# Health Assessment Position

DRAFT

**Development of a new reduced hazard 100g/l paraquat formulation A9409AL**

**Summary**

Syngenta propose to introduce a new formulation (A9409AL) of paraquat based on Syngenta's INTEON technology, which provides all the important agronomic and environmental benefits of paraquat in a reduced hazard formulation.

A9409AL shows very low levels of paraquat absorption in vitro following dermal exposure and is significantly less irritant to skin and eye compared with the current French formulation R-Bix.

Paraquat 100g/l A9409AL INTEON formulation offers a significant reduction in the gastrointestinal absorption of paraquat following oral ingestion. Doses up to 1414mg A9409AL formulation/kg are without overt toxicity in the dog. The highest dose 1414mg formulation/kg (equivalent to 128mg paraquat ion/kg bw.) represents more than 10 times the lethal dose of paraquat on an mg paraquat ion/kg basis. This demonstrates in the dog, a vomiting species, a substantial reduction in paraquat absorption, which could be expected to provide a significant reduction in the amount of paraquat absorbed in humans.

Syngenta consider that the available results for A9409AL reduces the potential risk to the operator and those accidentally or deliberately ingesting the formulation.

## 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 paraquat research programme and has led to the development of INTEON technology.

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). In this case we have selected an alginate, which specifically gels under low pH conditions (1-3).

The data presented in this document relate to a soluble liquid (SL) formulation of paraquat (A9409AL) that contains 100g/l paraquat ion) and is based on INTEON technology. This formulation is intended to replace R-Bix and Gramoxone Plus (100g paraquat ion/l) formulations currently registered in France. The formulation also contains a blue/green dye, an olfactory alert and the 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 A9409AL offers equivalent or superior herbicidal efficacy performance. The formulation is fully water-soluble and has not give rise to blocked spray nozzles or any other application difficulties.

paraquat formulations such as R-Bix and Gramoxone Plus. A comparison of the scores observed in the standard Regulatory tests is shown in the figures below.

### 2.1. Percutaneous absorption

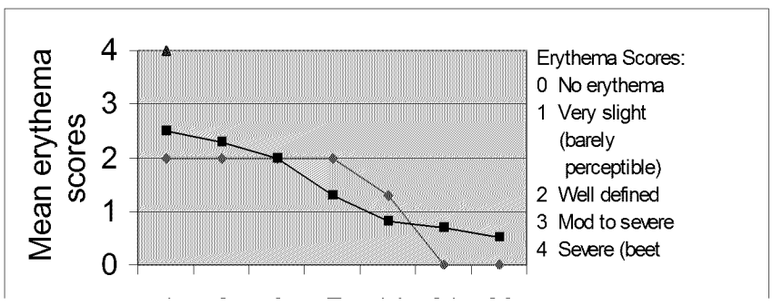
The results obtained in an in vitro percutaneous absorption study indicate that the absorption of paraquat from a 100g/l SL formulation (A9409AL) through human epidermis is very slow when compared with the absorption rates of other penetrants measured using *in vitro* techniques. (Dugard *et al*, 1984; Dugard and Scott, 1984).
The vast majority of the paraquat applied is likely to be removed from the surface of human skin by normal washing procedures for both the concentrate and spray dilution.

The small residual amounts of paraquat found in human skin, especially that recovered from the *stratum corneum*, is most likely to be lost by desquamation *in vivo*.
These data predict that the dermal absorption of paraquat from potential exposure to A9409AL formulation would be minimal from the concentrate and spray strength dilution. After 24 hours, the dermal absorption from the concentrate was 0.232% and from the dilution was 0.291% of the applied dose. It should be noted that since paraquat penetrates via the hair follicles much greater penetration has been observed in laboratory animals (Walker, M., Dugard, P.H. and Scott, R.C. 1983)

### 2.2. 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 A9409AL 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 undiluted A9409AL and R-Bix at 1in 25 dilution are given in Figures 1 and 2.

