

PQ products have been used successfully for over 40 years.

The toxicity of PQ has been extensively studied and when compared with most crop protection products, it has a very good toxicological profile. No chronic toxicity, reproductive toxicity, genetic toxicity, and it is not a skin sensitiser.

However, PQ can be acutely toxic if used incorrectly. The concentrate can irritate the skin/eyes and can be fatal if the undiluted product is swallowed.

Syngenta has therefore put major research effort into reducing the acute tox profile of its PQ products.

This presentation covers some of our recent toxicological research in this area, where we have been investigating alginate gels in modified formulations of Gramoxone, which contains 200g/l paraquat ion.



Scientists at Syngenta's Central Toxicology Laboratory in the UK have been studying the oral and skin toxicity of PQ for a number of years with the aim of improving still further the safety profile of our products. Ideally, we want to identify a formulation that is less well absorbed orally and also reduces the the risk of causing skin irritation, even if the concentrated product was left on the skin for several hours.

In order to be useful as a herbicidal product, any additives to a formulation must still allow the concentrate to be manufactured, diluted in water, sprayed and disposed of without significant problems that may affect its safety. It must also be effective as a crop protection product.

Bullet 1 - We explored a wide range of soluble polymer gels and noted that certain polymers are used in pharmaceutical products since they adhere and protect biological membranes, including the gastrointestinal tract and skin.

Bullet 2 – Alginates are soluble gel-type polymers used in Algicon/Gaviscon oral preparations for heartburn and in wound care skin dressings in order to promote healing.

Bullet 3 – Over several years we identified and optimised PQ formulations that contained different alginates. This involved primarily toxicology studies but also much formulation research and herbicidal efficacy studies.



Alginates are carbohydrates containing different ratios of sugar molecules (mainly polymannuronic and polyguluronic acid residues. Our research identified that one particular type of alginate had the ability to reduce the absorption of PQ from the GI tract and also the penetration of PQ through the skin.

Bullet 1 - These carbohydrates are extracted from seaweed and are used very widely in foods such as ice cream and confectionary. They are also extensively used in drug manufacture, often to slow the release and absorption of drugs.

Bullet 2 - Alginates are impregnated into skin dressings and have been shown to promote the re-growth of skin tissue following topical damage. The fibres allow new tissue to form a barrier and being natural, eventually dissolve leaving harmless sugar products in the skin.

Bullet 3 – Alginates have an affinity for surfaces and preferentially adhere to the skin and mucous membranes like the gastric mucosa.

Bullet 4 – We found that following application to the skin of the concentrate PQ product that the water evaporates leaving behind a film deposit of precipitated alginate gel on the surface.

Paraquat Alginate Wall Technology (AWT)

Theoretical mechanism for oral safening (gelling, emesis and purgation)

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Bullet 1 - The choice of alginate in our quest to improve oral toxicity of PQ was also based on its known properties in the gastrointestinal tract. Alginates are used to treat gastric heartburn, gastritis and are also used to slow the delivery of drugs into the absorptive small intestine. Interestingly, they are also used as stomach bulking agents to treat obesity.

Bullet 2 - The key chemical action of alginates, when swallowed, is the immediate transformation of the fully soluble and dispersed gelling agent into a thick gelatinous precipitate as its hits the acid environment of the stomach - a **Triggered Gelling** effect. In gastric fluid, the hydrated alginate salt is converted into a porous, insoluble alginic acid gel. This slows the dissolution of PQ, coats the membrane lining of the stomach and the bulking effect inhibits gastric emptying via a vagal nerve reflex. Consequently, PQ cannot get out of the stomach to its site of absorption in the small intestine.

Bullet 3 - All PQ products contain an emetic agent which is centrally acting. Once the emetic agent is absorbed it triggers the vomit centre in the brain and expels the contents of the stomach. Fortunately, the emetic agent is much more lipophilic than PQ so it prefers to partition into the lipid stomach wall and is absorbed effectively, even in the presence of the gel.

Bullet 4 – We have also explored the addition of the purgative agent, magnesium sulphate, to the AWT

formulation. This causes an osmotic influx of water into the small intestine to clear any PQ that has managed to reach the absorptive region of the gut prior to vomiting.



This slide shows a diagrammatic representation of the human stomach.

Each of the 3 processes; gelling, emesis and purgation would in their own right reduce the oral absorption of PQ. Together they would act synergistically and could potentially improve the oral toxicity following oral ingestion.

A key consideration is that a minimally lethal dose of product contains an effective dose of gelling agent, emetic and purgative.

An extensive research programme has investigated the potential of the AWT technology to deliver such a benefit following oral ingestion.



Site of absorption of paraquat from the gastrointestinal tract

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Published research at CTL demonstrated that PQ is primarily absorbed beyond the stomach. The main site for uptake is the small intestine, particularly the jejunum.

