Paraquat Alginate Wall Technology (AWT)

Dr Jon R Heylings

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Syngenta Central Toxicology Laboratory, Alderley Park, United Kingdom

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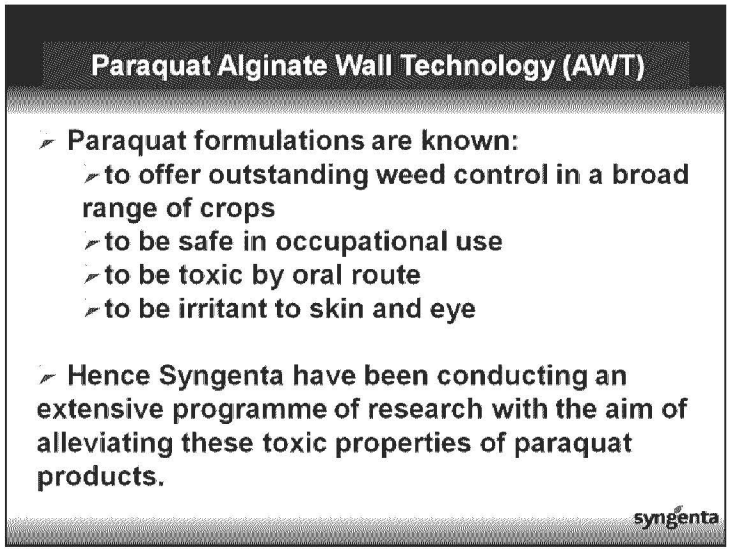


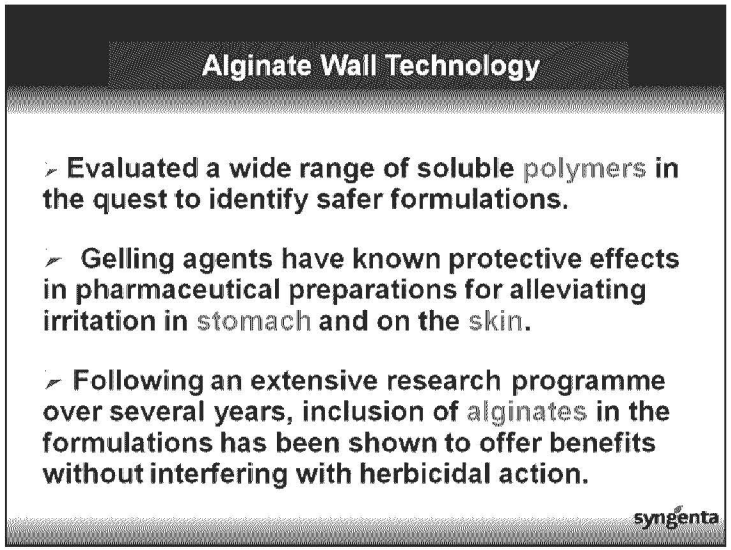
PQ products have been used successfully for over 40 years.

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.



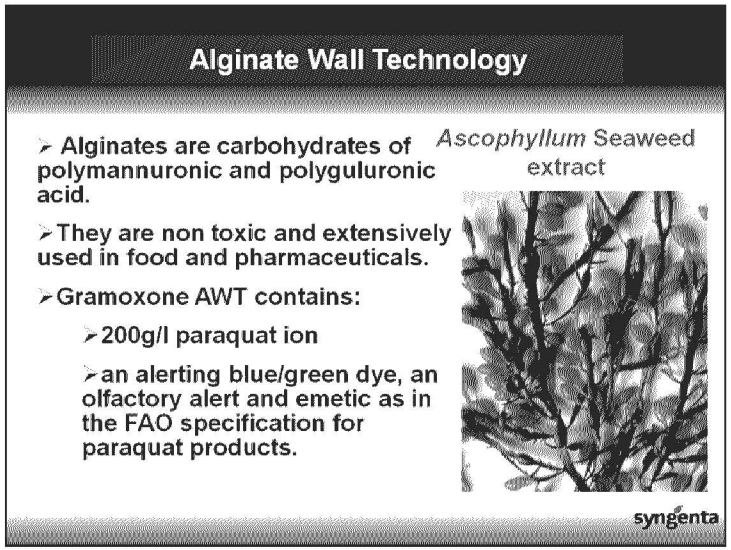
Scientists at Syngenta's Central Toxicology Laboratory in the UK have been studying the oral and skin toxicity of PQ for a number of years with the aim of improving still further the safety profile of our products. Ideally, we want to identify a formulation that is less well absorbed orally and also 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)

Site of absorption of paraquat from the gastrointestinal tract

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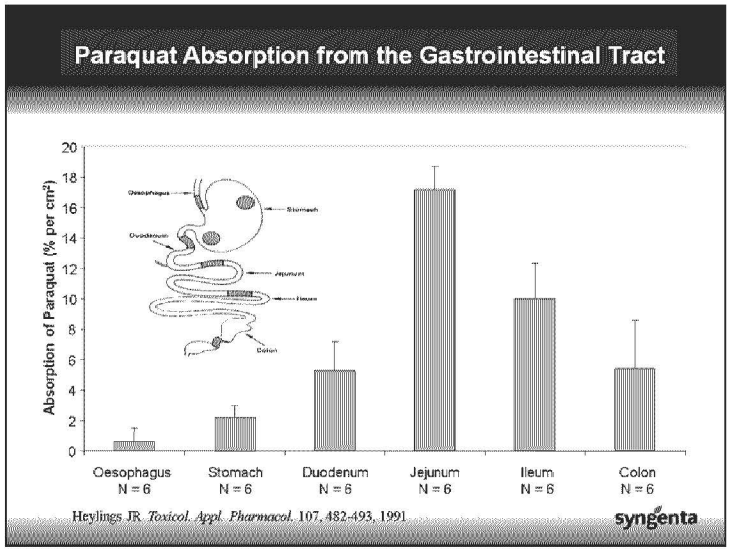


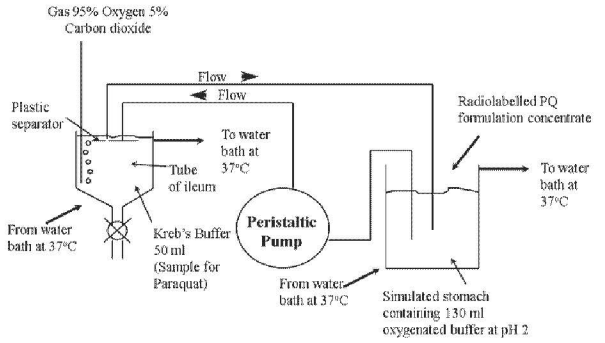
Published research at CTL demonstrated that PQ is primarily absorbed beyond the stomach. The main site for uptake is the small intestine, particularly the jejunum.

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.

