GRAMOXONE™ INTEON

A new Paraquat formulation

Dr Mike Clapp
Senior Product Toxicologist

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Paraquat

- **Existing paraquat formulations**
  - **offer outstanding weed control in a broad range of crops**
  - **Does not pose unreasonable risk when used according to the label.**
- **From toxicology studies**
  - **they show toxicity by oral route**
  - **they show irritancy to skin and eye**
- **Syngenta has therefore been conducting an extensive programme of research with the aim of reducing this toxicity**

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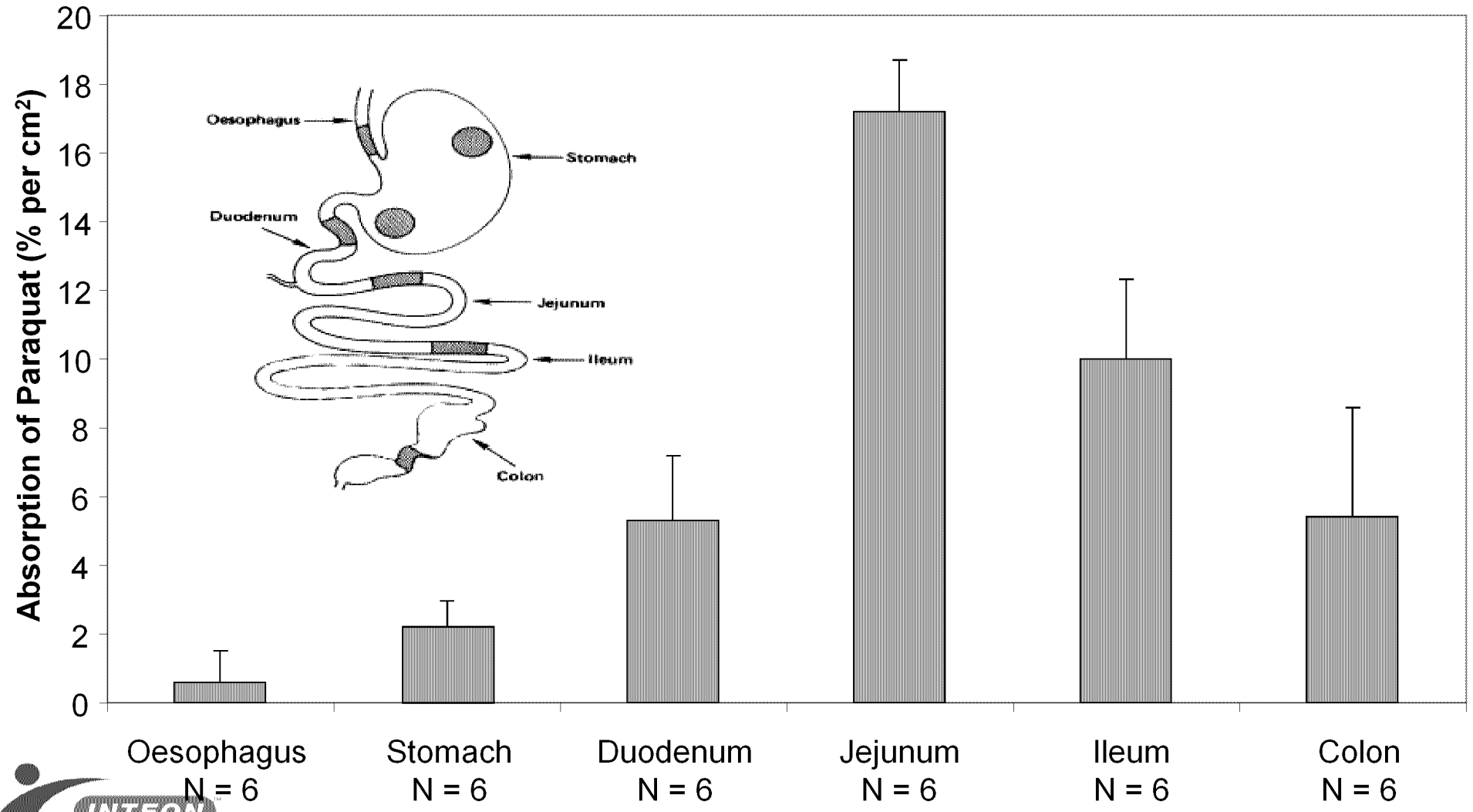
Site of absorption of paraquat from the gastrointestinal tract

Paraquat Absorption from the Gastrointestinal Tract



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

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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.

INTEON 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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INTEON Technology

- Alginates are carbohydrates of polymannuronic and polyguluronic acid
- They are non toxic and extensively used in the food and pharmaceutical industries
- Gramoxone INTEON contains:
  - 200 or 240 g/l paraquat ion
  - an alerting blue/green dye, an olfactory alert and the effective emetic as stipulated in the FAO specification for paraquat products
  - INTEON technology

***Ascophyllum* Seaweed extract**

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Paraquat INTEON technology

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

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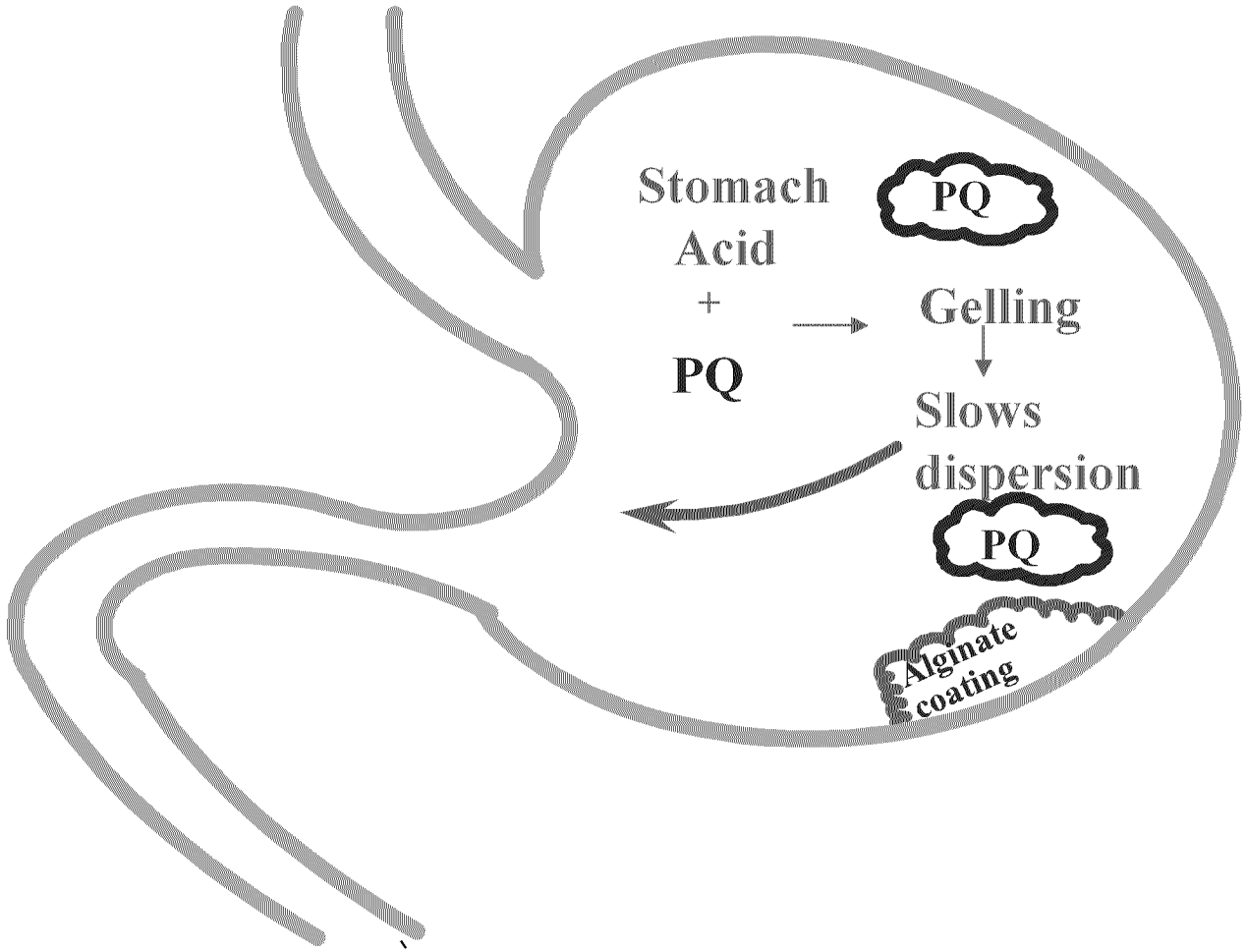
Gastrointestinal physiology



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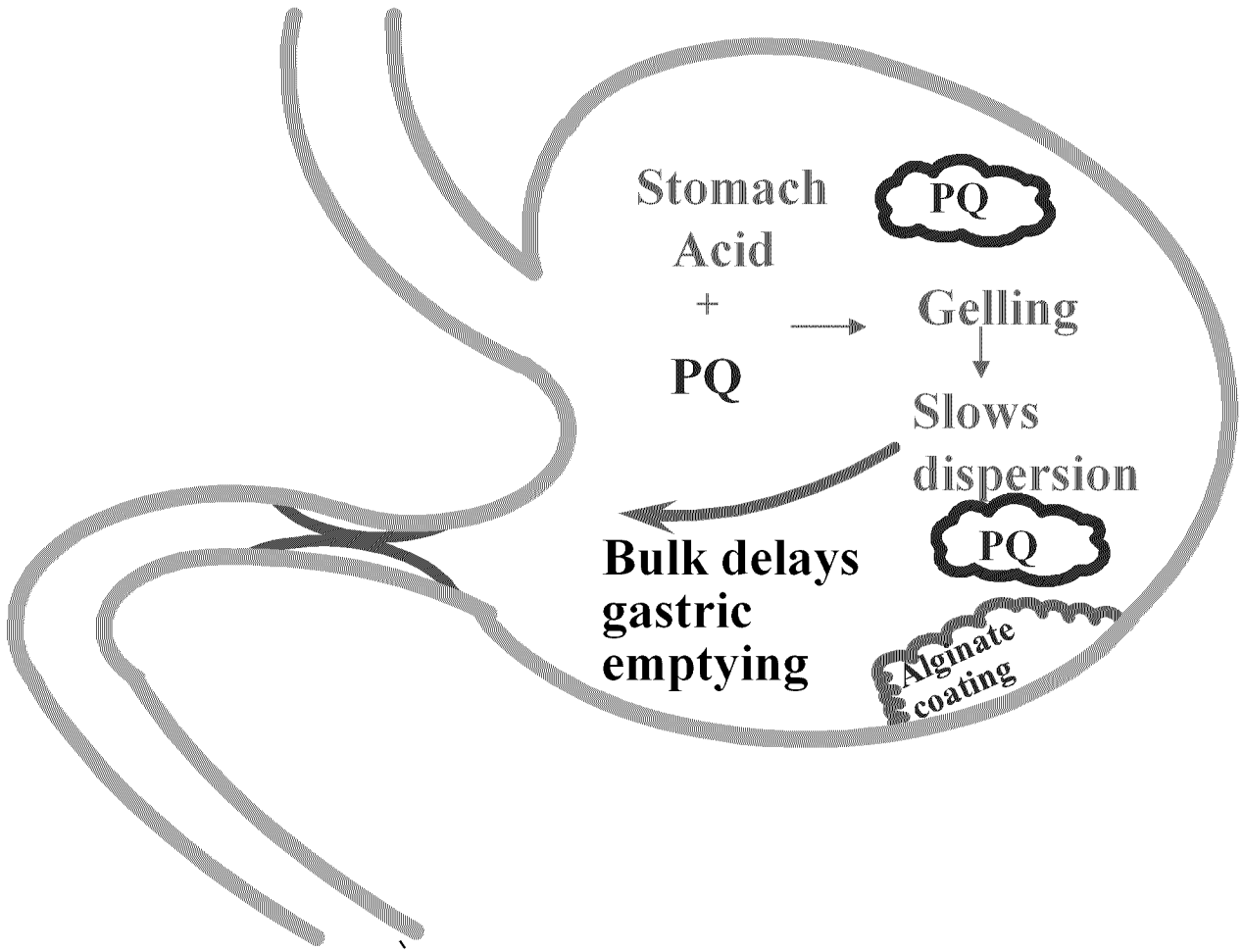
Gastrointestinal physiology