The chart shows the absorption of paraquat in rat isolated mucosa from different regions of the gut from oesophagus to colon. The concentration used represents a typical ingested dose.

Absorption of PQ is mainly a passive diffusional process with polar ions like PQ being mainly absorbed in the "leaky" epithelia of the small bowel.

The small intestine represents the major surface area of the total GI tract so prevention of PQ from entering this region (stomach gelling), coupled with faster transit of luminal contents through this region (purgation), results in less absorption into the blood.

Paraquat Alginate Wall Technology (AWT)

Evidence for reduced oral toxicity in the dog

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This chart shows the blood levels of PQ following an oral dose of 200g paraquat ion/l formulation as either Gramoxone or the AWT formulation (A3879BU) in the adult male dog.

As with human ingestion (suicide attempts) the increase in dose is achieved by administration of increasing volume and therefore the dose of any component in the formulation would be increased as the dose of paraquat increases, mg/kg bw.

The AWT formulation under identical conditions of dosing etc. caused no toxicity over the dose range 40-320mg formulation per kg bodyweight. There was no toxicity in any animal and no effect on kidney or liver function.

The additional gel, emetic and purgative is more than compensating for the extra PQ given. Consistent with acid triggered gelling in the stomach, the formulation remaining in the stomach longer and more productive emesis. (More of the dose being removed from the body prior to the dose reaching the small intestines.

Emesis occurred at approximately 53mins - low dose and approximately 25 mins high dose.

[Gramoxone contains 0.5g/l emetic, whereas A3879BU contains 1.5g/l, but it is the effectiveness of the emetic and gelling which is important rather than the level of emetic per se.]

Gramoxone control data was generated at CTL between 1987 and 1991. Mean of 7 independent groups of dogs (n = 3 each). The blood levels shown (in black) are well tolerated in this species with no acute toxicity.

How does this relate to a lethal dose in dogs? This is kinetic study but we use a criteria of a peak plasma paraquat level of $10\mu g/ml$ or a 24 hour AUC of 40 $\mu g/ml$ /h as the criteria for humane termination of test animals since it would lead to overt toxicity.



Same study as previous slide but showing the Area Under Curve (AUC). This is the integration of the blood level of PQ between zero and 1h, zero and 4h and zero and 24h. It represents, in kinetic terms, the systemic exposure to PQ at these time intervals.

Note that at all intervals the AWT performs better than Gramoxone with lower systemic exposure despite the greatly increased dose of product.

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The same dog study has been analysed at discrete time intervals of 15min, 30min and 1h. The actual blood levels never exceed that of Gramoxone.

Importantly, no animals have vomited in the first 15min so the improvement in performance of AWT over Gramoxone is independent of emesis.

We interpret this as confinement of the PQ in the stomach (with the gelling agent) and thus keeping the PQ away from the site of absorption in the small intestine.

By 1h, the emetic in the AWT formulation has removed the stomach contents so the resulting blood levels are lower than would be expected if this had not occurred.



Evidence that emesis occurs and AWT is better than emetic alone

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In the same dog study the blood levels of the emetic agent, PP796 are measured. This centrally acting emetic is absorbed rapidly, being much more lipophilic than PQ. Once it reaches the vomit centre in the brain, it triggers emesis and inhibition of stomach emptying.

As the dose of PQ is increased the vomit reflex occurs earlier as more emetic reaches the blood faster.



Historical data in this dog model puts the performance of AWT (A3879BU) into context.

AWT gives much lower blood levels than both Gramoxone containing the standard 0.5g/l emetic and a Gramoxone containing almost five times this level (2.4g/l).

Note that high (and potentially toxic) levels of PQ are achieved at a dose of 240mg formulation/kg. In contrast, AWT has a much lower and non toxic response at 320mg formulation/kg.

Conclusions from this comparison are that fortification of Gramoxone with a very high level of emetic is much less effective that when it is combined with a gelling agent as present in AWT.

This re-enforces the synergism hypothesis that the combined effect of gelling and effective emesis is much more effective at reducing oral toxicity in a vomiting species.



Reiterate this a kinetic study. Absorption of the emetic increases with dose but there is some indication this might reach a plateau at higher levels such that time to emesis is not shortened.

Productive emesis – refers to more of the ingested dose being removed from the body.

Since good protection (no increase in paraquat plasma levels across a range of doses) has been observed at doses tested to date there is little evidence of purgation contributing to the safening, but at higher doses this may play a more prominent role.



This is the summary used on the poster and less technical than slide 17, which is essentially for medical audience



Analysed data for 563 poisonings (Japan, Korea, Sri Lanka, Crete) and plotted mortality against dose ingested and then produced best fit curve

The mean amount ingested for the latest Korea data (n= \sim 120) was \sim 5g paraquat ion equivalent to \sim 25mls compared with patients in the Sri Lanka and Korea magnesium sulphate treatment trial (n=85) who ingested an average of 10.5g paraquat ion equivalent to approx 52.5mls.