Screening Model for Intestinal Absorption



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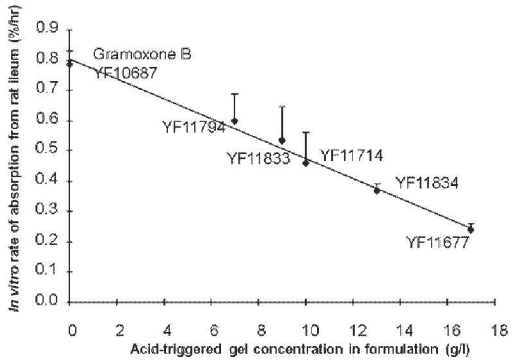
Optimisation of Alginate Wall Formulations



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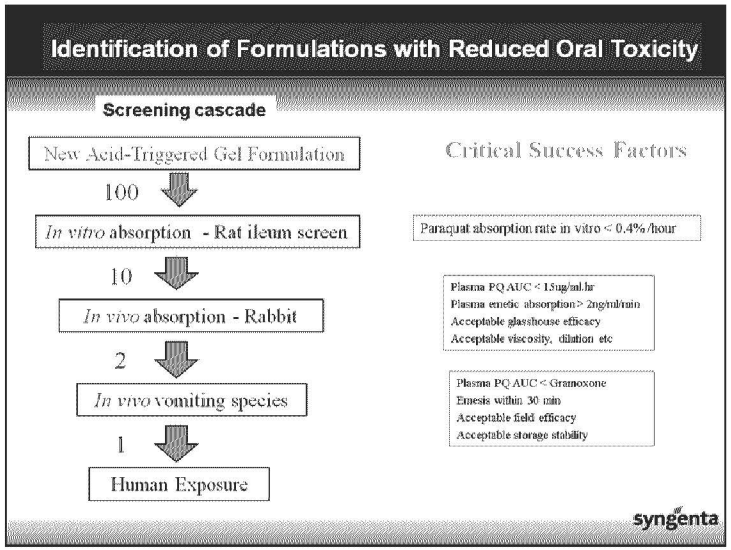
Identification of Formulations with Reduced Oral Toxicity
Screening cascade
New Acid-Triggered Gel Formulation
100
In vitro absorption - Rat ileum screen
10
In vivo absorption - Rabbit
2
In vivo vomiting species
1
Human Exposure
Critical Success Factors
Paraquat absorption rate in vitro < 0.4%/hour
Plasma PQ AUC < 15ug/ml.hr
Plasma enetic absorption > 2ng/ml/min
Acceptable glasshouse efficacy
Acceptable viscosity, dilution etc
Plasma PQ AUC < Gramoxone
Emesis within 30 min
Acceptable field efficacy
Acceptable storage stability
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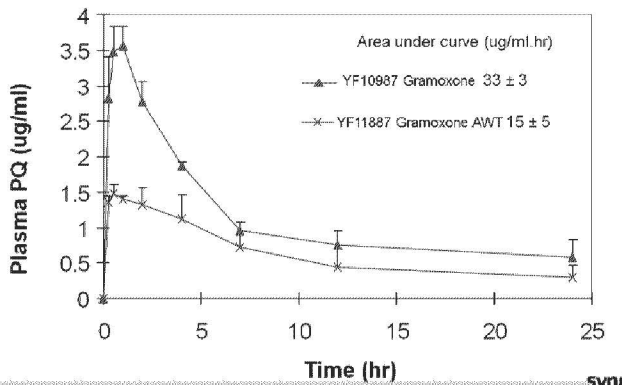
Paraquat Absorption in the Rabbit (200mg formulation/kg)



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Paraquat Alginate Wall Technology (AWT)

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

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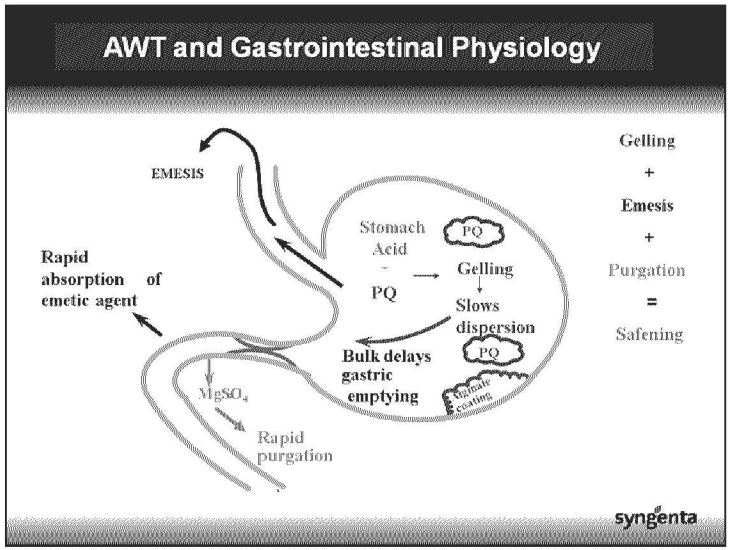

This slide shows a diagrammatic representation of the human stomach.

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.

Paraquat Alginate Wall Technology (AWT)

Evidence for reduced absorption and oral toxicity in the dog

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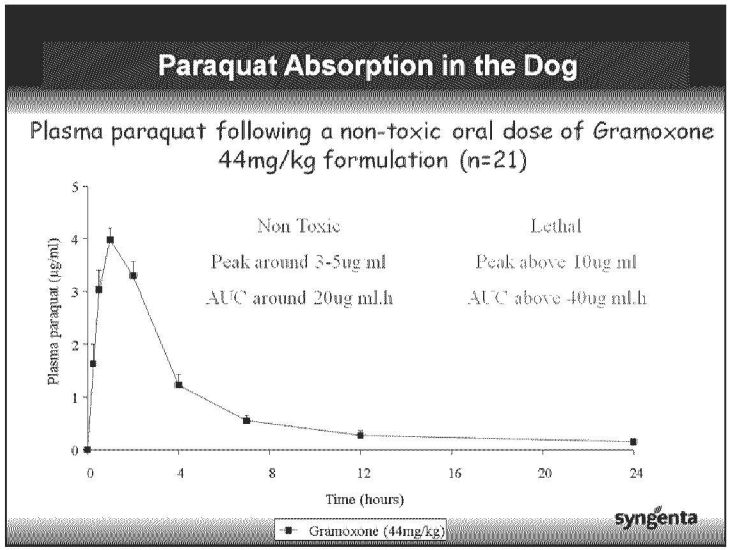


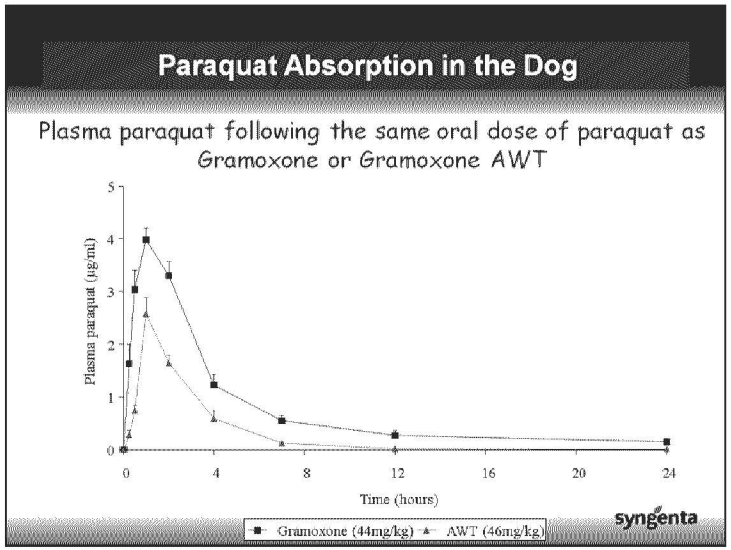
This chart shows the blood levels of PQ following an oral sub-lethal dose of Gramoxone 200g/l formulation in the adult male dog (when dosed at 40mg formulation/kg bodyweight, a lethal dose for dog would be 60 mg formulation /kg body weight.

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.

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 are well tolerated in this species with no acute toxicity.

Experience has shown that survival is related to the area below the curve: the smaller the area, the higher the % of survival.

The inclination of the curve during the first hour is also critical: the steepest it is, the worse.



This chart shows the blood levels of PQ following an oral sub-lethal dose of 200g paraquat ion/l formulation as either Gramoxone or the AWT formulation (A3879BU) in the adult male dog when dosed at 40mg formulation/kg bodyweight.