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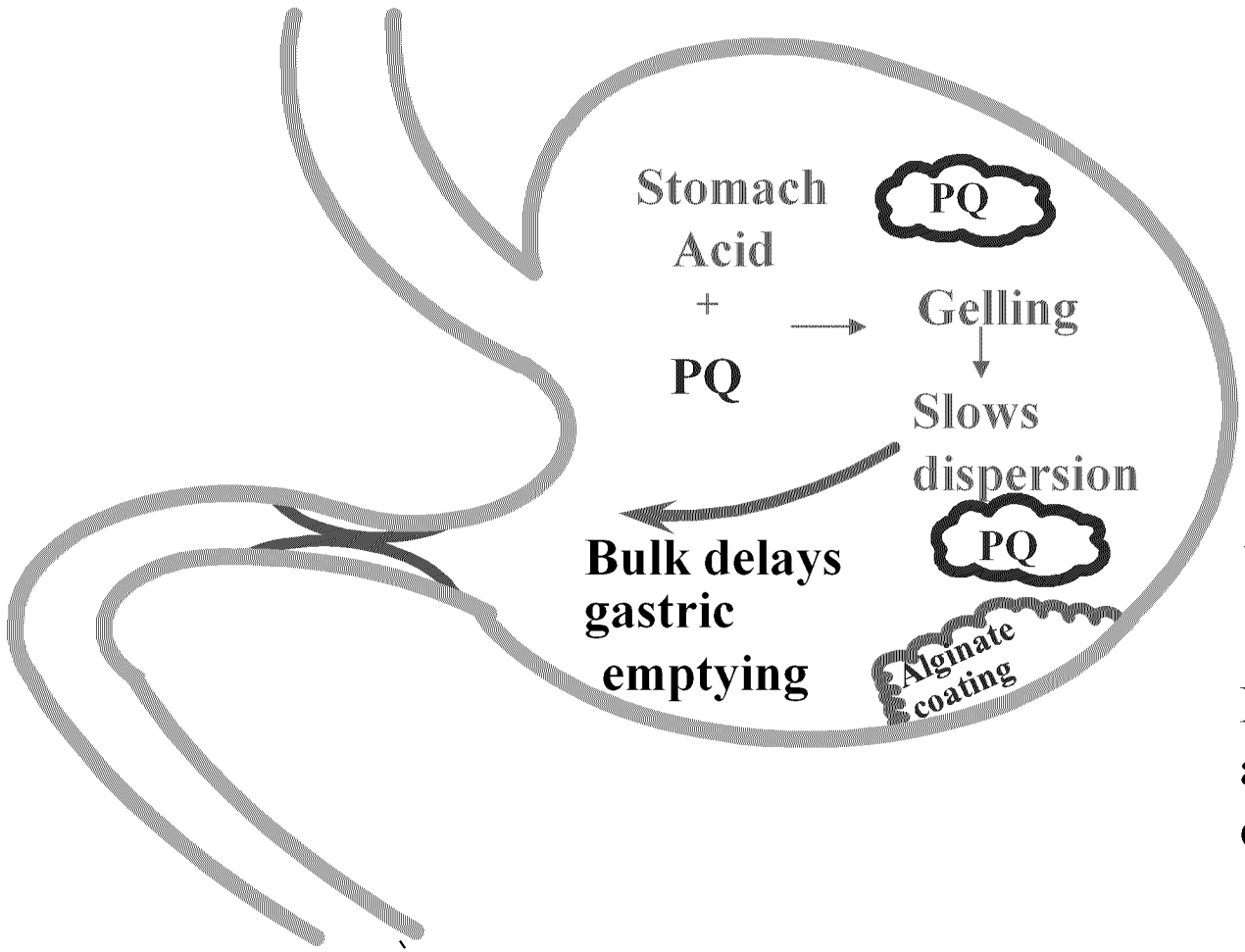
Gastrointestinal physiology



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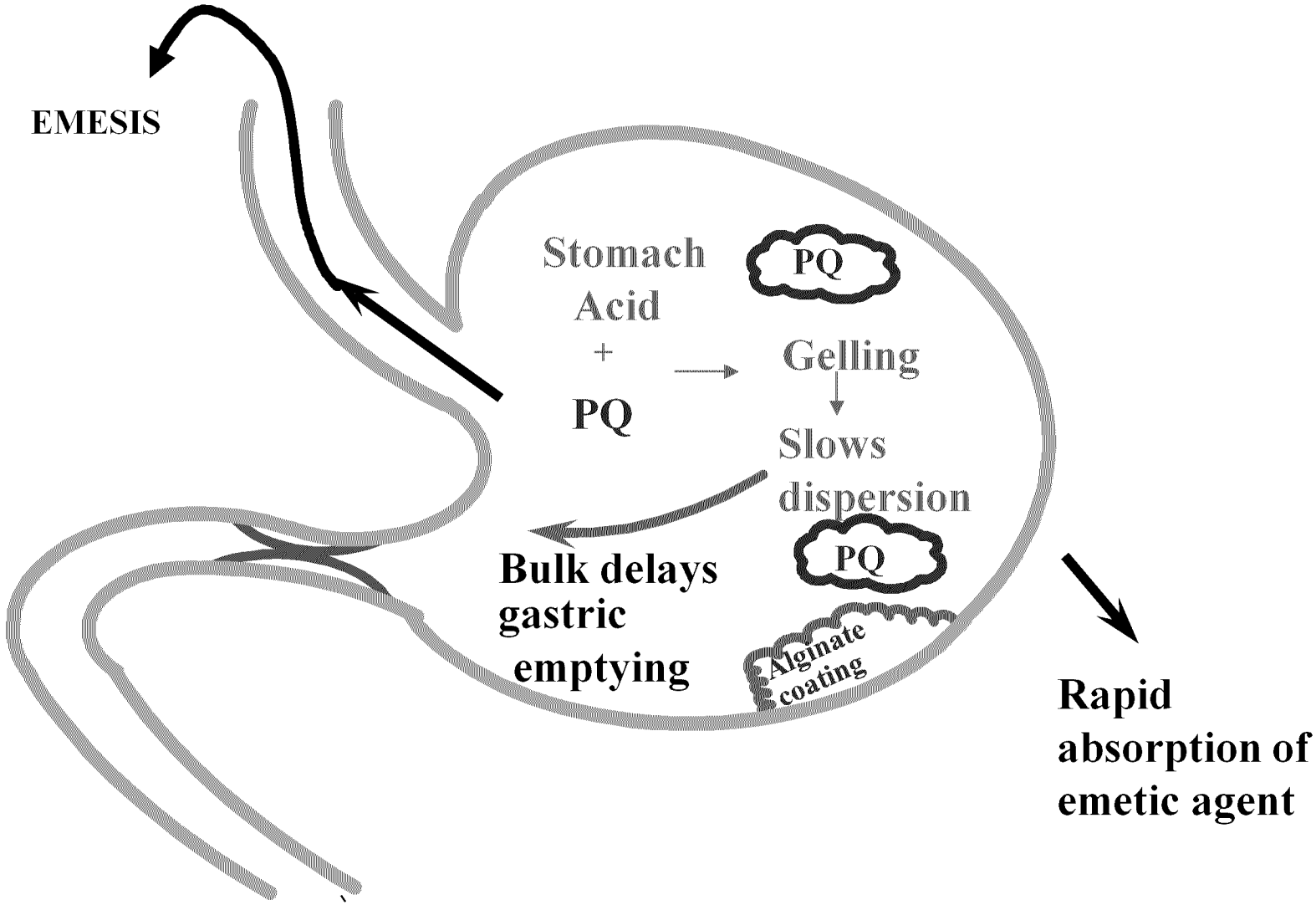
Gastrointestinal physiology



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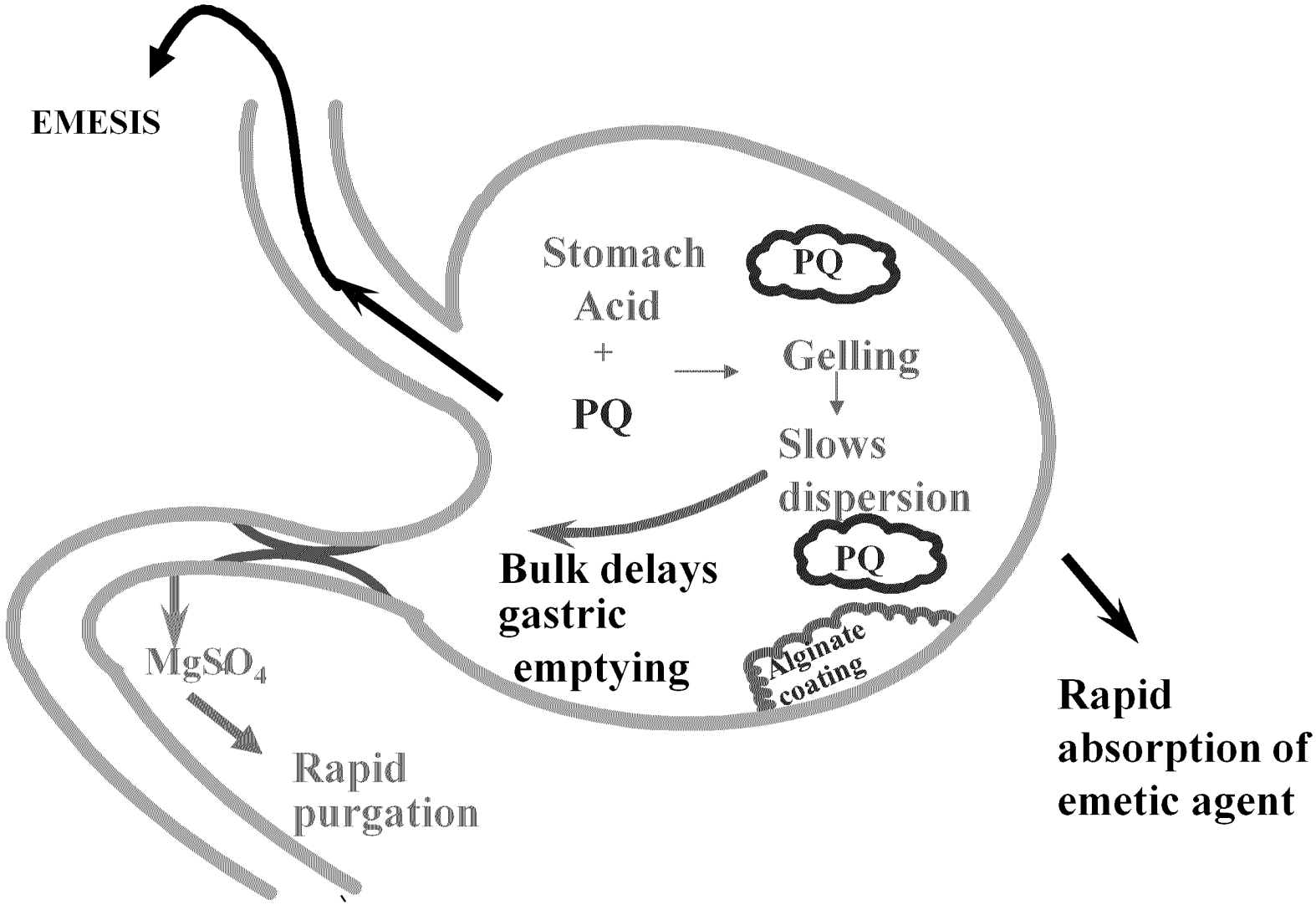
Gastrointestinal physiology



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Gastrointestinal physiology

Gelling

+

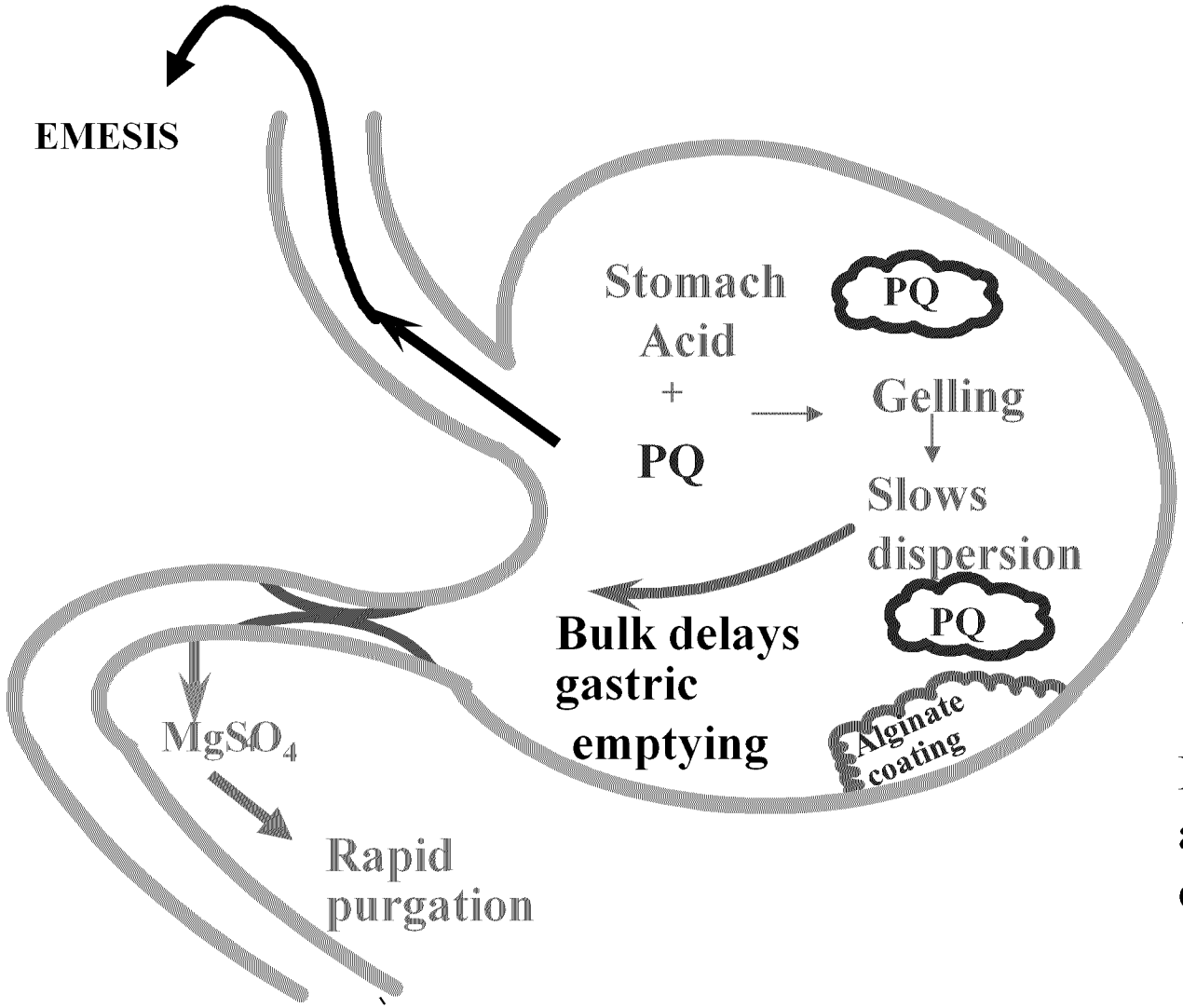
Emesis

+

Purgation

=

Safening



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Studies undertaken

1. Toxicokinetic study on a Gramoxone INTEON 200g/l formulation - A3879BU
    - Subsequently compared to historic data on a Gramoxone 200g/l formulation A3879D
2. Toxicokinetic study on a Gramoxone INTEON 240g/l formulation – A7813K
    - Comparison with a contemporaneous Gramoxone 200g/l formulation A3879D

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Toxicokinetic: Study design

- A group of three male beagle dogs
- Gramoxone Inteon 200g/l SL formulation (A3879BU)
- Oral doses by capsule
- On 5 occasions at monthly intervals
- The nominal dose levels used were 8, 16, 32, 64 and 128mg paraquat ion/kg bw.
- Achieved dose levels of 46, 92, 184, 368 and 736mg A3879BU formulation/kg bw.
- General clinical observations, bodyweights and food consumption were measured frequently throughout the study.