Variability in human data in estimating dose ingested, absorption and in treatment, but overall median lethal dose is about 3 - 3.5g paraquat ion (15 to 17.5mls of gramoxone 220g/l formulation) equivalent to ~50 - 60mg paraquat ion/kg bw for a 60kg human.

Given the uncertainties in human data should be precautionary in our predictions of benefits in human. 10 fold safening in dog translate to a 5 fold safening in man. The real evidence will be gained from epidemiology study – now at pilot stage in Sri Lanka. Collecting data before and after introduction of the AWT formulation.

5 fold safening in human moves the mortality curve to the right and results in an improvement in survival from 31% to 60+%. However this will vary depending on mean amounts ingested for that population – see earlier comment.

Assessment of Operator Hazard

> Several paraquat formulations are known to be skin irritants in concentrate form.

~Animal skin models are known to overestimate both irritation and penetration of chemicals.

➢ Particularly true for skin penetration of paraquat where the difference is 40 fold between rat and human.

> Therefore we have developed an in vitro screen to identify and optimise AWT formulation selection to give reduced irritation potential.

These benefits have then been confirmed in standard regulatory in vivo skin and eye irritation tests.

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In order to cause skin irritation, chemicals need to gain access to the living tissue below the epidermis. To do this the chemical has to cross the outer impermeable stratum corneum. Lipid soluble chemicals can do this relatively easily by simply dissolving in this lipid rich layer. PQ is very polar and cannot gain access through lipids. The only way water soluble molecules, like PQ, can get through the skin is via polar pathways, such as via the hair follicles.

This can be visualised as shown using a technique called autoradiography. Using radiolabelled PQ, added to Gramoxone, we have applied the product to the skin for 4h and then taken microscopic sections of the skin following freeze fixation of the tissue in liquid nitrogen. The black grains are the locations of the radioactive PQ that have been developed on a special photographic film.

The left panel show the skin surface (top left) with hair follicles protruding through the epidermis into the dermis (bottom right). PQ can be seen mainly on the surface and also in the hair follicles, but not in the dermis.

The right panel shows a high magnification of the dermis. The grains of radioactive PQ can be clearly seen in the cross sections of the hair follicles.

PQ absorption is therefore largely dependent on the follicle density of the skin. Human skin contains far fewer follicles than animal skin and consequently the skin penetration of PQ through human skin is very slow.

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This slide shows a microscopic picture of mouse skin following exposure to Gramoxone containing an alginate polymer. The skin was exposed to the concentrate for 4 hours prior to flash freezing.

The black dots are the nuclei of the cells in the epidermis (top) and underlying dermis. The section was stained for carbohydrate (shown in blue/green) which is clearly visible on the surface as an adherent gel wall.

The epidermis is completely normal following this treatment. Current research is investigating the localisation of PQ in the hair follicles following gel treatment to determine the mechanism by which the gel reduces skin penetration and irritation.



This slide shows the effectiveness the Alginate Wall Technology formulation to reduce the absorption of paraquat into mouse skin.

These autoradiograms are produced by exposure of mouse skin in vitro, placed in glass diffusion cells, to neat concentrate formulations of Gramoxone (Left panel) or Gramoxone AWT (Right panel). After 10min, the skin is frozen in liquid nitrogen and sections prepared for light microscopy. Photosensitive emulsion film is placed over the sections to locate the radioactivity in the whole skin section. The tissues are counterstained to show the location of the radioactivity.

In this hairy species, where penetration is via the polar routes, such as hair follicles, the amount of radioactive paraquat (shown by the red grains) that has reached the dermis is much higher, 10 minutes after exposure to the 200g/l Gramoxone concentrate. After penetrating through the barrier via hair follicles, paraquat can then diffuse freely in the living dermis.

At the same early time point, Gramoxone AWT has prevented paraquat from gaining entry to the living tissues of the dermis and radioactivity is minimal in both the epidermis and dermis. The paraquat is still largely external to the skin, remaining in the gel film on the epidermal surface.



Skin penetration of chemicals can be assessed in vitro (OECD Guideline 428). Discs of resected animal or human skin are mounted in glass diffusion chambers maintained at skin temperature.

The mouse was used in the screens since this has now become the standard species used in the SIFT(Skin Integrity Function Test) assay developed and now being validated Internationally as a predictive irritancy screen. Absorption was measured over a 4 hour period.

On human skin neat formulations and field-use dilutions are applied directly to the skin surface for 24 hours i.e a worst case situation, since it would be expected that the vast majority of workers would wash after a working day. The penetration of the active ingredient was measured over a 24 hour period in a complete balance study. In cases where application is for only 6 or 8 hours it is normal practise to include any present in the skin in the calculation of absorbed dose. Minimal amounts were found in the skin and this was compensated by the longer exposure period.