**Figure 1.**
**Comparative skin irritancy – Gramoxone INTEON formulation and Gramoxone formulation**



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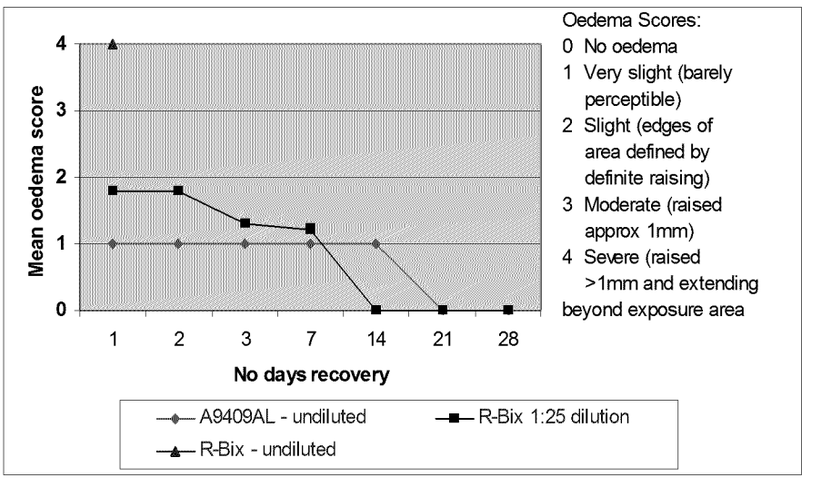
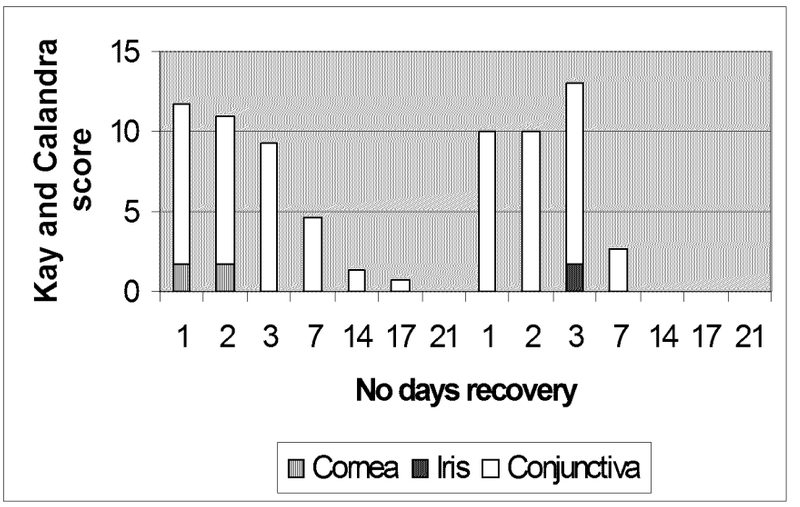



With undiluted R-Bix rabbits were terminated after 1 hour because of severe irritation. Therefore R-Bix when applied as the neat concentrate is a very severe skin irritant. On dilution to 1:25 in water the irritancy potential is significantly reduced. The INTEON (A9409AL) is much less irritant since the undiluted formulation shows a similar potential to that observed with a 1:25 dilution of the R-Bix formulation.

**2.3. Eye irritation.**

Studies were conducted according to OECD 405 protocol. A volume of 0.1 ml of A9409AL 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 3 shows a comparison on the total Kay and Calandra scores for the days after instillation for the INTEON formulation A9409AL compared with a 1:25 dilution of R-Bix. It also shows the scores for the different regions of the eye: cornea, iris and conjunctiva. There was minimal corneal and iris involvement with either the dilute R-Bix or undiluted A9409AL formulation. Since R-Bix was a very severe skin irritant it was not tested in the eye

**Figure 3.**
**Comparison on the total Kay and Calandra scores following instillation into the eye for a current Gramoxone INTEON formulation (A9409AL) compared with a 1:25 dilution of R-Bix.**




| | |
|---|---|
| A9409AL | 1:25 dilution R-Bix |

**3. The hypothe**

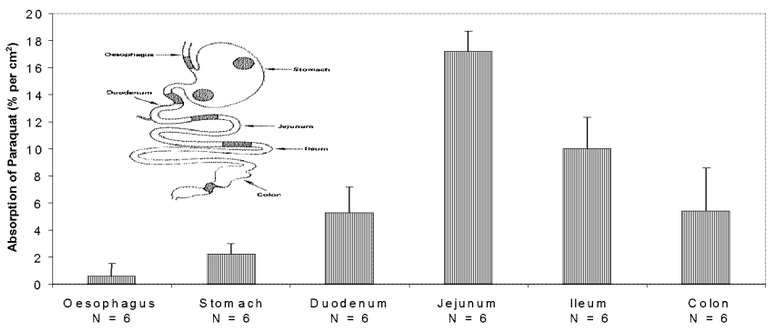
It was conceived that if alginates could be incorporated into a paraquat formulation and a pH-trigger used to ensure effective gelling in the stomach, this would slow the emptying of the formulation from the stomach, leading to more productive emesis (a greater amount of the formulation being emitted). 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.