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Toxicokinetic: Study design

- Following each dose dogs were:
  - observed continuously for 4 hours and frequently during the remainder of the day.
  - incidences of emesis were recorded and vomit and faeces were removed immediately to prevent possible re-ingestion
  - Blood samples were taken at intervals (0.5, 1, 2, 4, 7, 12 and 24h) 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
  - 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.

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Results

- All of the animals were clinically normal and remained in excellent clinical condition throughout the studies
- 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 inappetance, weight loss and decreased activity for several days following this dose.
- Kidney and liver function tests and veterinary examination have shown no adverse effects in any dog over this dose range (46 - 736mg A3879BU formulation/kg)
- 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.
- The other 2 dogs had no pathology of the lung.

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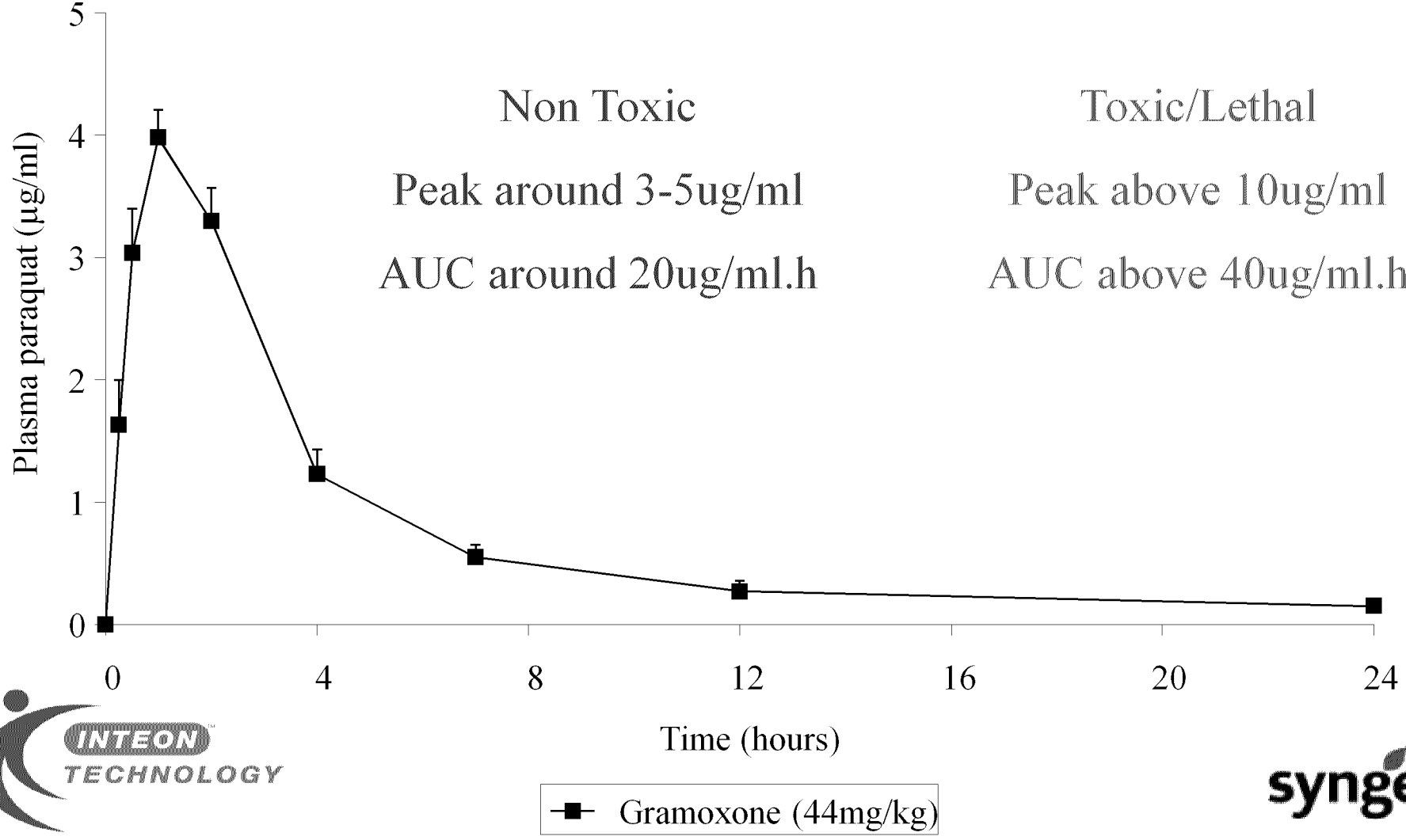
Evidence for reduced oral toxicity in the dog

Paraquat Absorption in the Dog

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



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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.

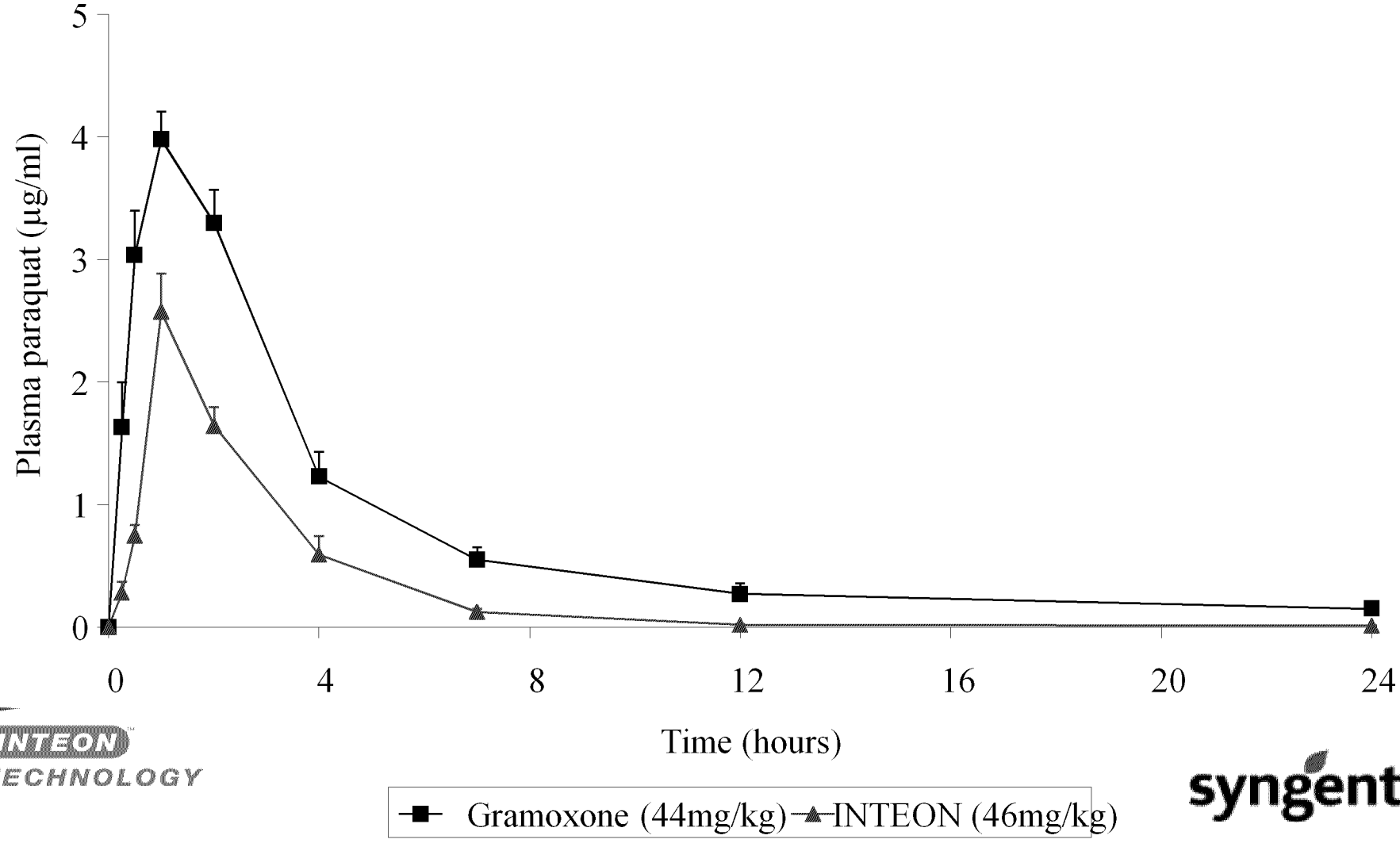
As with human ingestion (suicide attempts) the increase in dose is achieved by administration of increasing volume and therefore the dose of any component in the formulation would be increased as the dose of paraquat increases, mg/kg bw.
Gramoxone control data was generated at CTL between 1987 and 1991. 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.

Paraquat Absorption in the Dog

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



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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.

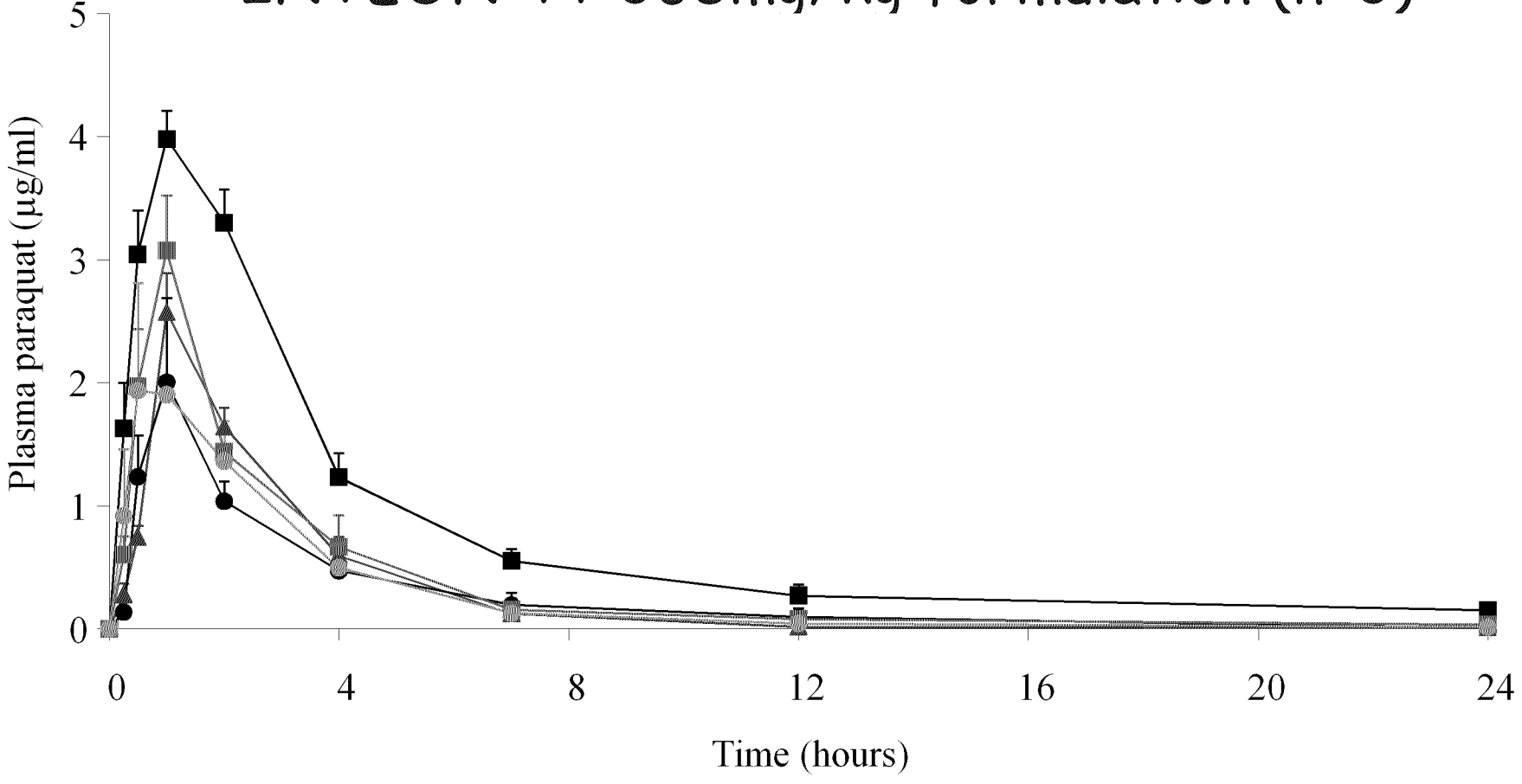
The AWT formulation under identical conditions of dosing etc. caused no toxicity. There was no toxicity in any animal and no effect on kidney or liver function.

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).

[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.]

Paraquat Absorption in the Dog

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



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 over a range of dose levels.

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.