This left hand panel slide shows the effectiveness of the Alginate Wall Technology concentrate formulation to

reduce the penetration of paraquat using the mouse skin. In this hairy species, the absorption of PQ through the skin, using our in vitro skin model, is typically reduced by more than 5-fold fold with AWT products, when compared with Gramoxone.

In contrast, the less hairy human skin is much less permeable to PQ. The amount of PQ absorbed through human skin is a fraction of that seen in rodent skin, since it gains access mainly via hair follicles. On the same scale you can see this large difference in penetration between mouse and human for PQ.

On the right panel we have enlarged the human skin penetration scale by 100X. AWT human skin absorption is similar (but numerically lower) than Gramoxone.



This slide shows a comparison of the skin absorption properties of Gramoxone and AWT in both concentrate form and as the 1:100spray strength solution.

Two studies (1994 and 1999) have been performed with Gramoxone. These had different protocols due to emerging OECD guidelines. However, both studies gave similar results.

Although, the AWT formulation A3879BU is similar to the 1999 |Gramoxone study, there is an indication that the concentrate form is generally less well absorbed through human skin than the Gramoxone product.

In dilute form, PQ skin absorption is much lower than from the concentrate and no significant differences are seen.



The percentage percutaneous absorption values obtained in vitro for Gramoxone and A3879BU have been applied to the French operator exposure scenarios and show an improvement in the model calculations. Any improvement in practice is dependent on a number of factors, but it would be expected that the new formulation would result in a reduced operator risk.



In addition to the research programme aimed at identifying improved safety of PQ products, it is important that we also check key toxicological end points with any new formulation.

The alginate polymer itself is safe and an accepted food, cosmetic and pharmaceutical additive.

When formulated with PQ, it has no detrimental effect on the acute toxicity profile already established for Gramoxone.



The benefits of the AWT PQ formulation can be clearly seen in in vivo skin irritation studies.

Regulatory studies that define the classification of products are undertaken in the rabbit where the redness (erythema) and swelling (oedema) following application of the concentrated product are assessed using an internationally accepted scoring system.

The erythema response with the AWT formulation (A3879BU) is much lower and quicker to recover compared with Gramoxone.


Likewise, the oedema response is much better and reduced to zero faster with the AWT formulation.



In descriptive terms the AWT formulations do not show necrosis in the skin and the damage is restricted to the surface of the tissue.

The response is not absent but it is fully reversible and there is no evidence of scarring.

By visual assessment, the skin has fully recovered in 5 weeks, a week earlier than other Gramoxone products studied in the same protocol.



Eye irritation is improved with the AWT formulation compared with Gramoxone.



Eye irritation studies in the rabbit are also conducted on all new formulations as part of the registration process. The results with the AWT formulations are very promising.

The internationally accepted Draize scoring system assessed the AWT as less irritant to the eye. In particular, the iris and cornea effects seen with Gramoxone are much improved with AWT formulations.

The gelling component may therefore also prevent the dissolution and penetration of PQ into these tissues. It may also allow better decontamination of the eye surface by lacrimation.



Simpler overhead for EU based on only standard 200g/l formulation – Gramoxone vs. A3879BU.

Classification of skin and eye irritation is different around the world. This slide is based on the EU classification of formulated products and is a summary of the previous rabbit skin and eye studies on our lead AWT formulation, A3879BU.

With regard to the skin the new AWT formulation is still a skin irritant but the degree of irritation is much less and the A3879BU formulation concentrate would fall into the "non-irritant" classification within the EU. Current standard Gramoxone 200g paraquat ion/l product is R38 (veretical red arrow).

Similarly, with the eye studies, the AWT formulation A3879BU formulation would be classified as "non-irritant" in the EU, a clear improvement on other PQ products in the market. Current standard Gramoxone 200g paraquat ion/l product is R36 (vertical red arrow)

The selection of alginate polymers may therefore prove to be even safer in-use that our current products.



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-	Scientific rationale for AWT reducing the dermal and oral toxicity of paraquat formulations.
j.	Experimental evidence shows AWT formulations to be less irritant to the skin and eye.
*	Recent experimental data in dogs has shown a reduction in the gastrointestinal absorption of paraquat from an AWT formulation compared with Gramoxone.
*	It is anticipated this will eliminate fatalities from accidental ingestion and significantly increase survival following deliberate ingestions.

Bullets self explanatory.

Paraquat Alginate Wall Technology (AWT)

Paraquat formulations are known to be:
toxic by oral route
irritant to skin and eye

Hence Syngenta have been conducting an extensive programme of research with the aim of alleviating these toxic properties of paraquat products.



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The toxicity of PQ has been extensively studied and when compared with most crop protection products, it has a very good toxicological profile. No chronic toxicity, reproductive toxicity, genetic toxicity, and it is not a skin sensitiser.