The AWT formulation under identical conditions of dosing etc. caused no toxicity. There was no toxicity in any animal and no effect on kidney or liver function.

Consistent with acid triggered gelling in the stomach, the formulation remains in the stomach longer and there is a more productive emesis (more of the dose being removed from the body prior to the dose reaching the small intestines).

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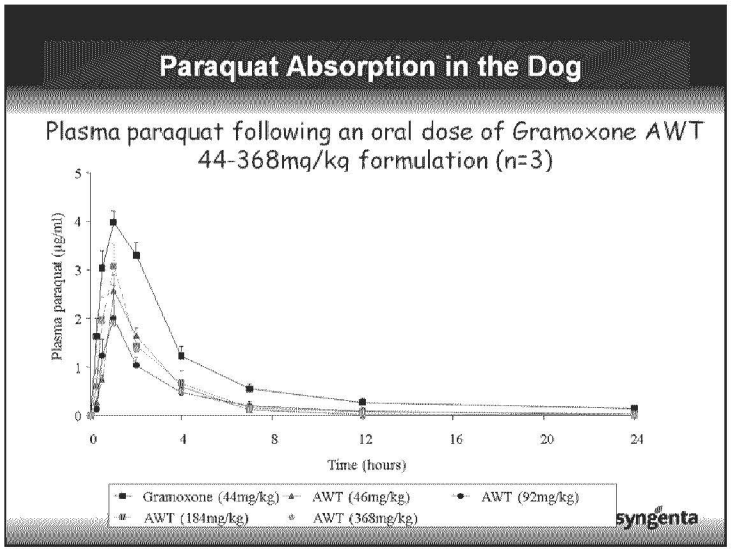

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The AWT formulation under identical conditions of dosing etc. caused no toxicity over the dose range 40-320mg formulation per kg bodyweight. There was no toxicity in any animal and no effect on kidney or liver function.

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.

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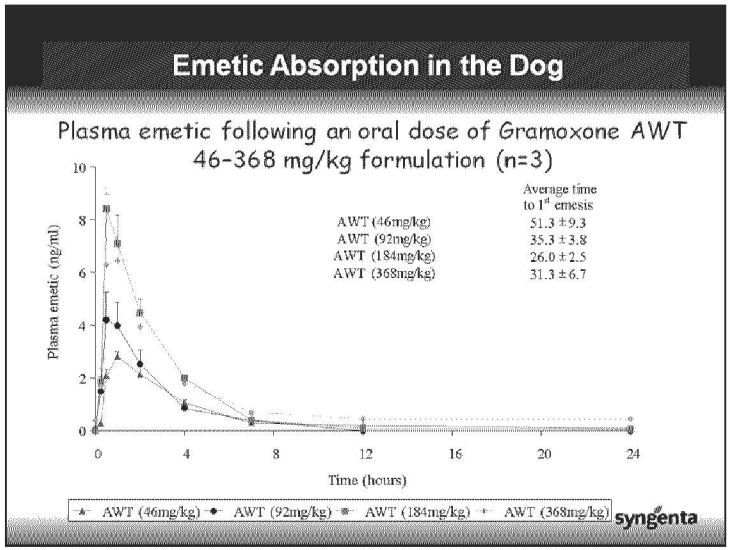
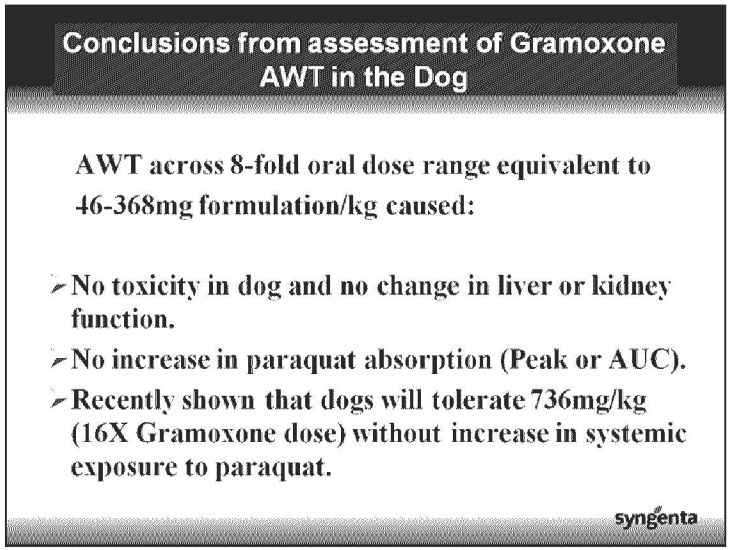
Gramoxone control data was generated at CTL between 1987 and 1991. Mean of 7 independent groups of dogs (n = 3 each). The blood levels shown (in black) are well tolerated in this species with no acute toxicity.

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 10µg/ml or a 24 hour AUC of 40 µg/ml /h as the criteria for humane termination of test animals since it would lead to overt toxicity.



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.

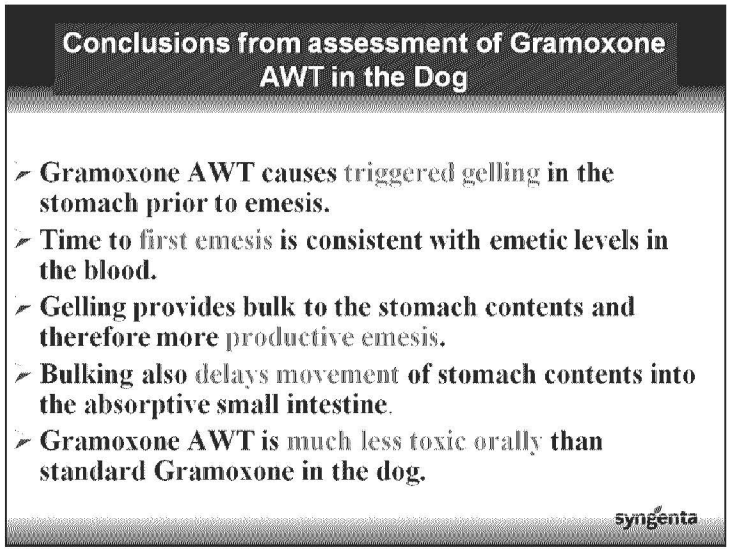
As the dose of PQ is increased the vomit reflex occurs earlier as more emetic reaches the blood faster.



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.



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Extrapolation to likely outcome in man

- **Results indicate that the technology would be expected to provide at a** significant 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.**

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

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Paraquat Alginate Wall Technology (AWT)

- Paraquat formulations are known:
  - to offer outstanding weed control in a broad range of crops
  - to be safe in occupational use
  - to be toxic by oral route
  - to be 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.

Speaker Notes:
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.

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Alginate Wall Technology

- Evaluated 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 on the 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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Speaker Notes:
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.
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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.

Alginate Wall Technology

- Alginates are carbohydrates of polymannuronic and polyguluronic acid.
- They are non toxic and extensively used in food and pharmaceuticals.
- Gramoxone AWT contains:
  - 200g/l paraquat ion
  - an alerting blue/green dye, an olfactory alert and emetic as in the FAO specification for paraquat products.