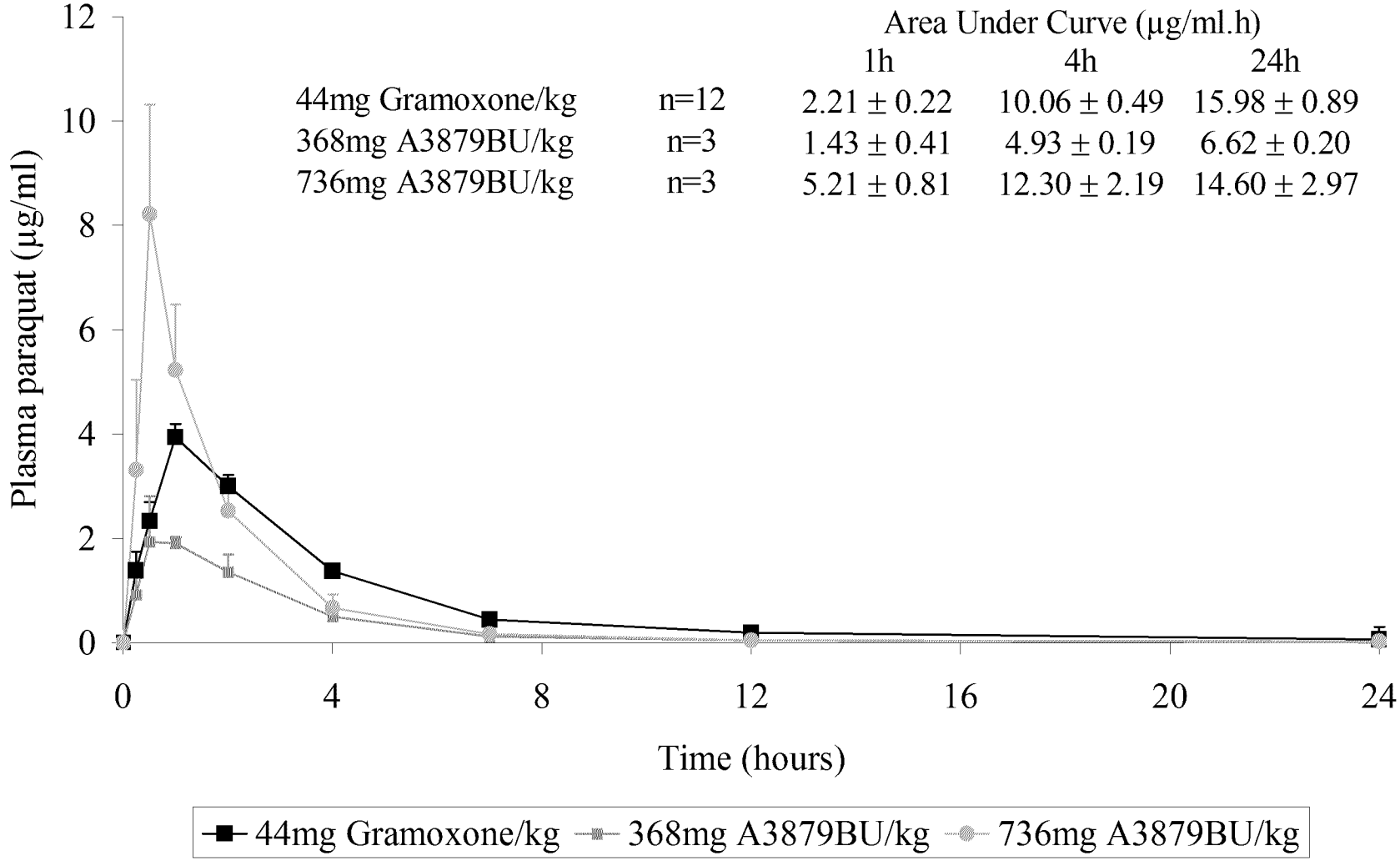
[Gramoxone contains 0.5g/l emetic, whereas A3879BU contains 1.5g/l, but it is the effectiveness of the emetic and gelling which is important rather than the level of emetic per se.]

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 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.

Plasma paraquat following an oral dose of Gramoxone or A3879BU (44-736mg formulation/kg) in male dogs

		Area Under Curve (µg/ml.h) 1h	4h	24h
44mg Gramoxone/kg	n=12	2.21 ± 0.22	10.06 ± 0.49	15.98 ± 0.89
368mg A3879BU/kg	n=3	1.43 ± 0.41	4.93 ± 0.19	6.62 ± 0.20
736mg A3879BU/kg	n=3	5.21 ± 0.81	12.30 ± 2.19	14.60 ± 2.97



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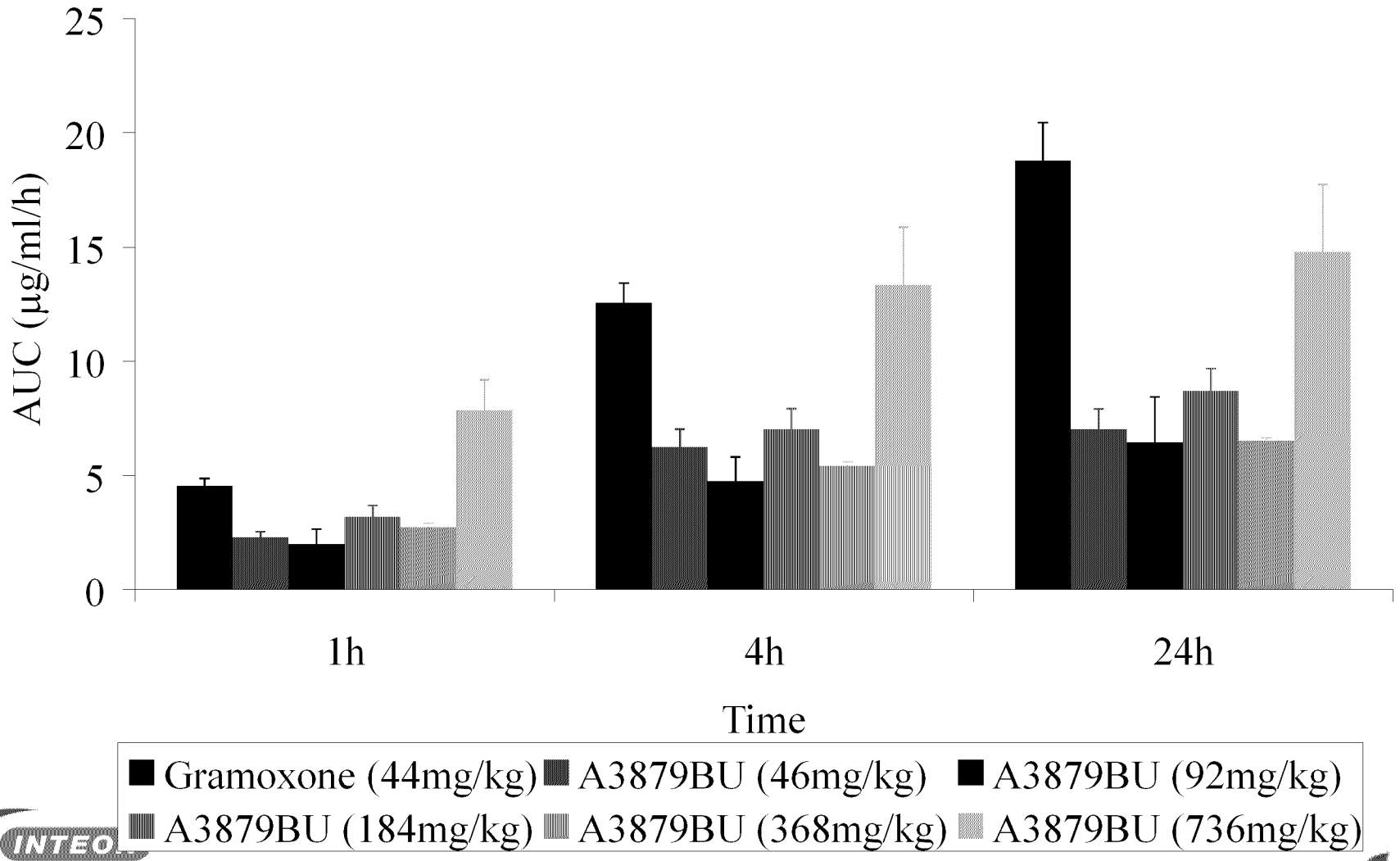
Plasma paraquat AUC values following an oral dose of A3879BU (46 - 736mg formulation/kg b wt.) in dogs (n = 3)



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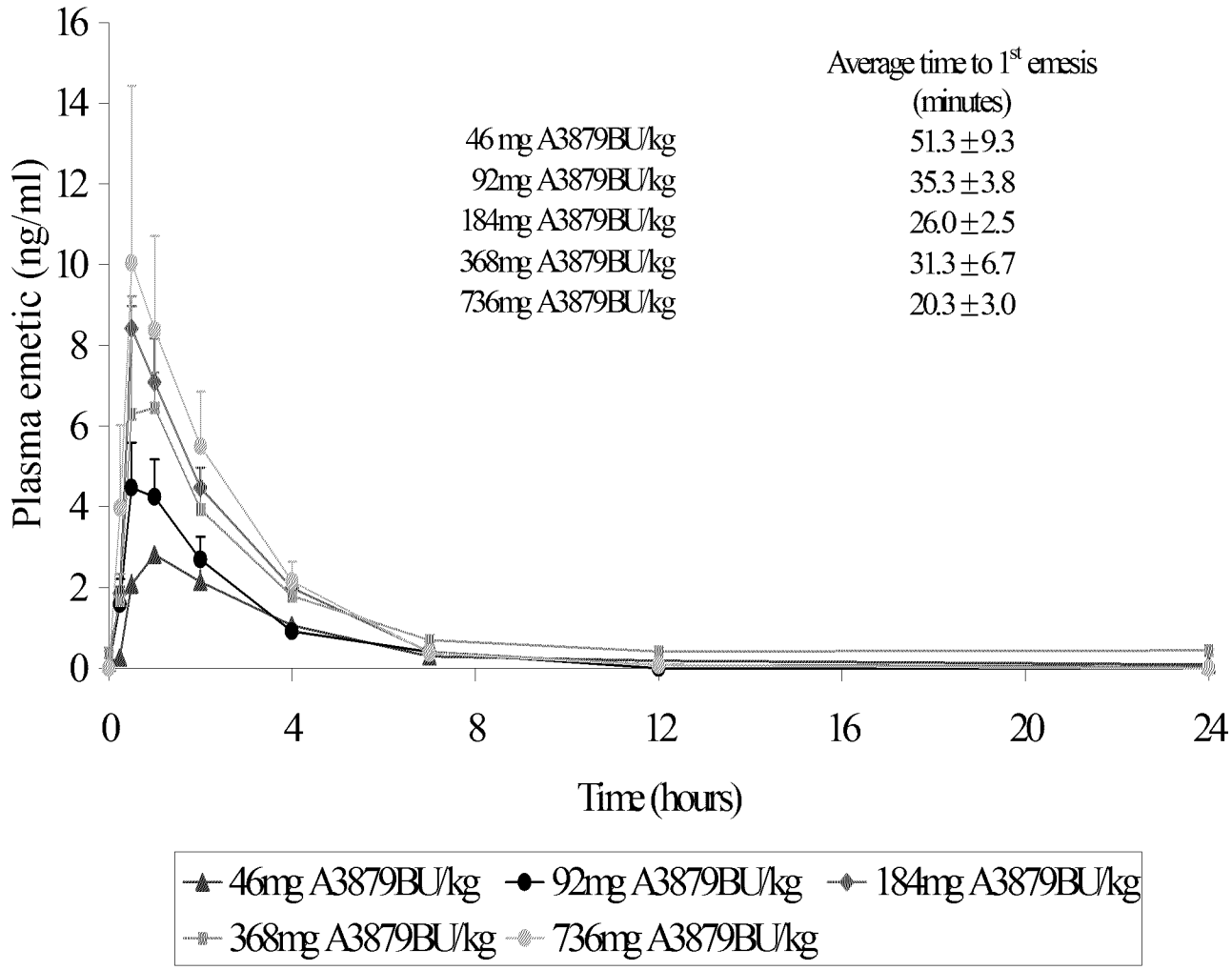
Same study as previous slide but showing the Area Under Curve (AUC). This is the integration of the blood level of PQ between zero and 1h, zero and 4h and zero and 24h. It represents, in kinetic terms, the systemic exposure to PQ at these time intervals.

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

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.

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



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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.

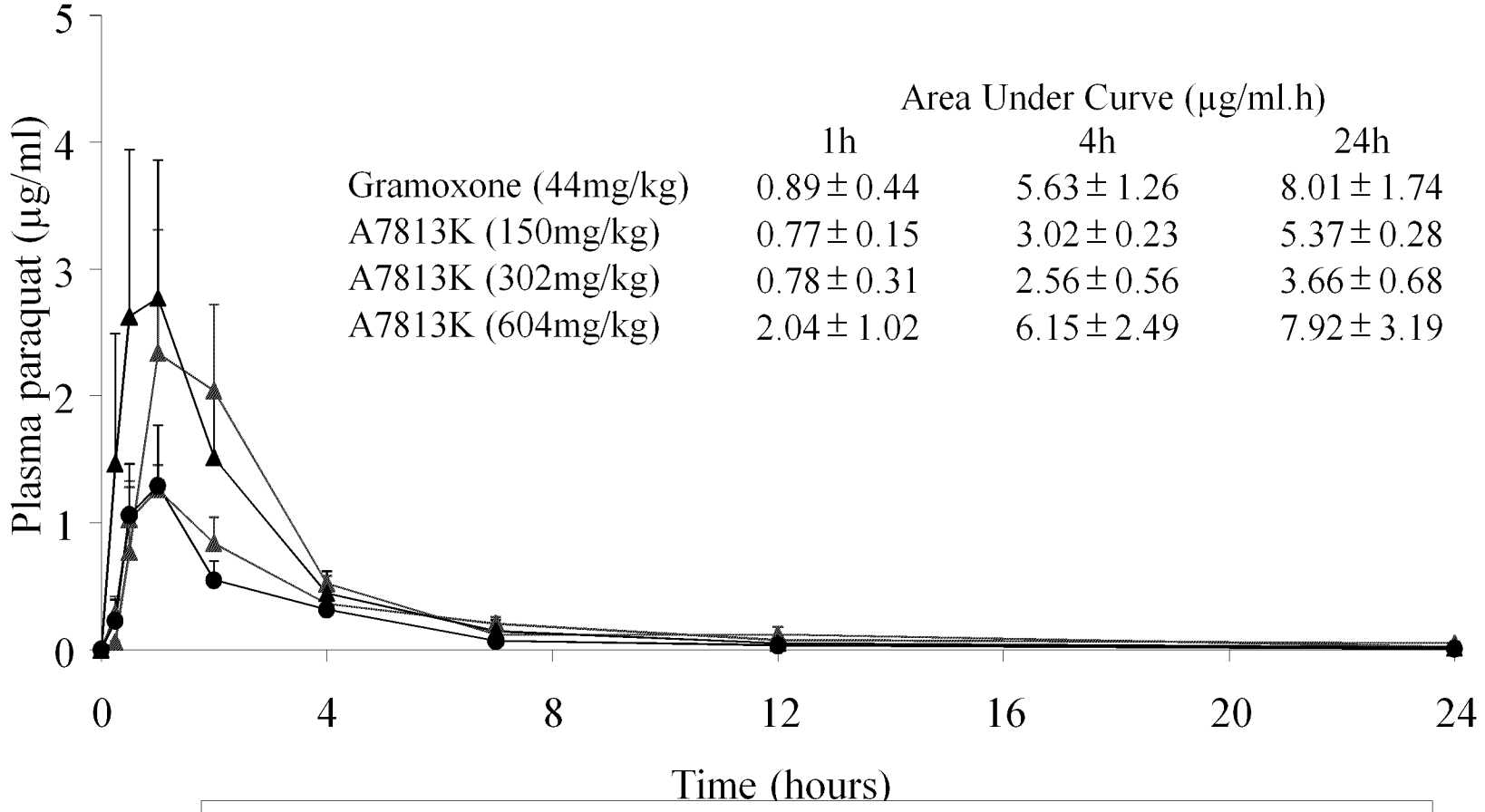
As the dose of PQ is increased the vomit reflex occurs earlier as more emetic reaches the blood faster.