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 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)

Figure 4. Absorption of paraquat from different areas of the gastrointestinal tract




Heylings JR *Toxicol. Appl. Pharmacol.* 107, 482-493, 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 (pH2-3) 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 raises the intraluminal pressure and clears the small intestine. It also inhibits gastric emptying via a hormonal reflex.

**3.1. Experimental data.**

This hypothesis has been tested in a toxicokinetic study on A9409AL in the dog (Brammer et al, 2005).

A9409AL formulation offers a significant reduction in the absorption of paraquat from the gastrointestinal tract into the blood following oral ingestion. Doses of 354 – 1414mg A9409AL formulation/kg, were well tolerated in the dog and did not give rise to overt toxicity. The highest dose used represents more than 20 times the lethal dose of Gramoxone (200g/l), approximately 55mg formulation/kg (Widdop et al, 1977). This demonstrates in the dog, a vomiting species, a substantial reduction in the toxicity of A9409AL formulation. Syngenta consider that the available

removed immediately to prevent possible re-ingestion. Blood samples were taken at frequent intervals following each dose to enable a 24hour plasma profile of paraquat and PP796 (the emetic) to be 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) for paraquat and emetic were calculated.

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, lung samples along with any abnormal tissue were taken for histopathological examination.

**3.3. Results - Plasma paraquat and emetic toxicokinetic profiles for A9409AL.**

The toxicokinetic profiles obtained with the A9409AL formulation are presented below. Administration of A9409AL was well tolerated by all dogs at all dose levels assessed, up to 1414mg A9409AL formulation/kg (equivalent to 128mg paraquat ion/kg).

- Increasing the dose of A9409AL did not significantly increase the plasma paraquat levels (Figure 4)
- This formulation caused prompt and effective emesis with no clinical signs of toxicity.
- There were no effects on food consumption or bodyweights and there were no abnormalities detected at veterinary examination.
- Kidney and liver function tests showed no adverse effects in any dog
- Generally peak plasma levels were observed at 30 mins with significant elimination after 2 and 4 hours and almost complete elimination by 12hours.
- Mean peak plasma paraquat levels were less than 3µg/ml (Figure 4)and the mean 24h AUC was less than 5µg/ml.h (Figure 5)
- There were no pathological changes observed in the lung at termination (14 days after the highest dose).
- The reduction in time to first emesis with increasing dose of A9409AL formulation is consistent with the plasma emetic kinetics, which shows increased levels of absorbed emetic with increasing dose. (Figure 6)
- A9409AL 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 and or the pharmacological action of the emetic, leading to significant reduction in paraquat absorption over the first 15 to 30 mins following dosing.

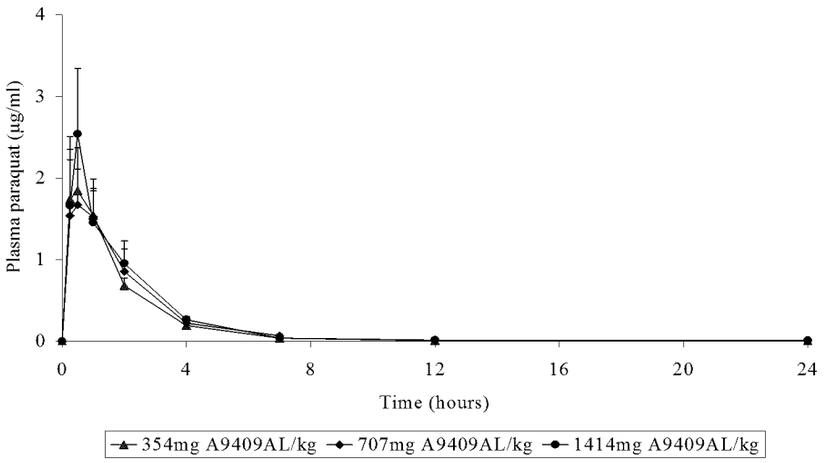



**Figure 5**
**Plasma paraquat AUC values following an oral dose of A9409AL (354 - 1414mg formulation/kg b wt.) in dogs (mean and SEM, n=3).**