***Ascophyllum* Seaweed extract**

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Speaker Notes:
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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)

Site of absorption of paraquat from the gastrointestinal tract

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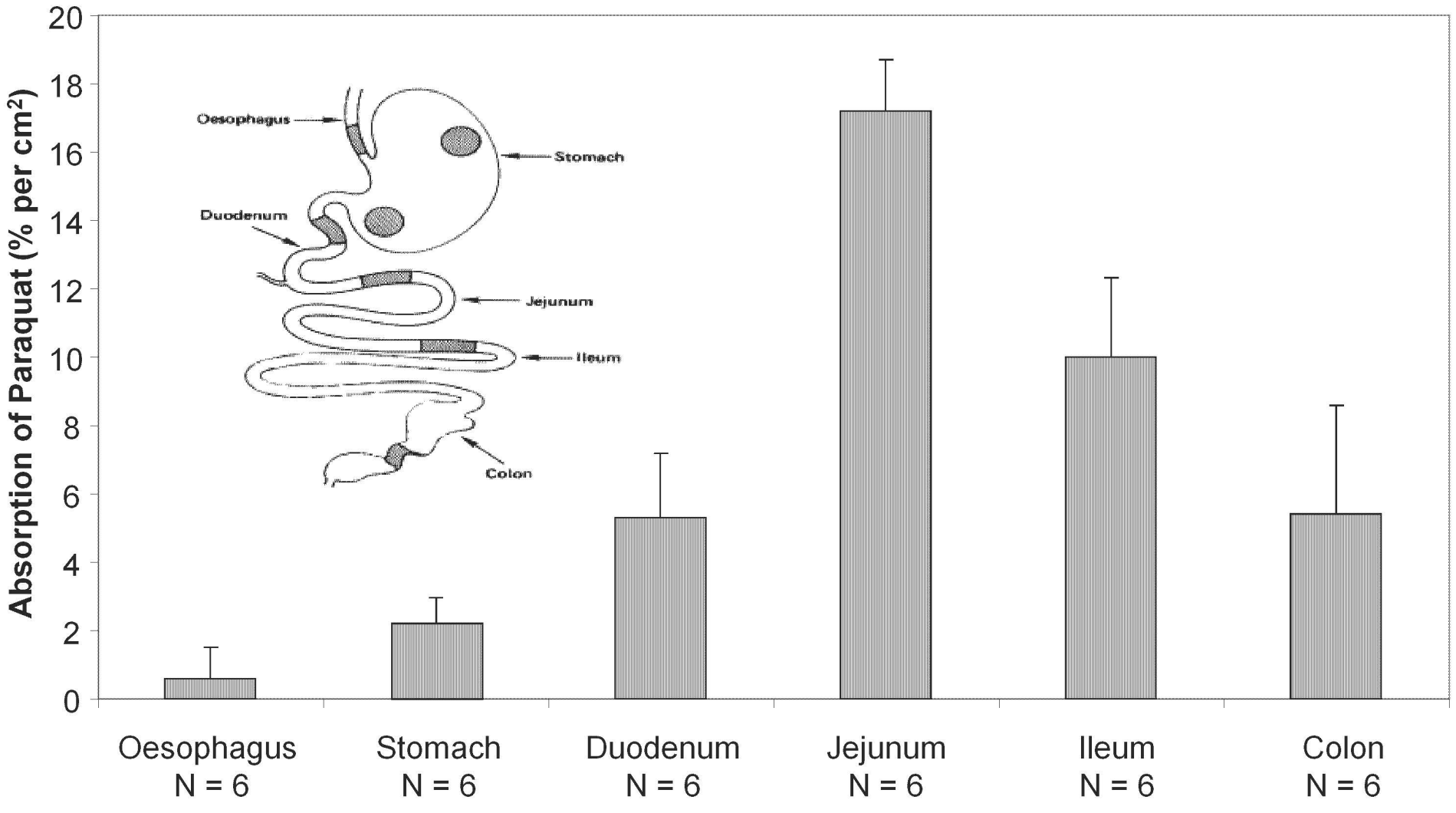
Paraquat Absorption from the Gastrointestinal Tract



Heylings JR *Toxicol. Appl. Pharmacol.* 107, 482-493, 1991

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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.