NB Data for 64mg/kg is for only two not three dogs, due to omission of one dog which had slower absorption of paraquat and emetic due to eating faeces prior to dosing.

Second study

2. Toxicokinetic study on a Gramoxone INTEON 240g/l formulation – A7813K
   - Comparison with a contemporaneous Gramoxone 200g/l formulation A3879D

Plasma paraquat levels following an oral dose of Gramoxone or A7813K (150-604mg formulation/kg) in male dogs



	Area Under Curve (µg/ml.h)		
	1h	4h	24h
Gramoxone (44mg/kg)	0.89 ± 0.44	5.63 ± 1.26	8.01 ± 1.74
A7813K (150mg/kg)	0.77 ± 0.15	3.02 ± 0.23	5.37 ± 0.28
A7813K (302mg/kg)	0.78 ± 0.31	2.56 ± 0.56	3.66 ± 0.68
A7813K (604mg/kg)	2.04 ± 1.02	6.15 ± 2.49	7.92 ± 3.19

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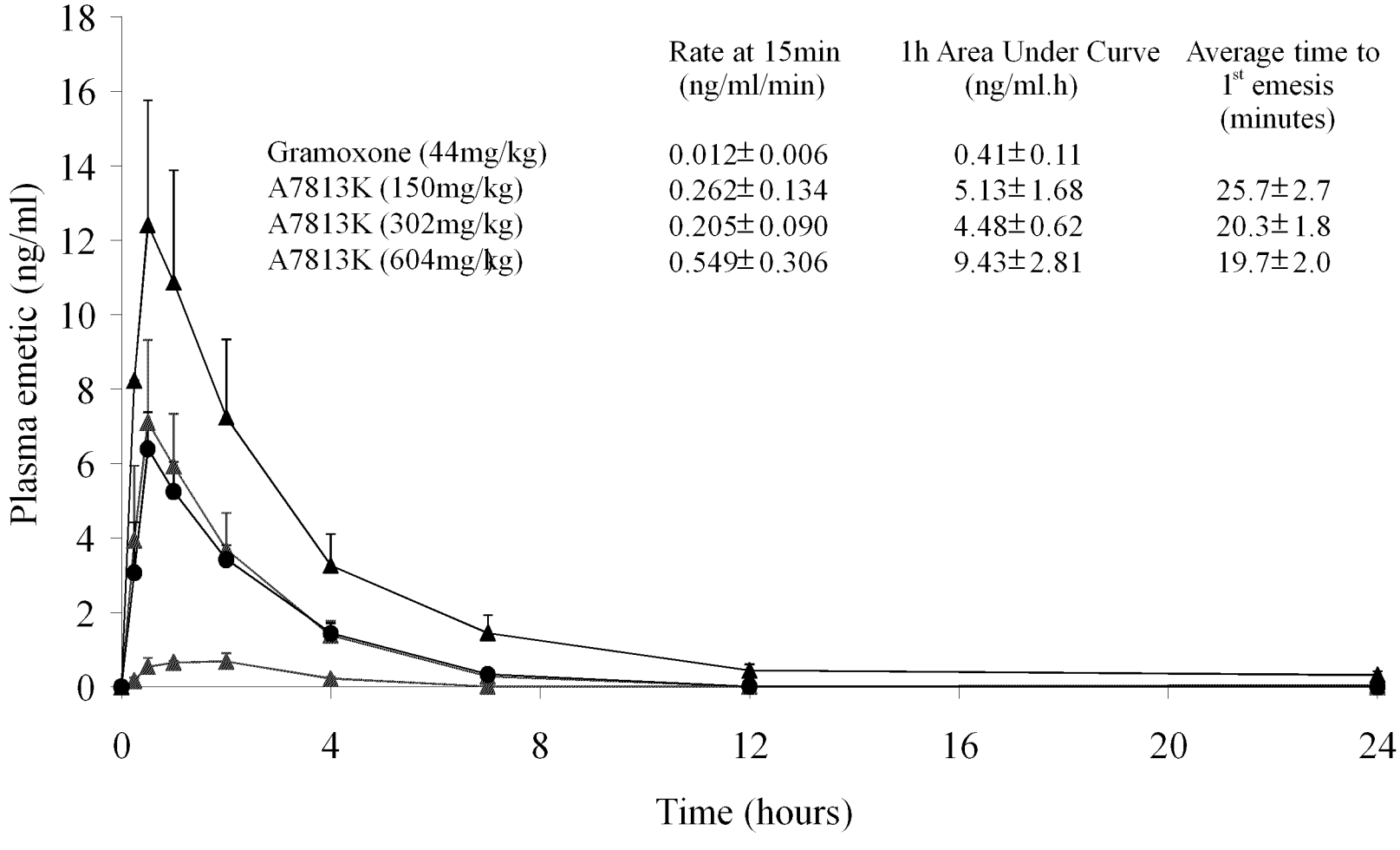
Plasma Emetic following an oral dose of Gramoxone or Gramoxone INTEON(a7813K) to male dogs

	Rate at 15min (ng/ml/min)	1h Area Under Curve (ng/ml.h)	Average time to 1st emesis (minutes)
Gramoxone (44mg/kg)	0.012± 0.006	0.41± 0.11	
A7813K (150mg/kg)	0.262± 0.134	5.13± 1.68	25.7± 2.7
A7813K (302mg/kg)	0.205± 0.090	4.48± 0.62	20.3± 1.8
A7813K (604mg/kg)	0.549± 0.306	9.43± 2.81	19.7± 2.0



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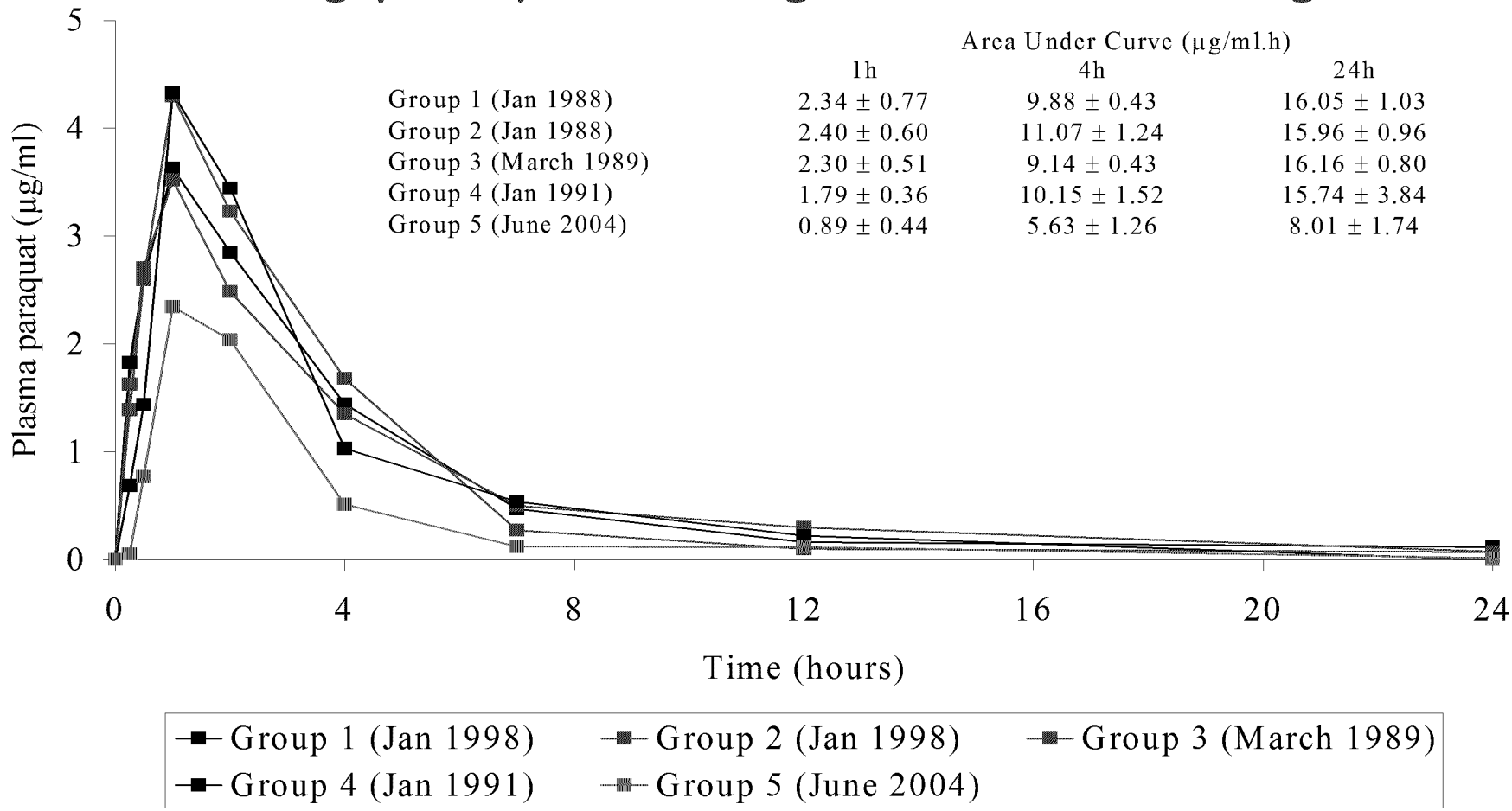
Gramoxone 200g/l formulation (A3879D)

Plasma paraquat concentration following an oral dose of 8mg paraquat ion/kg to adult male dogs

	Area Under Curve (µg/ml.h)		
	1h	4h	24h
Group 1 (Jan 1988)	2.34 ± 0.77	9.88 ± 0.43	16.05 ± 1.03
Group 2 (Jan 1988)	2.40 ± 0.60	11.07 ± 1.24	15.96 ± 0.96
Group 3 (March 1989)	2.30 ± 0.51	9.14 ± 0.43	16.16 ± 0.80
Group 4 (Jan 1991)	1.79 ± 0.36	10.15 ± 1.52	15.74 ± 3.84
Group 5 (June 2004)	0.89 ± 0.44	5.63 ± 1.26	8.01 ± 1.74



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All dogs received APE-containing Gramoxone 200g/l (YF7697A= A3879D).

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Conclusions in the dog

- These data are consistent with acid triggered gelling in the stomach and productive emesis prior to any movement into the small intestines.
  - Earlier emesis occurs with increasing dose
- Shown reduced absorption with Gramoxone INTEON (A3879BU and A7813K) formulation across a 16 fold dose range
- Lethal dose of Gramoxone in dogs is just above dose used in toxicokinetic study - approximately 55 to 66 mg formulation/kg
- Therefore Gramoxone INTEON (A3879BU and A7813K) formulations have shown a 10x safening in the dog

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Assessment of Operator Hazard

- Several paraquat formulations are known to be skin irritants in concentrate form.
- Animal models are known to overestimate penetration of chemicals.
- Particularly true for skin penetration of paraquat where the difference is 40 fold between rat and human.
- Paraquat is known to penetrate the skin via the hair follicle and Gramoxone INTEON has been shown to reduce penetration.
- Gramoxone INTEON has also been shown to have a reduced irritation potential in standard regulatory in vivo skin and eye irritation tests.