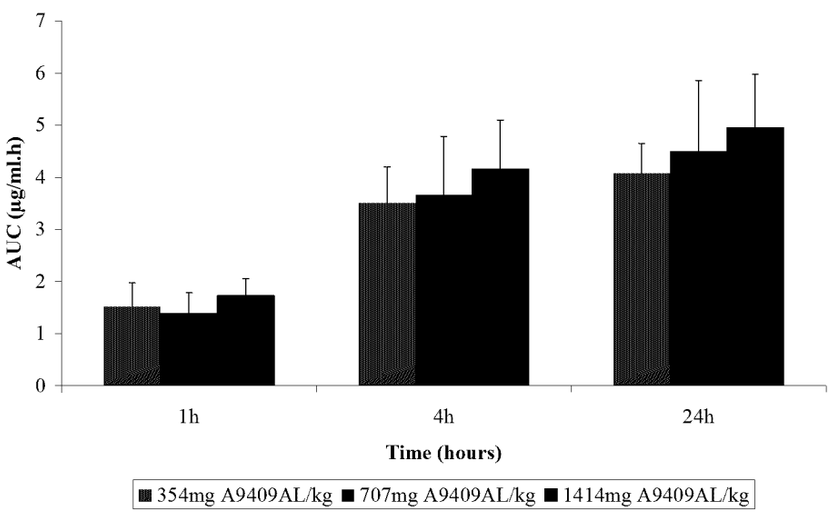
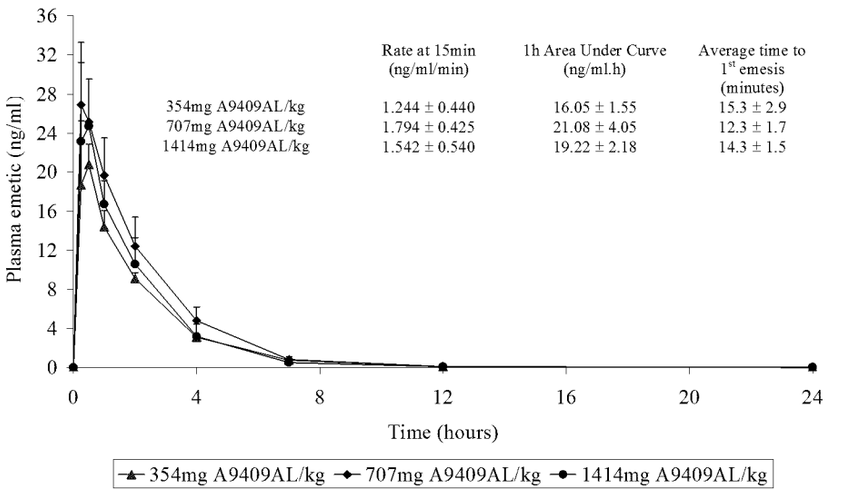



**Figure 6**
**Plasma emetic levels following an oral dose of A9409AL (354 - 1414mg formulation/kg b wt.) in dogs (mean and SEM, n=3).**




| | Rate at 15min (ng/ml/min) | 1h Area Under Curve (ng/ml.h) | Average time to 1st emesis (minutes) |
|---|---|---|---|
| 354mg A9409AL/kg | 1.244 ± 0.440 | 16.05 ± 1.55 | 15.3 ± 2.9 |
| 707mg A9409AL/kg | 1.794 ± 0.425 | 21.08 ± 4.05 | 12.3 ± 1.7 |
| 1414mg A9409AL/kg | 1.542 ± 0.540 | 19.22 ± 2.18 | 14.3 ± 1.5 |

## 4.0. Conclusion.

Syngenta's have developed a new paraquat formulation (A9409AL) based on INTEON technology which shows very low levels of paraquat absorption in vitro following dermal exposure and is significantly less irritant to skin and eye compared with the current French formulation R-Bix.

Paraquat 100g/l A9409AL INTEON formulation offers a significant reduction in the gastrointestinal absorption of paraquat following oral ingestion. Doses up to 1414mg A9409AL formulation/kg are without overt toxicity in the dog. The highest dose 1414mg formulation/kg (equivalent to 128mg paraquat ion/kg bw.) represents more than 10 times the lethal dose of paraquat on an mg paraquat ion/kg basis. This demonstrates in the dog, a vomiting species, a substantial reduction in paraquat absorption, which could be expected to provide a significant reduction in the amount of paraquat absorbed in humans.

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M J L Clapp and J R Heylings
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