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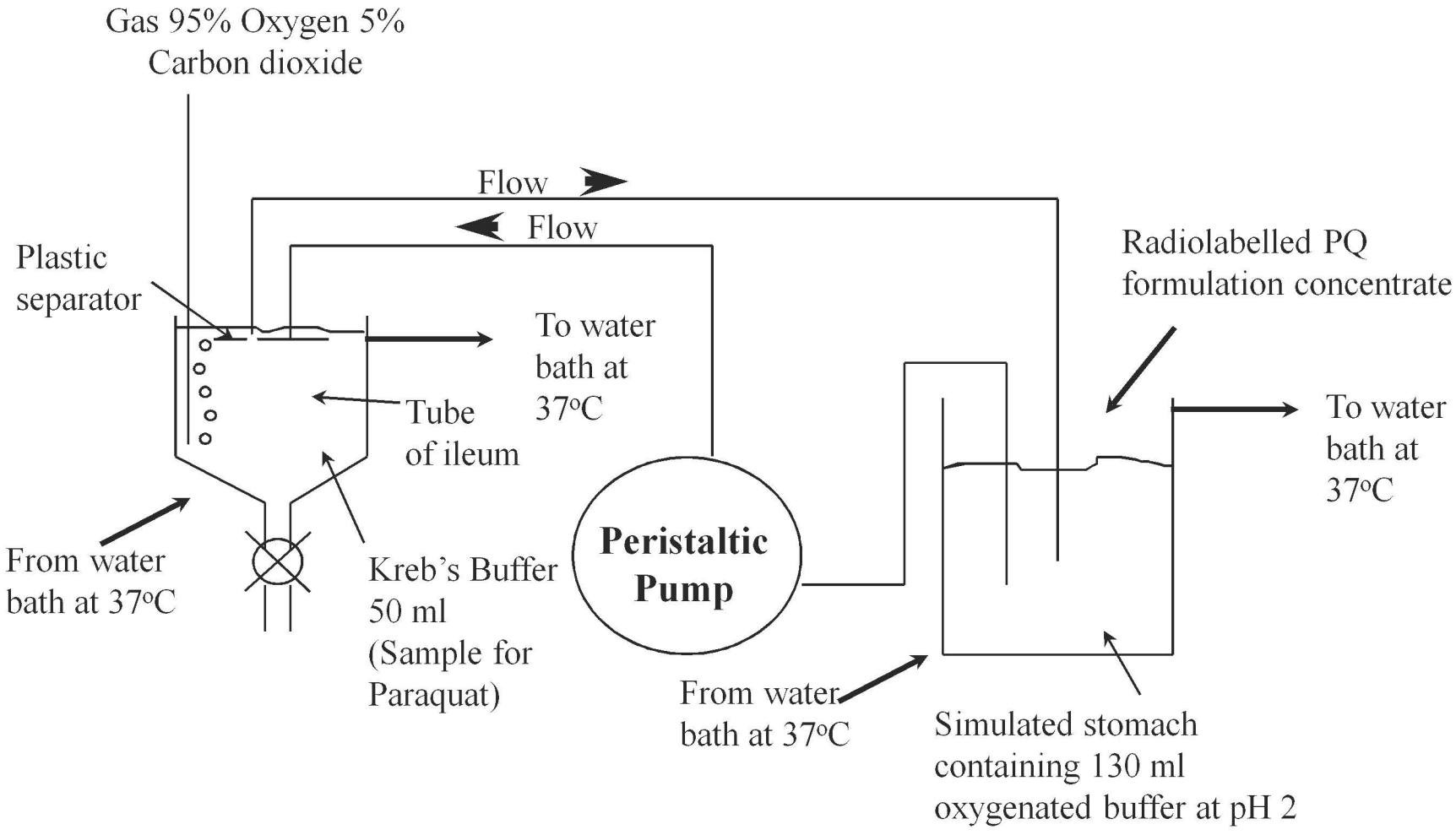
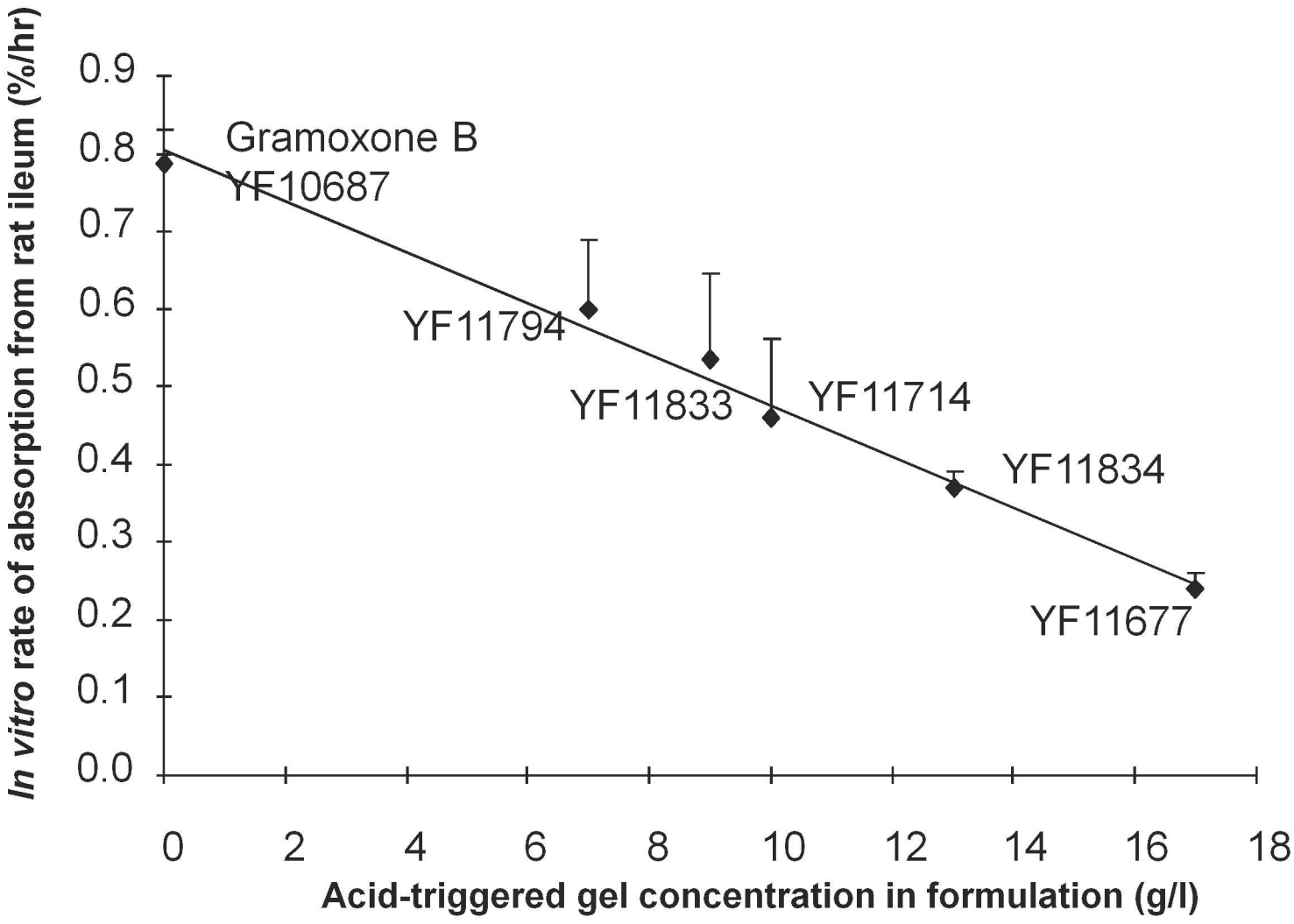
The small intestine represents the major surface area of the total GI tract so prevention of PQ from entering this region (stomach gelling), coupled with faster transit of luminal contents through this region (purgation), results in less absorption into the blood.

Screening Model for Intestinal Absorption



Optimisation of Alginate Wall Formulations



Identification of Formulations with Reduced Oral Toxicity

Screening cascade

New Acid-Triggered Gel Formulation

100

In vitro absorption - Rat ileum screen

10

In vivo absorption - Rabbit

2

In vivo vomiting species

1

Human Exposure

Critical Success Factors

Paraquat absorption rate in vitro < 0.4% /hour

Plasma PQ AUC < 15ug/ml.hr
Plasma emetic absorption > 2ng/ml/min
Acceptable glasshouse efficacy
Acceptable viscosity, dilution etc

Plasma PQ AUC < Gramoxone
Emesis within 30 min
Acceptable field efficacy
Acceptable storage stability

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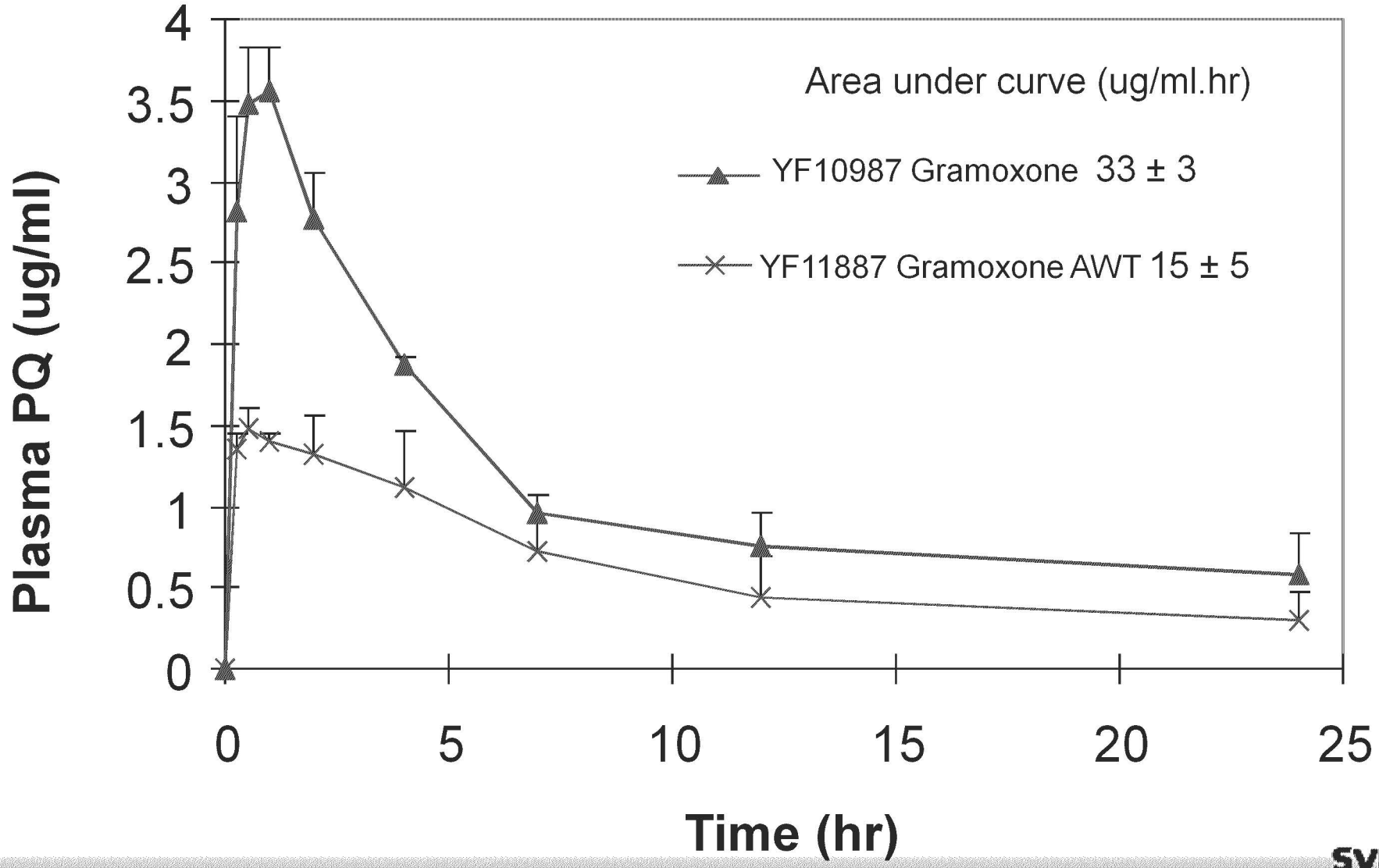
Paraquat Absorption in the Rabbit (200mg formulation/kg)