However, PQ can be acutely toxic if used incorrectly. The concentrate can irritate the skin/eyes and can be fatal if the undiluted product is swallowed.

Syngenta has therefore put major research effort into reducing the acute tox profile of its PQ products.

This presentation covers some of our recent toxicological research in this area, where we have been investigating alginate gels in modified formulations of Gramoxone, which contains 200g/l paraquat ion.

Alginate Wall Technology

Syngenta have been evaluating a wide range of soluble polymers in the quest to identify safer formulations.

Gelling agents have known protective effects in pharmaceutical preparations for alleviating irritation in stomach and skin.

Following an extensive research programme over several years, inclusion of alginates in the formulations has been shown to offer benefits without interfering with herbicidal action.



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Bullet 1 – We explored a wide range of soluble polymer gels and noted that certain polymers are used in pharmaceutical products since they adhere and protect biological membranes, including the gastrointestinal tract and skin.

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Alginate Wall Technology

Ascophyllum Seaweed extract

Polysaccharide extensively used in the food industry.

Used in wound care and skin bandages. Fibres promote healing and eventually dissolve.

Preferentially binds to membrane surfaces like skin.

Forms protective membrane on surface that reduces skin irritancy and aids recovery.





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Paraquat Alginate Wall Technology (AWT)

Theoretical mechanism for oral safening (gelling, emesis and purgation)



Alginate Wall Technology

Syngenta have been investigating the acute oral toxicity of these AWT formulations.

They form gels in the acidic environment of the stomach, slowing the delivery of paraquat to the absorptive small intestine.

The emetic is more effective since the gelled formulation remains in the stomach longer.

> The addition of a soluble purgative agent, magnesium sulphate should remove any ingested paraquat that has reached the small intestine.



Bullet 1 - The choice of alginate in our quest to improve oral toxicity of PQ was also based on its known properties in the gastrointestinal tract. Alginates are used to treat gastric heartburn, gastritis and are also used to slow the delivery of drugs into the absorptive small intestine. Interestingly, they are also used as stomach bulking agents to treat obesity.

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AWT and Gastrointestinal Physiology



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Paraquat Alginate Wall Technology (AWT)

Site of absorption of paraquat from the gastrointestinal tract



Paraquat Absorption from the Gastrointestinal Tract



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Paraquat Alginate Wall Technology (AWT)

Evidence for reduced oral toxicity in the dog



Plasma paraquat following an oral dose of A3879BU (40 - 320mg formulation/kg bwt.) in dogs (n = 3)



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Plasma paraquat AUC values following an oral dose of A3879BU (40 - 320mg formulation/kg bwt.) in dogs (n = 3)



Same study as previous slide but showing the Area Under Curve (AUC). This is the integration of the blood level of PQ between zero and 1h, zero and 4h and zero and 24h. It represents, in kinetic terms, the systemic exposure to PQ at these time intervals.

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Paraquat Alginate Wall Technology (AWT)

Evidence for gelling



Plasma paraquat values following an oral dose of A3879BU (40 - 320mg formulation/kg bwt.) in dogs (n = 3)



The same dog study has been analysed at discrete time intervals of 15min, 30min and 1h. The actual blood levels never exceed that of Gramoxone.

Importantly, no animals have vomited in the first 15min so the improvement in performance of AWT over Gramoxone is independent of emesis.

We interpret this as confinement of the PQ in the stomach (with the gelling agent) and thus keeping the PQ away from the site of absorption in the small intestine.

By 1h, the emetic in the AWT formulation has removed the stomach contents so the resulting blood levels are lower than would be expected if this had not occurred.

Paraquat Alginate Wall Technology (AWT)

Evidence that emesis occurs and AWT is better than emetic alone



Plasma emetic values following an oral dose of A3879BU (40 - 320mg formulation/kg bwt.) in dogs (n = 3)



In the same dog study the blood levels of the emetic agent, PP796 are measured. This centrally acting emetic is absorbed rapidly, being much more lipophilic than PQ. Once it reaches the vomit centre in the brain, it triggers emesis and inhibition of stomach emptying.

As the dose of PQ is increased the vomit reflex occurs earlier as more emetic reaches the blood faster.

Plasma PQ following oral doses in the dog (n=3)

Comparison of different formulations with high emetic (Gramoxone with 2.4g/l and A3879BU with 1.5g/l)



Historical data in this dog model puts the performance of AWT (A3879BU) into context.

AWT gives much lower blood levels than both Gramoxone containing the standard 0.5g/l emetic and a Gramoxone containing almost five times this level (2.4g/l). Note that high (and potentially toxic) levels of PQ are achieved at a dose of 240mg formulation/kg. In contrast, AWT has a much lower and non toxic response at 320mg formulation/kg.

Conclusions from this comparison are that fortification of Gramoxone with a very high level of emetic is much less effective that when it is combined with a gelling agent as present in AWT.