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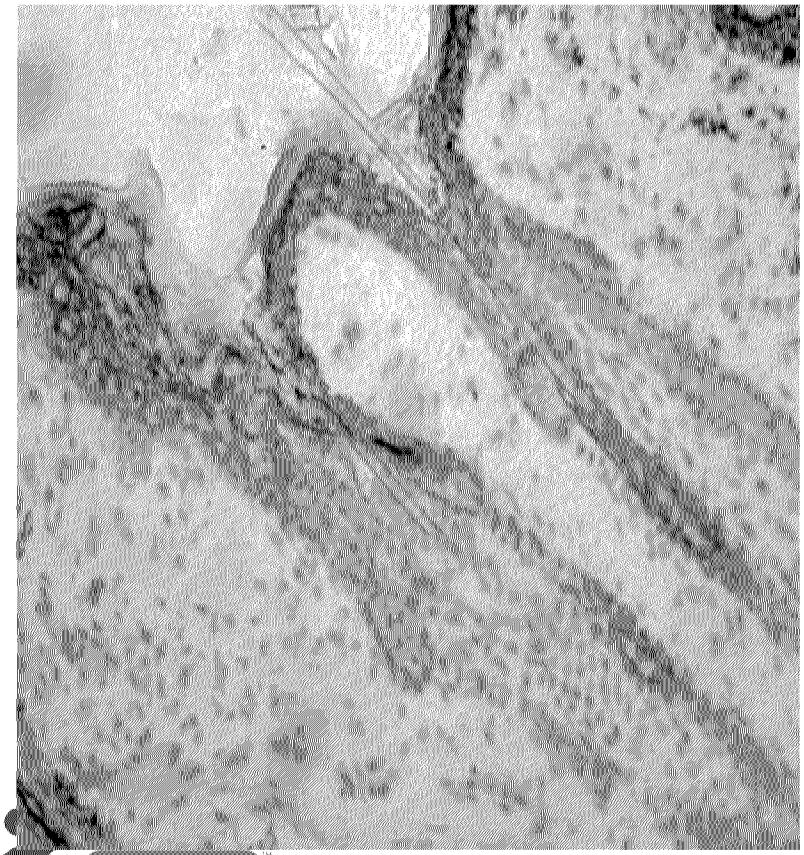
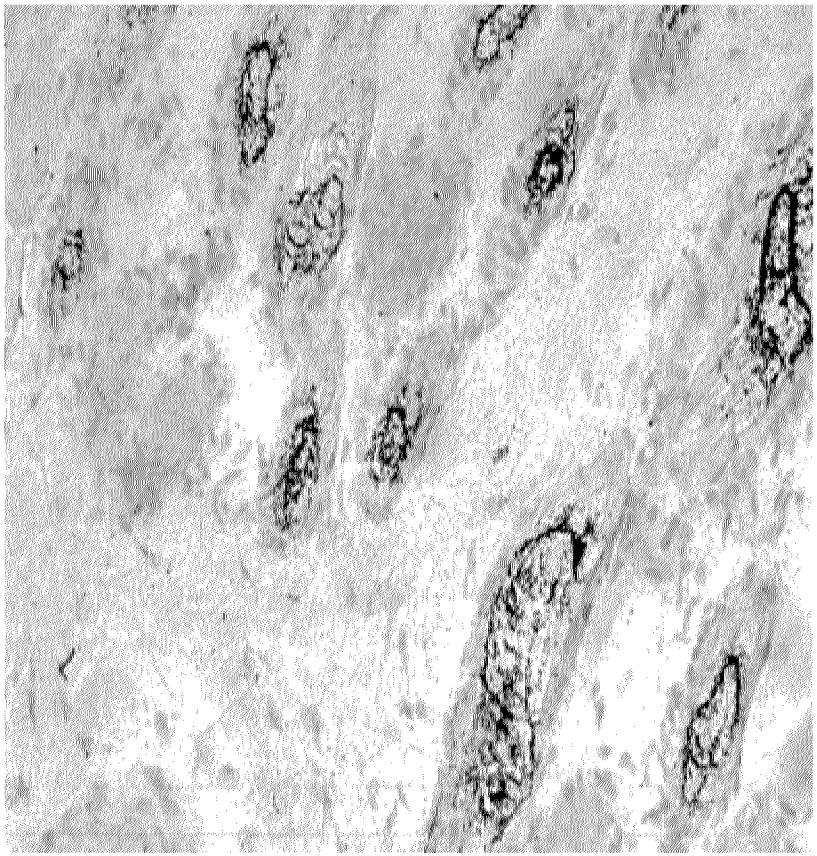
Paraquat penetrates the skin through hair follicles

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

Radioactivity mainly on surface



Radioactivity also in hair follicles



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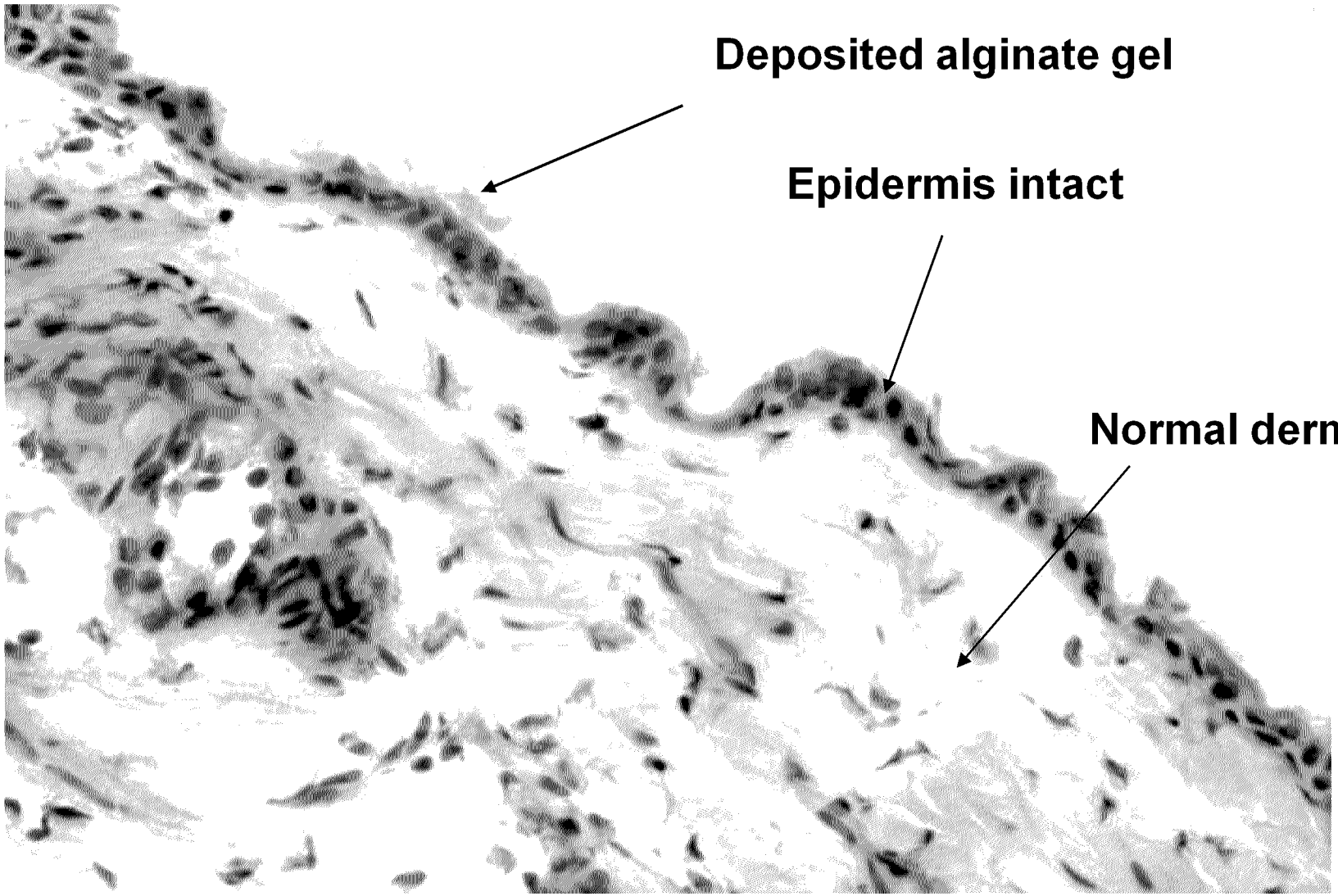
In order to cause skin irritation, chemicals need to gain access to the living tissue below the epidermis. To do this the chemical has to cross the outer impermeable stratum corneum. Lipid soluble chemicals can do this relatively easily by simply dissolving in this lipid rich layer. PQ is very polar 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.

Skin Morphology following INTEON Exposure



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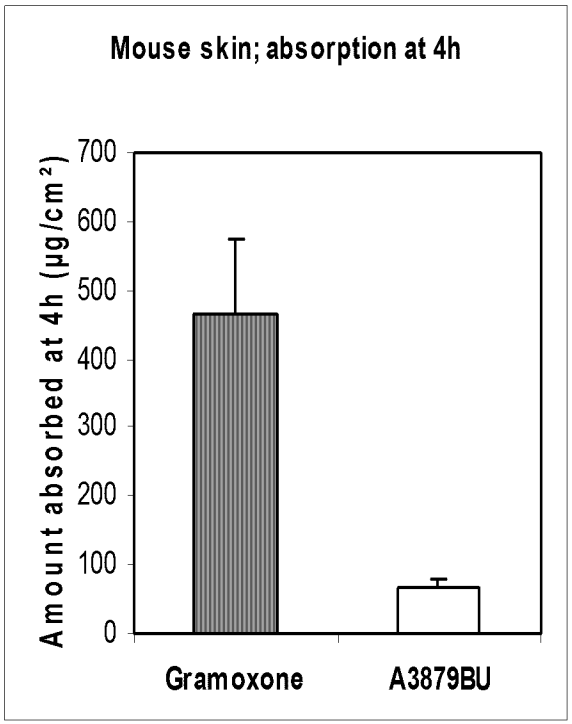
This slide shows a microscopic picture of mouse skin following exposure to Gramoxone containing an alginate polymer. The skin was exposed to the concentrate for 4 hours prior to flash freezing.

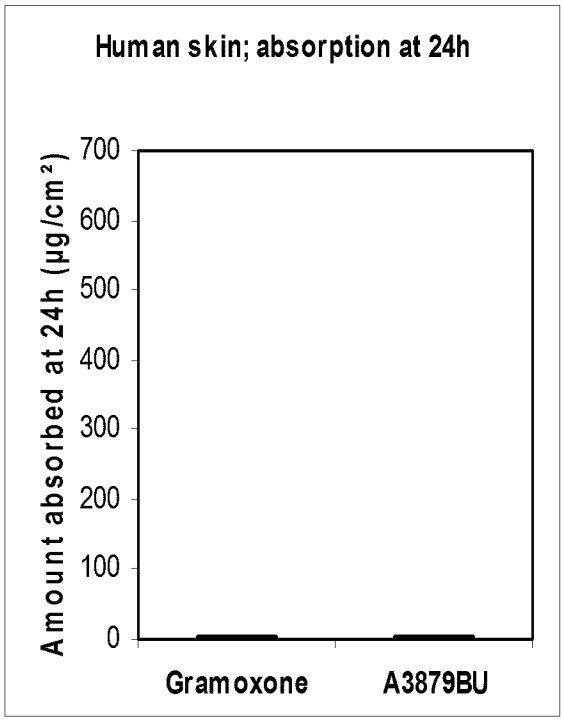
The black dots are the nuclei of the cells in the epidermis (top) and underlying dermis. The section was stained for carbohydrate (shown in blue/green) which is clearly visible on the surface as an adherent gel wall.

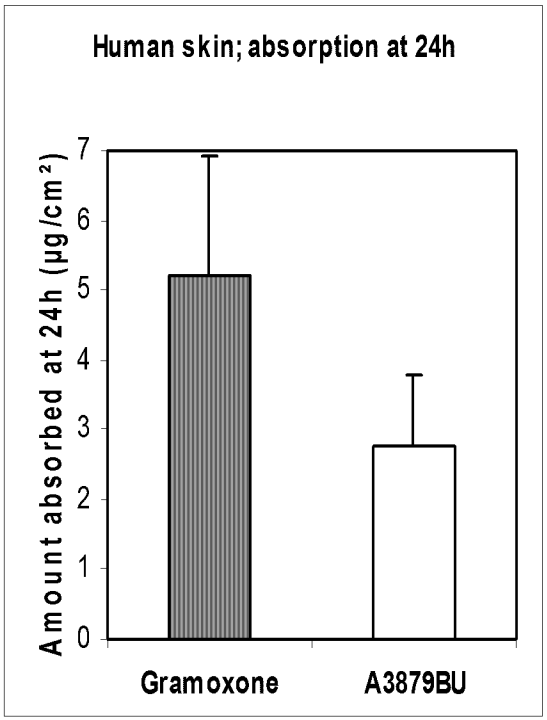
The epidermis is completely normal following this treatment. Current research is investigating the localisation of PQ in the hair follicles following gel treatment to determine the mechanism by which the gel reduces skin penetration and irritation.

Skin Absorption: Comparison Gramoxone and INTEON formulations

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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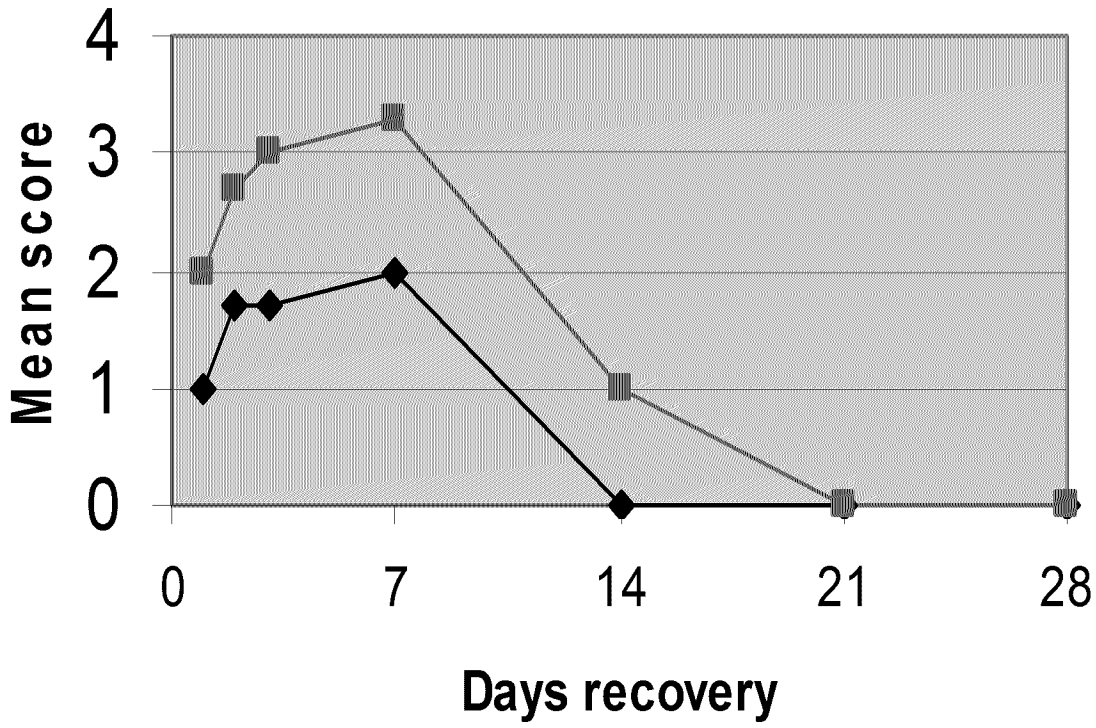
Comparative skin irritancy – Gramoxone INTEON and Gramoxone 200g/l formulations