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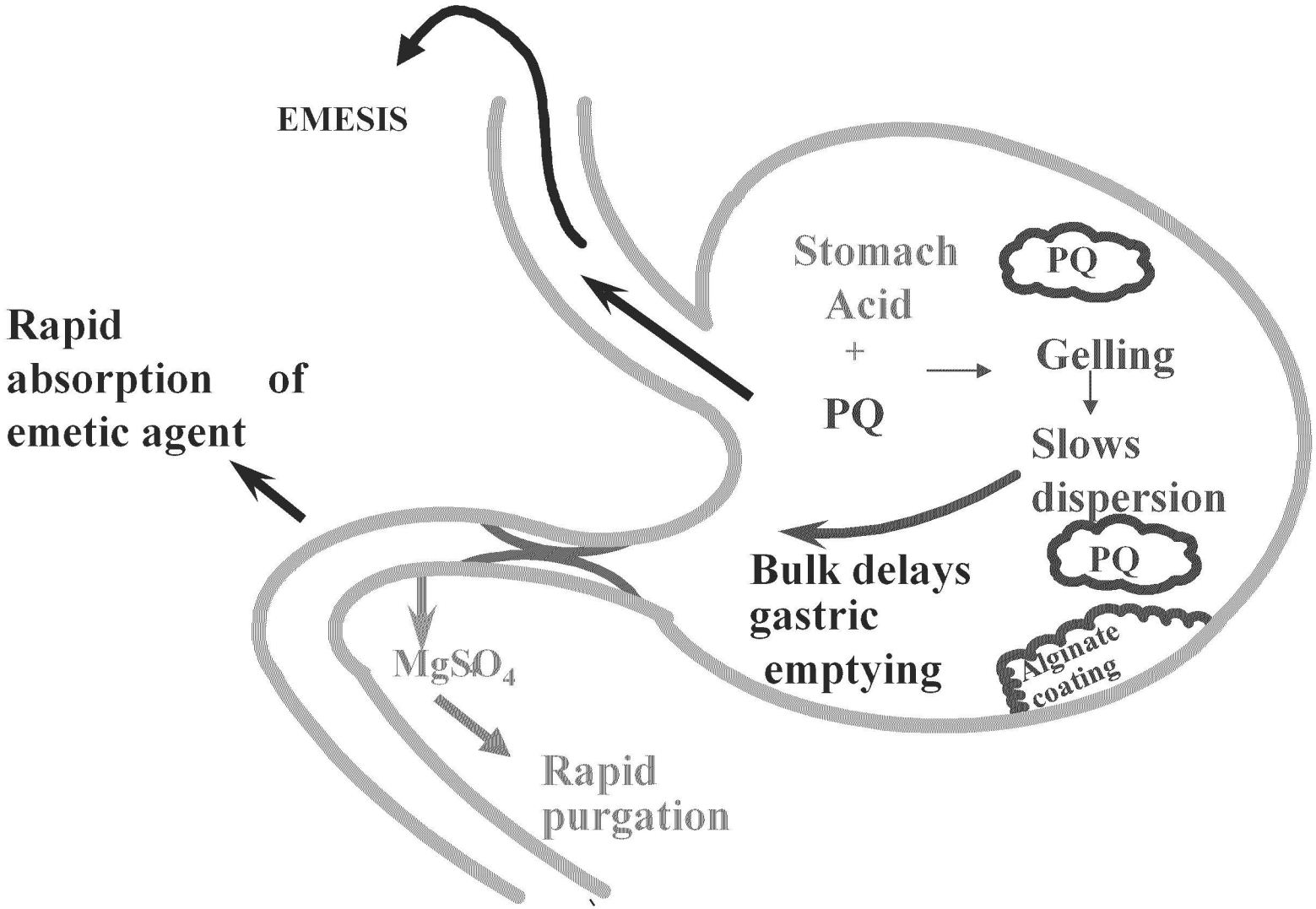
Paraquat Alginate Wall Technology (AWT)

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

AWT and Gastrointestinal Physiology



Gelling

+

Emesis

+

Purgation

=

Safening

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Speaker Notes:
This slide shows a diagrammatic representation of the human stomach.

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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.

Paraquat Alginate Wall Technology (AWT)

Evidence for reduced absorption and oral toxicity in the dog

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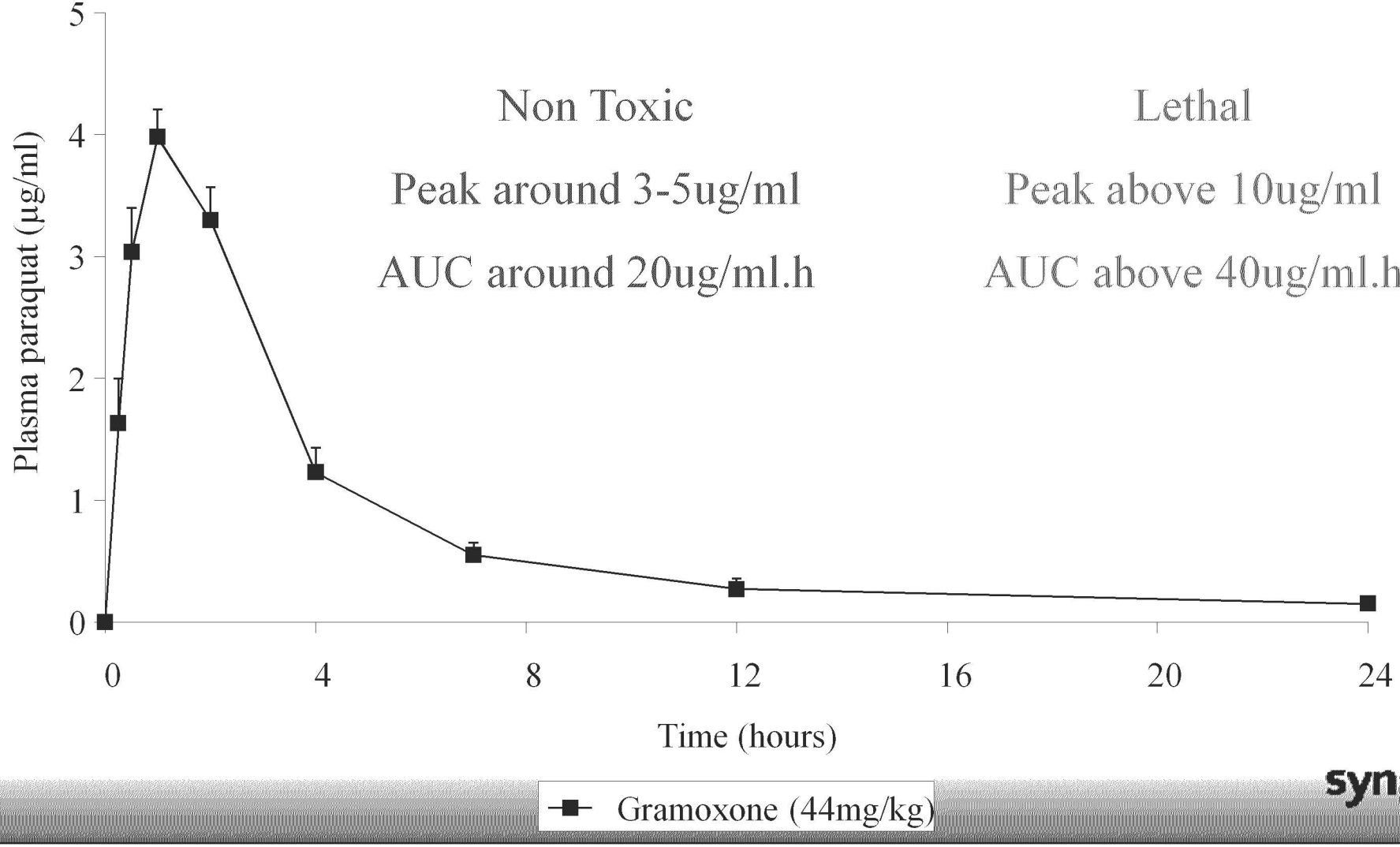
Paraquat Absorption in the Dog