This re-enforces the synergism hypothesis that the combined effect of gelling and effective emesis is much more effective at reducing oral toxicity in a vomiting species.

Conclusions from assessment of oral toxicity of AWT formulation in a vomiting species

- AWT formulation across a 8-fold oral dose range (40-320mg formulation/kg) produced:
 - > No toxicity in dog and no change in liver or kidney function
 - > No increase in paraquat absorption
 - > The full extent of this safening is still under investigation.
- > Consistent with triggered gelling in the stomach prior to emesis.
- Reduction in time to first emesis with increasing dose is consistent with plasma emetic kinetics.
- AWT formulation provides more opportunity for productive emesis.
- Consistent with acid triggered gelling in the stomach delaying the movement of stomach contents into the small intestine.
Reiterate this a kinetic study. Absorption of the emetic increases with dose but there is some indication this might reach a plateau at higher levels such that time to emesis is not shortened.

Productive emesis – refers to more of the ingested dose being removed from the body.

Since good protection (no increase in paraquat plasma levels across a range of doses) has been observed at doses tested to date there is little evidence of purgation contributing to the safening, but at higher doses this may play a more prominent role.

Extrapolation to likely outcome in man

- The extent of the reduction is still being quantified, but the available results indicate that the technology would be expected to provide at least a 5-fold safening for humans
- Anticipate that the AWT formulation will eliminate fatal accidents of mistaken ingestion
- Anticipate that the AWT formulation will significantly increase the survival rate following intentional ingestion



This is the summary used on the poster and less technical than slide 17, which is essentially for medical audience

Extrapolation to likely outcome in man

- Data from human poisoning surveys have enabled us to produce a mortality probability curve for paraquat.
- Regional differences in the amount of paraquat deliberately ingested.
- Available human data is much more variable compared with dog.
- Prudent to assume that a shift in the absorption curve for paraquat of 10 fold in the dog would shift the human mortality probability curve 5 fold.
- > Overall survival rate in man is approximately 31%.
- 5-fold shift in the mortality probability curve is likely to double the percentage survival.



Analysed data for 563 poisonings (Japan, Korea, Sri Lanka, Crete) and plotted mortality against dose ingested and then produced best fit curve

The mean amount ingested for the latest Korea data (n=~120) was ~5g paraquat ion equivalent to ~25mls compared with patients in the Sri Lanka and Korea magnesium sulphate treatment trial (n=85) who ingested an average of 10.5g paraquat ion equivalent to approx 52.5mls.

Variability in human data in estimating dose ingested, absorption and in treatment, but overall median lethal dose is about 3 – 3.5g paraquat ion (15 to 17.5mls of gramoxone 220g/l formulation) equivalent to ~50 - 60mg paraquat ion/kg bw for a 60kg human.

Given the uncertainties in human data should be precautionary in our predictions of benefits in human. 10 fold safening in dog translate to a 5 fold safening in man. The real evidence will be gained from epidemiology study – now at pilot stage in Sri Lanka. Collecting data before and after introduction of the AWT formulation.

5 fold safening in human moves the mortality curve to the right and results in an improvement in survival from 31% to 60+%. However this will vary depending on mean amounts ingested for that population – see earlier comment.

Assessment of Operator Hazard

Several paraquat formulations are known to be skin irritants in concentrate form.

Animal skin models are known to overestimate both irritation and penetration of chemicals.

➢ Particularly true for skin penetration of paraquat where the difference is 40 fold between rat and human.

> Therefore we have developed an in vitro screen to identify and optimise AWT formulation selection to give reduced irritation potential.

These benefits have then been confirmed in standard regulatory in vivo skin and eye irritation tests.



Paraquat penetrates the skin through hair follicles

Autoradiograms of mouse skin following 4h Gramoxone exposure containing ¹⁴C-paraquat

Radioactivity mainly on surface



Radioactivity also in hair follicles





In order to cause skin irritation, chemicals need to gain access to the living tissue below the epidermis. To do this the chemical has to cross the outer impermeable stratum corneum. Lipid soluble chemicals can do this relatively easily by simply dissolving in this lipid rich layer. PQ is very polar and cannot gain access through lipids. The only way water soluble molecules, like PQ, can get through the skin is via polar pathways, such as via the hair follicles.

This can be visualised as shown using a technique called autoradiography. Using radiolabelled PQ, added to Gramoxone, we have applied the product to the skin for 4h and then taken microscopic sections of the skin following freeze fixation of the tissue in liquid nitrogen. The black grains are the locations of the radioactive PQ that have been developed on a special photographic film.

The left panel show the skin surface (top left) with hair follicles protruding through the epidermis into the dermis (bottom right). PQ can be seen mainly on the surface and also in the hair follicles, but not in the dermis.