Erythema Scores:

0 No erythema

1 Very slight (barely perceptible)

2 Well defined

3 Mod to severe

4 Severe (beet redness)

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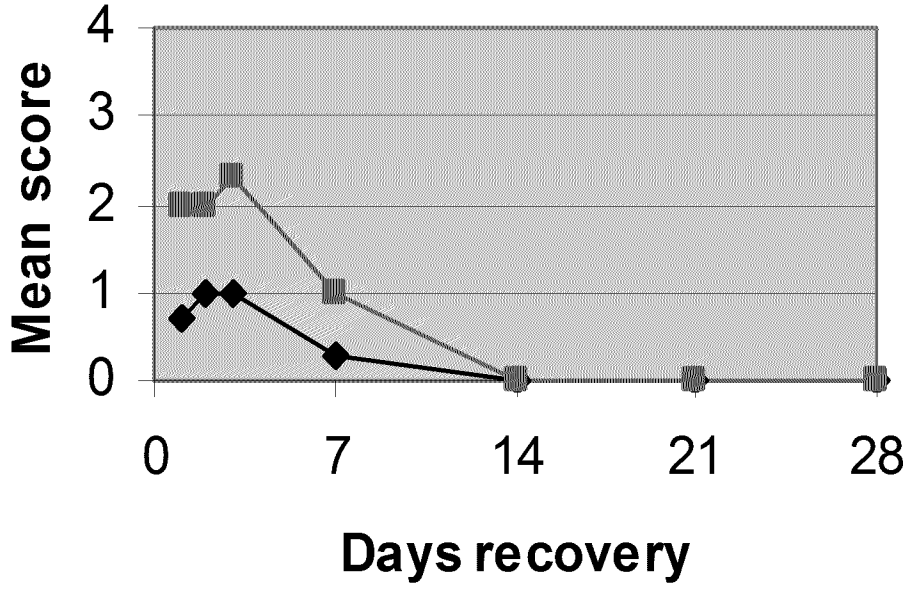
Comparative skin irritancy – Gramoxone INTEON and Gramoxone 200g/l formulations



Oedema Scores:

0 No oedema

1 Very slight (barely perceptible)

2 Slight (edges of area defined by definite raising)

3 Moderate (raised approx 1mm)

4 Severe (raised >1mm and extending beyond exposure area

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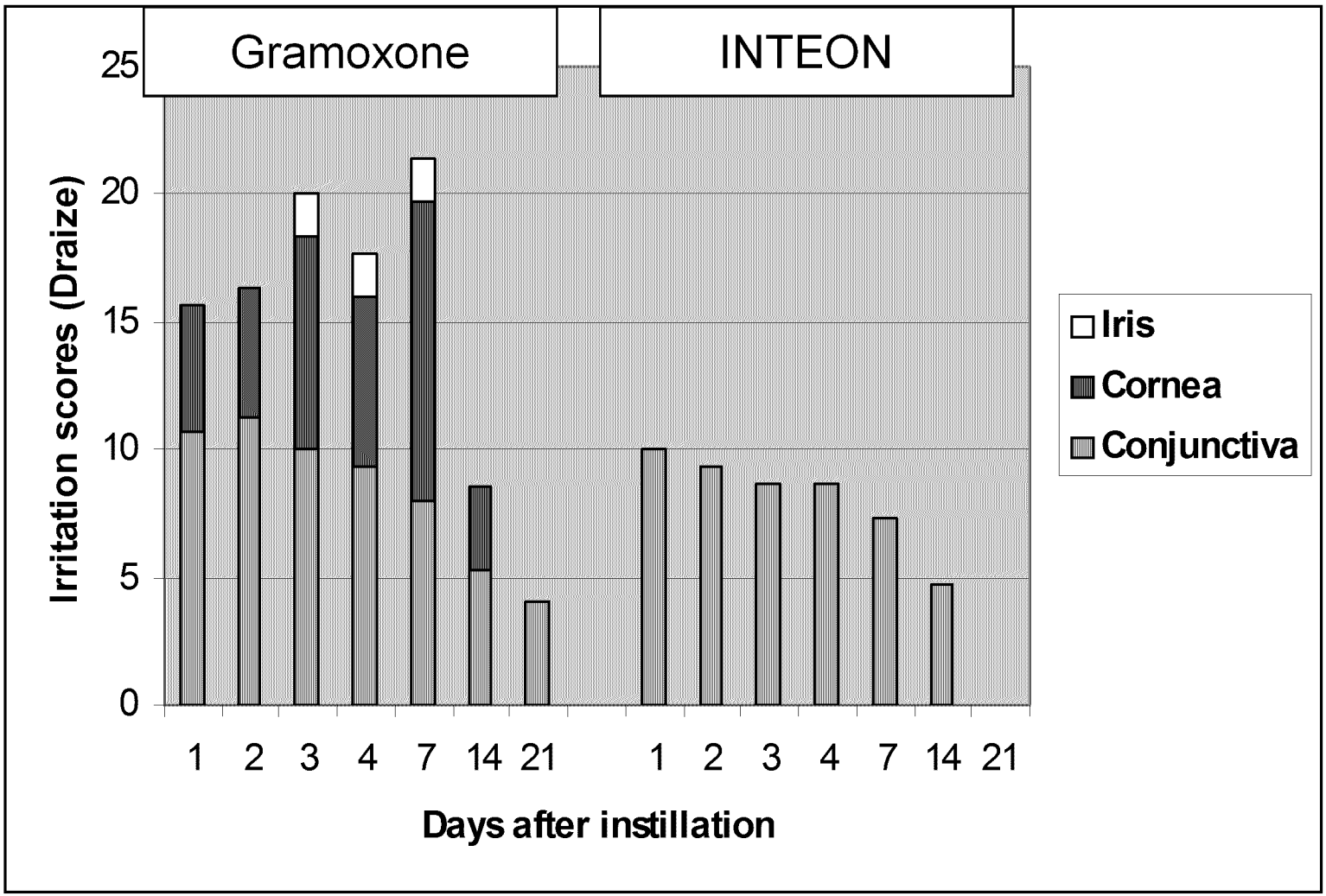
Comparative eye irritancy of 200g/l formulations: Gramoxone INTEON and Gramoxone



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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 INTEON Technology: Conclusions

Gramoxone INTEON provides unique features that enhance its safety profile

- **Scientific rationale for INTEON technology reducing the oral toxicity of paraquat formulations**
- **Recent experimental data in dogs have shown a reduction in the gastrointestinal absorption of paraquat from an INTEON formulation compared with Gramoxone**
- **Experimental evidence shows INTEON formulations to be less irritant to the skin and eye**

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Bullets self explanatory.

GRAMOXONE™ INTEON *

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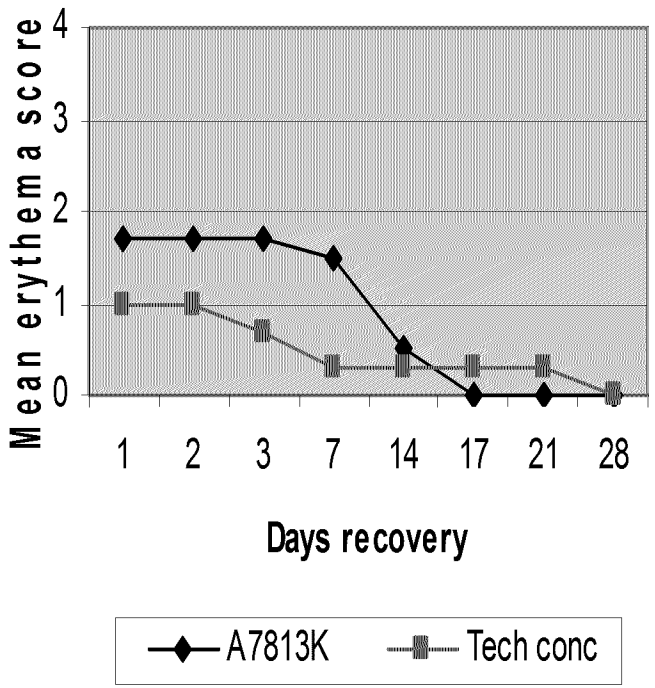
SYNG-PQ-01021167_R

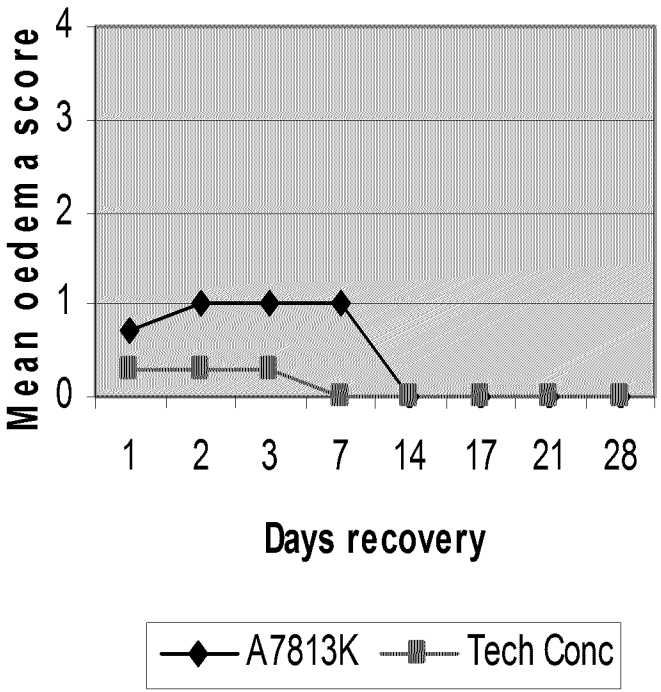
Gramoxone INTEON 240g/l formulation (A7813K)

Skin irritation A7813K 240g/l: Erythema



Erythema Scores:
0 No erythema
1 Very slight (barely perceptible)
2 Well defined
3 Mod to severe
4 Severe (beet redness)

Skin irritation A7813K 240g/l: Oedema



Oedema Scores:
0 No oedema
1 Very slight (barely perceptible)
2 Slight (edges of area defined by definite raising)
3 Moderate (raised approx 1mm)
4 Severe (raised >1mm and extending beyond

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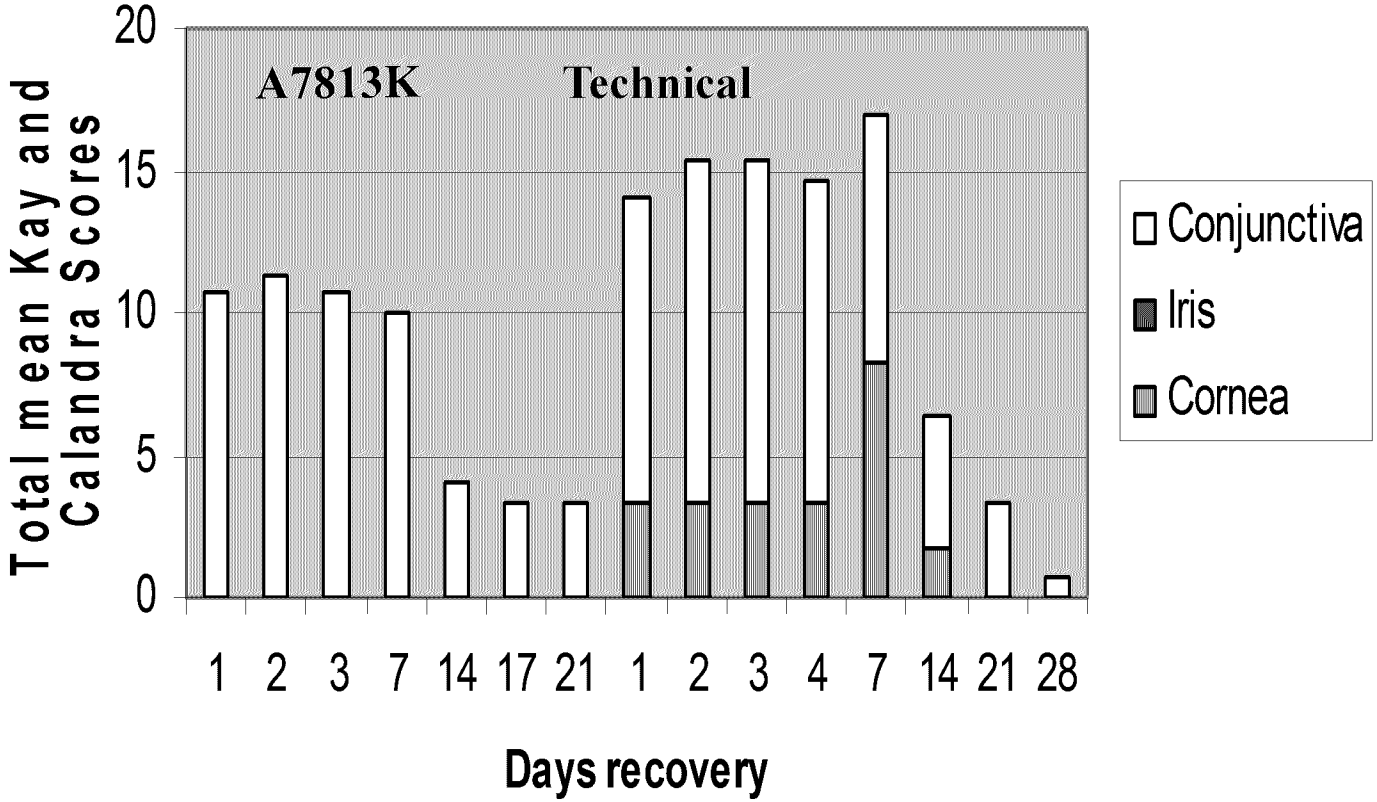
Gramoxone INTEON 240g/l formulation (A7813K)



