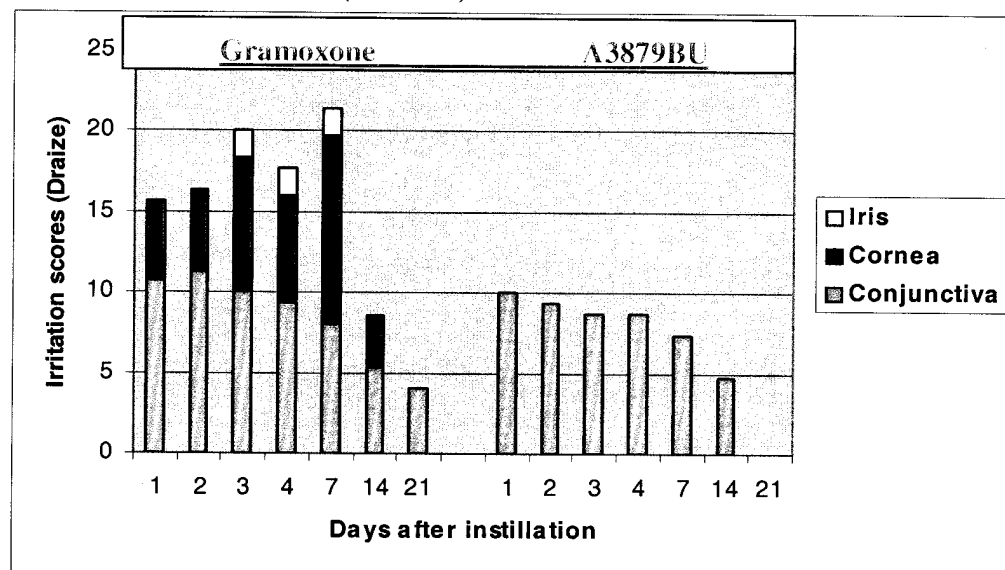


Figure 8.

Comparison on the total Kay and Calandra scores for the different regions of the eye; cornea, iris and conjunctiva following instillation for a current Gramoxone formulation compared with Gramoxone Inteon formulation (A3879BU)



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According to a modified form of the Kay and Calandra system, under the conditions of this study, Gramoxone Inteon formulation (A3879BU) showed less irritation with no involvement of cornea and iris compared with current Gramoxone and a slightly faster recovery time in the unrinsed eye.

**5.0. Conclusion.**

It is concluded that the Gramoxone Inteon 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, 736mg A3879BU formulation/kg, was well tolerated and represents more than 10 times the lethal dose of Gramoxone, approximately 55mg formulation/kg. This demonstrates in the dog, a vomiting species, a substantial improvement in the safety of Gramoxone Inteon (A3879BU) formulation, compared to the current 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 Gramoxone Inteon 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 centrally acting emetic, mistaken ingestion of paraquat is now extremely rare. The Gramoxone Inteon formulation contains an alerting blue/green dye, an olfactory alert and the effective emetic, PP796, as stipulated in the FAO specification for paraquat products. The Gramoxone Inteon technology provides a significant further safening, since accidental ingestion invariably involves relatively small quantities of product. The available experimental data demonstrate a reduction in the gastrointestinal absorption of paraquat from the Gramoxone Inteon formulation compared with Gramoxone at toxic doses. We therefore anticipate that the Gramoxone Inteon 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 Gramoxone Inteon formulation offers a positive contribution to addressing suicide as a public health issue. The available experimental data demonstrates a reduction in the gastrointestinal absorption of paraquat from Gramoxone Inteon formulation compared with current Gramoxone formulations. We therefore anticipate that the Gramoxone Inteon formulation will significantly increase the survival rate following intentional ingestion in human.

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17<sup>th</sup> March 2004

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**6.0. References:**

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