The right panel shows a high magnification of the dermis. The grains of radioactive PQ can be clearly seen in the cross sections of the hair follicles.

PQ absorption is therefore largely dependent on the follicle density of the skin. Human skin contains far fewer follicles than animal skin and consequently the skin penetration of PQ through human skin is very slow.

Skin Morphology following AWT Exposure





This slide shows a microscopic picture of mouse skin following exposure to Gramoxone containing an alginate polymer. The skin was exposed to the concentrate for 4 hours prior to flash freezing.

The black dots are the nuclei of the cells in the epidermis (top) and underlying dermis. The section was stained for carbohydrate (shown in blue/green) which is clearly visible on the surface as an adherent gel wall.

The epidermis is completely normal following this treatment. Current research is investigating the localisation of PQ in the hair follicles following gel treatment to determine the mechanism by which the gel reduces skin penetration and irritation.

Mouse Skin Micro-Autoradiography

AWT 200g/l Gramoxone 200g/l **Epidermis Epidermis** Dermis Dermis

Radioactive PQ (red grains) in the epidermis and dermis 10 min following Gramoxone (left panel). PQ is only in the external gel 10 min following the AWT concentrate (right panel).

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This slide shows the effectiveness the Alginate Wall Technology formulation to reduce the absorption of paraquat into mouse skin.

These autoradiograms are produced by exposure of mouse skin in vitro, placed in glass diffusion cells, to neat concentrate formulations of Gramoxone (Left panel) or Gramoxone AWT (Right panel). After 10min, the skin is frozen in liquid nitrogen and sections prepared for light microscopy. Photosensitive emulsion film is placed over the sections to locate the radioactivity in the whole skin section. The tissues are counterstained to show the location of the radioactivity.

In this hairy species, where penetration is via the polar routes, such as hair follicles, the amount of radioactive paraquat (shown by the red grains) that has reached the dermis is much higher, 10 minutes after exposure to the 200g/I Gramoxone concentrate. After penetrating through the barrier via hair follicles, paraquat can then diffuse freely in the living dermis.

At the same early time point, Gramoxone AWT has prevented paraquat from gaining entry to the living tissues of the dermis and radioactivity is minimal in both the epidermis and dermis. The paraquat is still largely external to the skin, remaining in the gel film on the epidermal surface.

Skin Absorption: AWT Formulation A3879BU

Concentrate formulation: mouse and human skin



Note that there is a marked difference in skin absorption between mouse and human skin

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Skin penetration of chemicals can be assessed in vitro (OECD Guideline 428). Discs of resected animal or human skin are mounted in glass diffusion chambers maintained at skin temperature.

The mouse was used in the screens since this has now become the standard species used in the SIFT(Skin Integrity Function Test) assay developed and now being validated Internationally as a predictive irritancy screen. Absorption was measured over a 4 hour period.

On human skin neat formulations and field-use dilutions are applied directly to the skin surface for 24 hours i.e a worst case situation, since it would be expected that the vast majority of workers would wash after a working day. The penetration of the active ingredient was measured over a 24 hour period in a complete balance study. In cases where application is for only 6 or 8 hours it is normal practise to include any present in the skin in the calculation of absorbed dose. Minimal amounts were found in the skin and this was compensated by the longer exposure period.

This left hand panel slide shows the effectiveness of the Alginate Wall Technology concentrate formulation to reduce the penetration of paraquat using the mouse skin. In this hairy species, the absorption of PQ through the skin, using our in vitro skin model, is typically reduced by more than 5-fold fold with AWT products, when compared with Gramoxone.

In contrast, the less hairy human skin is much less permeable to PQ. The amount of PQ absorbed through human skin is a fraction of that seen in rodent skin, since it gains access mainly via hair follicles. On the same scale you can see this large difference in penetration between mouse and human for PQ.

On the right panel we have enlarged the human skin penetration scale by 100X. AWT human skin absorption is similar (but numerically lower) than Gramoxone.

Skin Absorption: Gramoxone and AWT Formulation

Concentrate and diluted formulations: human skin





This slide shows a comparison of the skin absorption properties of Gramoxone and AWT in both concentrate form and as the 1:100spray strength solution.

Two studies (1994 and 1999) have been performed with Gramoxone. These had different protocols due to emerging OECD guidelines. However, both studies gave similar results.

Although, the AWT formulation A3879BU is similar to the 1999 |Gramoxone study, there is an indication that the concentrate form is generally less well absorbed through human skin than the Gramoxone product.

In dilute form, PQ skin absorption is much lower than from the concentrate and no significant differences are seen.

Operator Exposure estimates using original POEM calculations and gloves for mixing loading



The percentage percutaneous absorption values obtained in vitro for Gramoxone and A3879BU have been applied to the French operator exposure scenarios and show an improvement in the model calculations. Any improvement in practice is dependent on a number of factors, but it would be expected that the new formulation would result in a reduced operator risk.