Kay and Calandra ratings (based on mean total score days 1-4):

0 to 0.5: None to practically non-irritating

0.5 to 2.5: Practically non-irritating

2.5 to 15: Slight to mild irritant

15 to 25: Mild to moderate irritant

25 to 100: Moderate to severe

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Comparison of Handlers toxicology of the new and current formulation

Study	Gramoxone Max	Gramoxone INTEON (A7813K)
Oral MLD mg/kg	344/283 Cat II	Approx 310 (OECD 425) Cat II
Dermal MLD mg/kg	>2000 - Cat IV	>2000 - Cat III
Dermal irritancy	Moderate Cat IV	Mild Cat IV
Eye irritancy	Moderate –Severe Cat II	Moderate Cat II
Sensitisation	Not a sensitiser (x3)	Not a sensitiser (x3)

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Comparison of Handlers toxicology of the new and current formulation

Study	Gramoxone (YF7697A)	Gramoxone INTEON (A3879BU)
Oral MLD mg/kg	612 (f), 707 (m)	Approx 550 (OECD 425)
Dermal MLD mg/kg	590 (m), 735 (f)	805 (m) 1231 (f)
Dermal irritancy	Moderate to severe	Less irritant, Mild
Eye irritancy	Moderate	Less irritant – no corneal involvement , Moderate
Sensitisation	Not a sensitiser (x3)	Not a sensitiser (x9)

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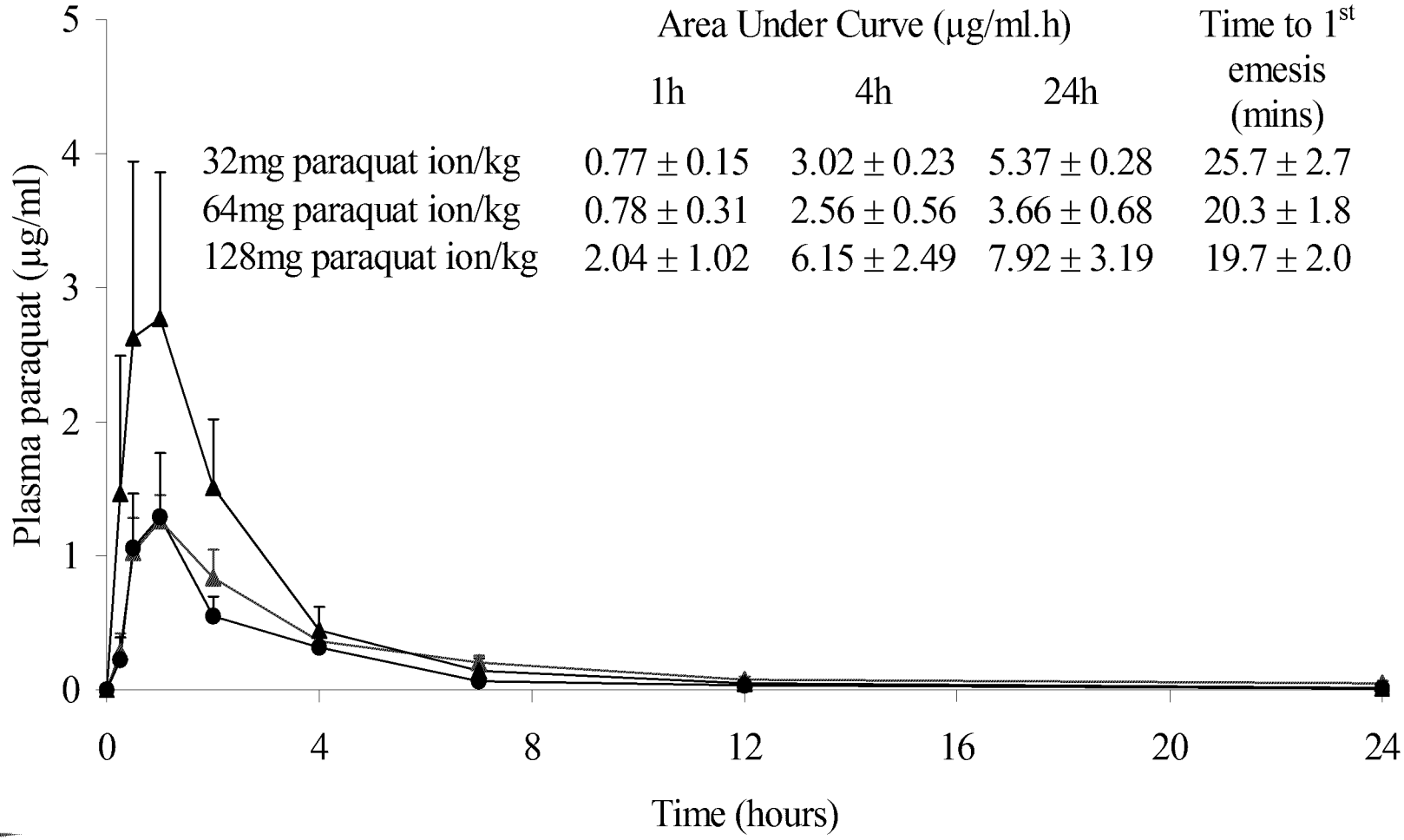
Gramoxone INTEON 240g/l formulation (A7813K)

Plasma paraquat following an oral dose of paraquat ion to

Performance of the US AWT Formulation (A7813K)

	Area Under Curve (µg/ml.h)			Time to 1st emesis (mins)
	1h	4h	24h	
32mg paraquat ion/kg	0.77 ± 0.15	3.02 ± 0.23	5.37 ± 0.28	25.7 ± 2.7
64mg paraquat ion/kg	0.78 ± 0.31	2.56 ± 0.56	3.66 ± 0.68	20.3 ± 1.8
128mg paraquat ion/kg	2.04 ± 1.02	6.15 ± 2.49	7.92 ± 3.19	19.7 ± 2.0



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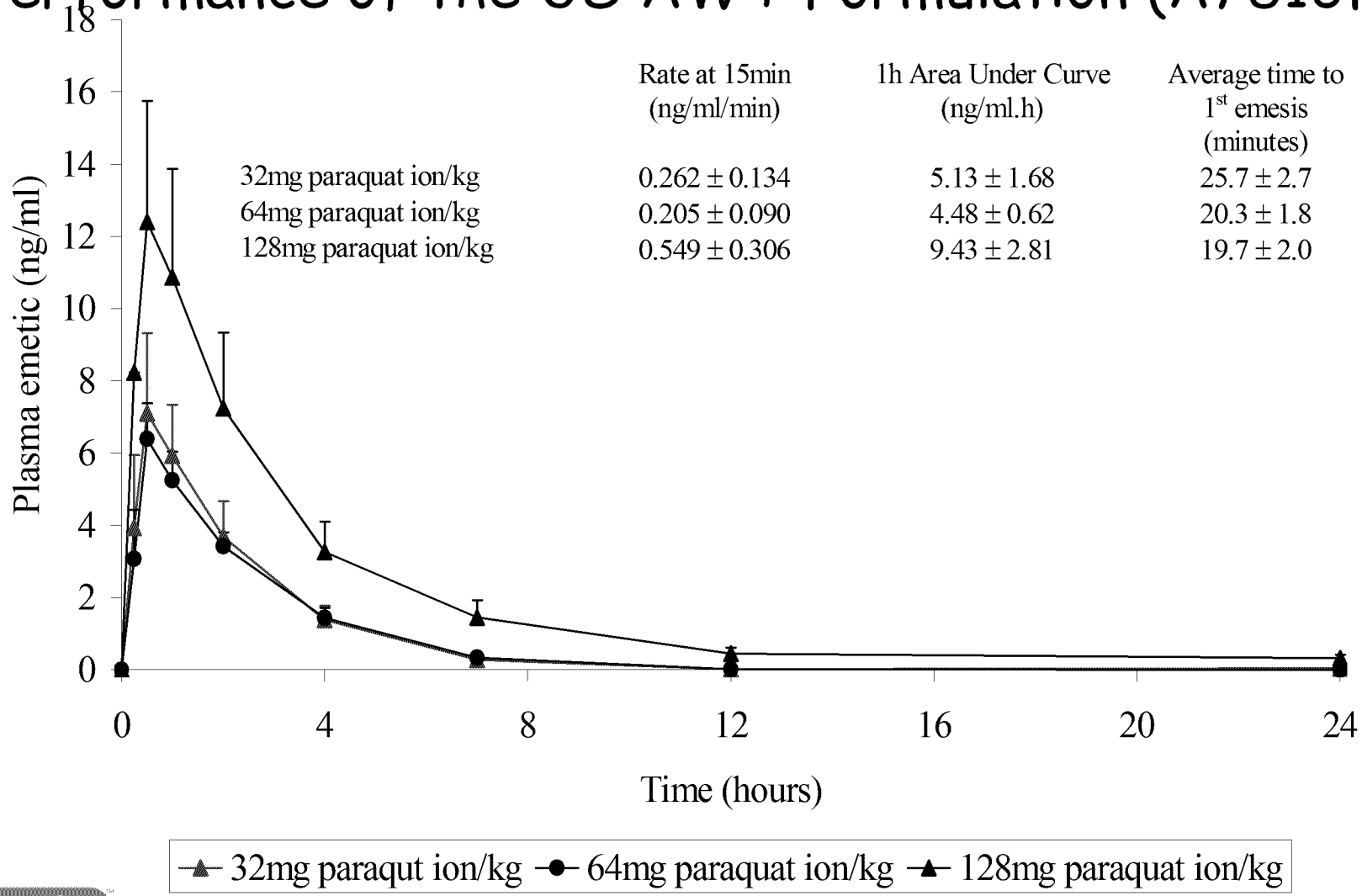
Gramoxone INTEON 240g/l formulation (A7813K)

Plasma emetic following an oral dose of paraquat ion to male

Performance of the US AWT Formulation (A7813K)

	Rate at 15min (ng/ml/min)	1h Area Under Curve (ng/ml.h)	Average time to 1st emesis (minutes)
32mg paraquat ion/kg	0.262 ± 0.134	5.13 ± 1.68	25.7 ± 2.7
64mg paraquat ion/kg	0.205 ± 0.090	4.48 ± 0.62	20.3 ± 1.8
128mg paraquat ion/kg	0.549 ± 0.306	9.43 ± 2.81	19.7 ± 2.0



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Paraquat AWT – Key Targets for Development

- pH trigger causing gelling in the stomach improving effective emesis
- Potential for improving dermal irritation
- Excellent weed efficacy

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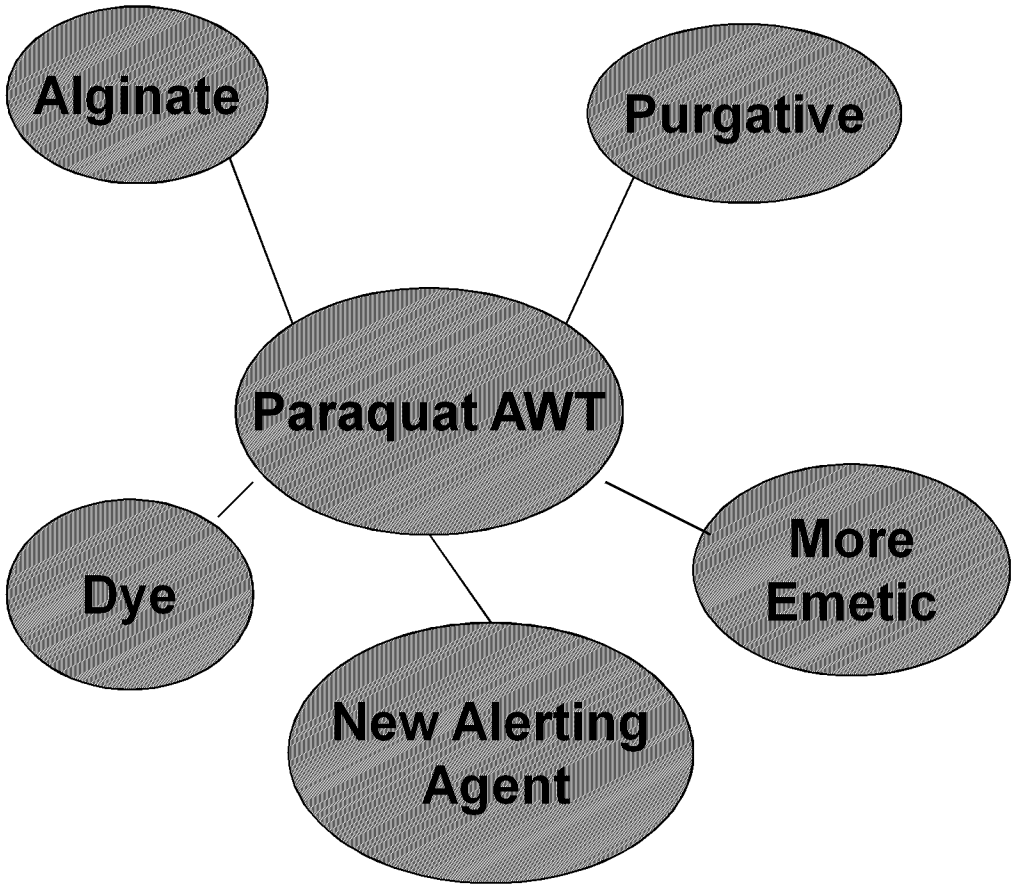
Paraquat AWT Formula



Goals

- Improve oral toxicity in humans
- Improve dermal irritation
- Improve odor while keeping alerting properties

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Acute Oral Testing

- Must use vomiting species
- Dog study designed and completed (3 dogs)
- Literature / previous studies indicate 55 mg/kg is a lethal dose in dogs
- Baseline data from previous studies used for non-AWT formulation (at 44 mg/kg, just below lethal dose)
- Doses were 46, 92, 184, 368, and 736 mg/kg of formulated product

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