Plasma paraquat following a non-toxic oral dose of Gramoxone 44mg/kg formulation (n=21)



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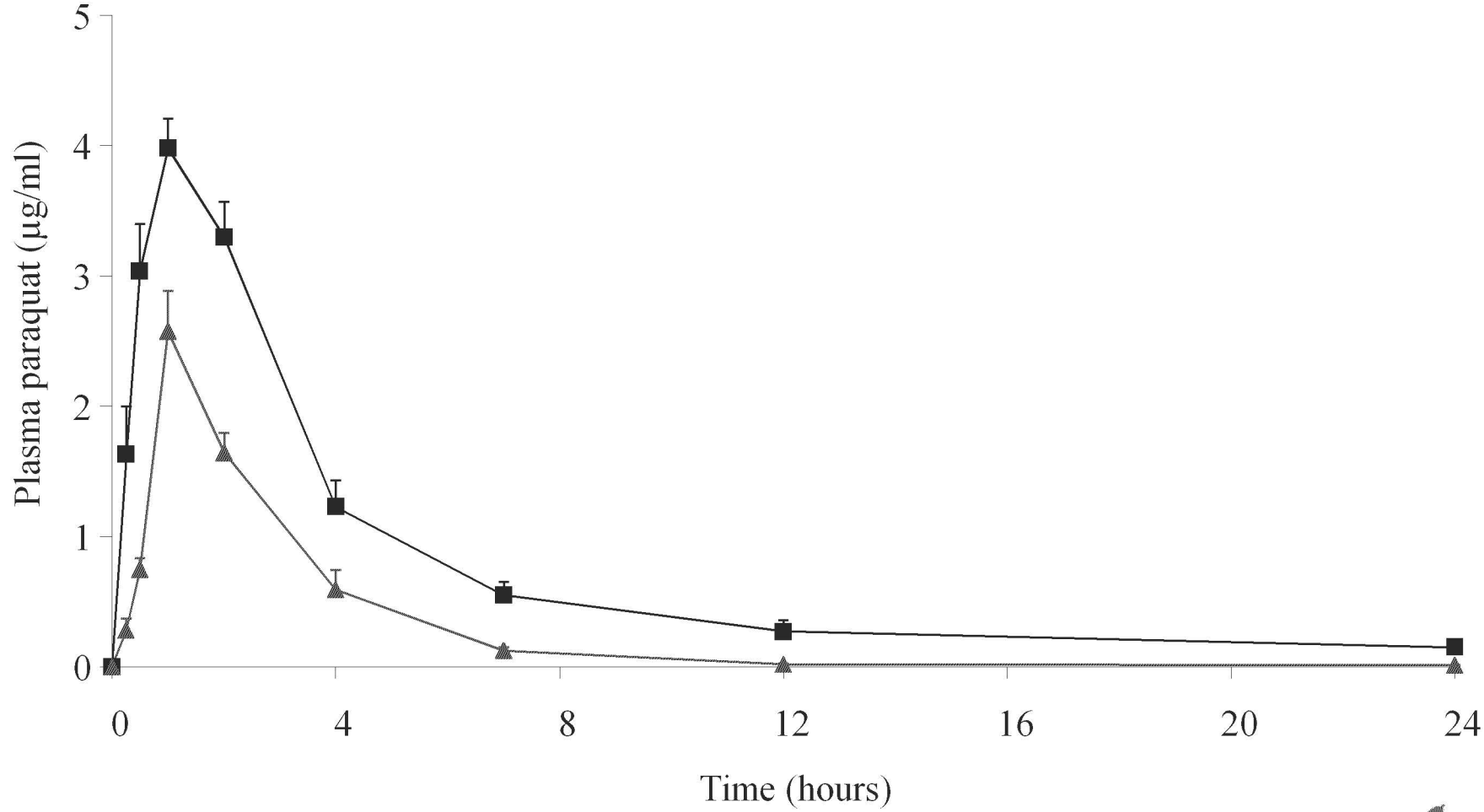
Experience has shown that survival is related to the area below the curve: the smaller the area, the higher the % of survival.

The inclination of the curve during the first hour is also critical: the steepest it is, the worse.

Paraquat Absorption in the Dog

Plasma paraquat following the same oral dose of paraquat as Gramoxone or Gramoxone AWT