Acute toxicology of AWT formulations compared with Gramoxone 200g/l

No detrimental effect on: Oral toxicity (rat)

- Dermal toxicity (rat 24 hr occluded)
- Inhalation
- ➢Not a sensitiser



In addition to the research programme aimed at identifying improved safety of PQ products, it is important that we also check key toxicological end points with any new formulation.

The alginate polymer itself is safe and an accepted food, cosmetic and pharmaceutical additive.

When formulated with PQ, it has no detrimental effect on the acute toxicity profile already established for Gramoxone.

Comparative skin irritancy of 200g/l formulations: AWT (A3879BU) and Gramoxone



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The benefits of the AWT PQ formulation can be clearly seen in in vivo skin irritation studies.

Regulatory studies that define the classification of products are undertaken in the rabbit where the redness (erythema) and swelling (oedema) following application of the concentrated product are assessed using an internationally accepted scoring system.

The erythema response with the AWT formulation (A3879BU) is much lower and quicker to recover compared with Gramoxone.

Comparative skin irritancy of 200g/l formulations: AWT (A3879BU) and Gramoxone





Likewise, the oedema response is much better and reduced to zero faster with the AWT formulation.

Paraquat AWT formulation: Skin irritation (rabbit)

Gramoxone products:

- Evidence of necrosis full depth skin damage
- Scarring
- Recovery 43 days

New AWT formulations:

- e.g. A3879BU
- No necrosis
- Only superficial skin damage
- No scarring
- Full recovery in 35 days



In descriptive terms the AWT formulations do not show necrosis in the skin and the damage is restricted to the surface of the tissue.

The response is not absent but it is fully reversible and there is no evidence of scarring.

By visual assessment, the skin has fully recovered in 5 weeks, a week earlier than other Gramoxone products studied in the same protocol.

Comparative eye irritancy of 200g/l formulations: AWT (A3879BU) and Gramoxone





Speaker Notes: Eye irritation is improved with the AWT formulation compared with Gramoxone.

Comparative eye irritancy of 200g/l formulations: AWT (A3879BU) and Gramoxone



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Eye irritation studies in the rabbit are also conducted on all new formulations as part of the registration process. The results with the AWT formulations are very promising.

The internationally accepted Draize scoring system assessed the AWT as less irritant to the eye. In particular, the iris and cornea effects seen with Gramoxone are much improved with AWT formulations.

The gelling component may therefore also prevent the dissolution and penetration of PQ into these tissues. It may also allow better decontamination of the eye surface by lacrimation.

Paraquat Alginate Wall Technology (AWT)

EU Classification of skin and eye irritancy (A3879BU)



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Simpler overhead for EU based on only standard 200g/l formulation - Gramoxone vs. A3879BU.

Classification of skin and eye irritation is different around the world. This slide is based on the EU classification of formulated products and is a summary of the previous rabbit skin and eye studies on our lead AWT formulation, A3879BU.

With regard to the skin the new AWT formulation is still a skin irritant but the degree of irritation is much less and the A3879BU formulation concentrate would fall into the "non-irritant" classification within the EU. Current standard Gramoxone 200g paraquat ion/I product is R38 (veretical red arrow).

Similarly, with the eye studies, the AWT formulation A3879BU formulation would be classified as "non-irritant" in the EU, a clear improvement on other PQ products in the market. Current standard Gramoxone 200g paraquat ion/I product is R36 (vertical red arrow)

The selection of alginate polymers may therefore prove to be even safer in-use that our current products.

Paraquat Alginate Wall Technology (AWT)

EU Classification of skin and eye irritancy (A3879BU)





Classification of skin and eye irritation is different around the world. This slide is based on the EU classification of formulated products and is a summary of the previous rabbit skin and eye studies on our lead AWT formulation, A3879BU.

With regard to the skin the new AWT formulation is still a skin irritant but the degree of irritation is much less and the A3879BU formulation concentrate would fall into the "non-irritant" classification within the EU. Current PQ products are either R34 or R38 (horizontal arrows).

Similarly, with the eye studies, the AWT formulation A3879BU formulation would be classified as "non-irritant" in the EU, a clear improvement on other PQ products in the market. Current PQ products are either R41 or R36 (horizontal arrows).

The selection of alginate polymers may therefore prove to be even safer in-use that our current products.

Paraquat Alginate Wall Technology: Conclusions

- Scientific rationale for AWT reducing the dermal and oral toxicity of paraquat formulations.
- Experimental evidence shows AWT formulations to be less irritant to the skin and eye.
- Recent experimental data in dogs has shown a reduction in the gastrointestinal absorption of paraquat from an AWT formulation compared with Gramoxone.
- It is anticipated this will eliminate fatalities from accidental ingestion and significantly increase survival following deliberate ingestions.



Speaker Notes: Bullets self explanatory.