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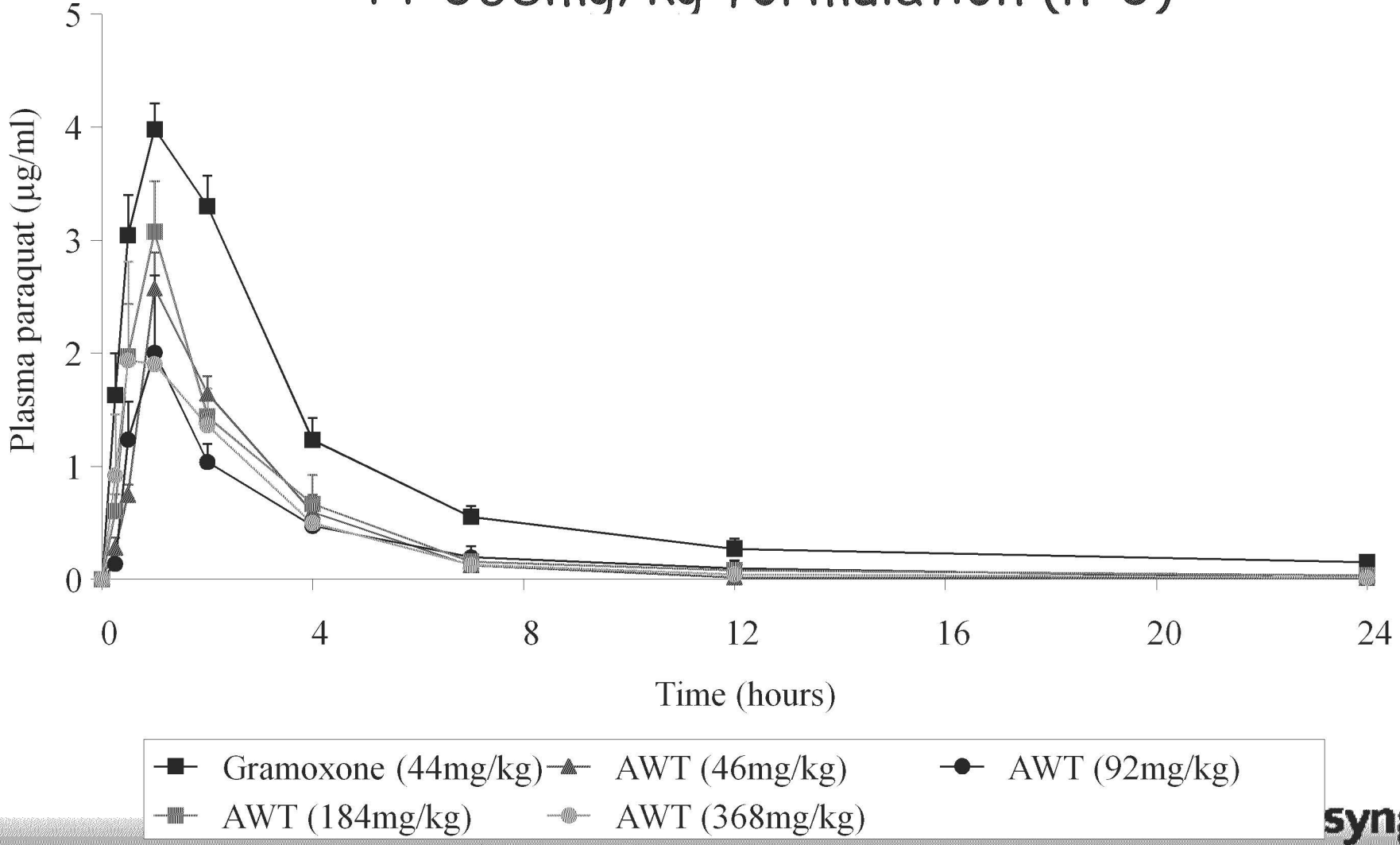
Paraquat Absorption in the Dog

Plasma paraquat following an oral dose of Gramoxone AWT 44-368mg/kg formulation (n=3)



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

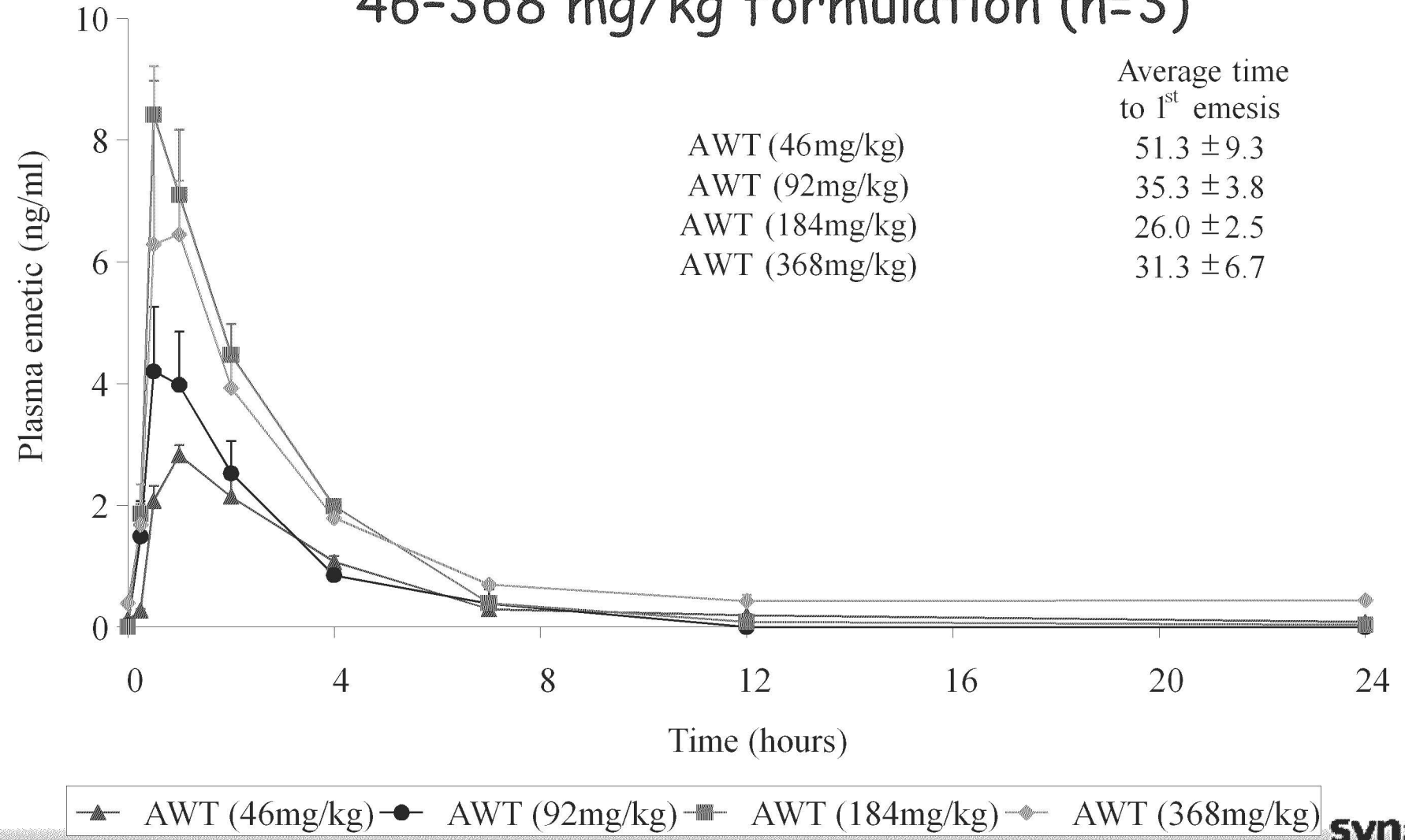
[Gramoxone contains 0.5g/l emetic, whereas A3879BU contains 1.5g/l, but it is the effectiveness of the emetic and gelling which is important rather than the level of emetic per se.]

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 10µg/ml or a 24 hour AUC of 40 µg/ml /h as the criteria for humane termination of test animals since it would lead to overt toxicity.

Emetic Absorption in the Dog

Plasma emetic following an oral dose of Gramoxone AWT 46-368 mg/kg formulation (n=3)



	Average time to 1st emesis
AWT (46mg/kg)	51.3 ±9.3
AWT (92mg/kg)	35.3 ±3.8
AWT (184mg/kg)	26.0 ±2.5
AWT (368mg/kg)	31.3 ±6.7

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Speaker Notes:
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.

Conclusions from assessment of Gramoxone AWT in the Dog

AWT across 8-fold oral dose range equivalent to 46-368mg formulation/kg caused:

- **No toxicity in dog and no change in liver or kidney function.**
- **No increase in paraquat absorption (Peak or AUC).**
- **Recently shown that dogs will tolerate 736mg/kg (16X Gramoxone dose) without increase in systemic exposure to paraquat.**

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Speaker Notes:
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.

Conclusions from assessment of Gramoxone AWT in the Dog

- Gramoxone AWT causes triggered gelling in the stomach prior to emesis.
- Time to first emesis is consistent with emetic levels in the blood.
- Gelling provides bulk to the stomach contents and therefore more productive emesis.
- Bulking also delays movement of stomach contents into the absorptive small intestine.
- Gramoxone AWT is much less toxic orally than standard Gramoxone in the dog.

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Speaker Notes:
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

- **Results indicate that the technology would be expected to provide at a significant 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.**

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