Gramoxone Inteon Improvement in Oral Toxicity Experimental Evidence

Michael Clapp, PhD

Two questions posed by EPA in Discussion Piece on Paraquat Inteon Products, received 4-20-06

1. **Is the paraquat Inteon formulation safer for human than the paraquat non-Inteon formulation?**
   - ✓ **Clear evidence in the dog**
   - ✓ **Dog model is relevant to human**

2. Is the paraquat Inteon formulation safe for humans at the high exposures achieved after accidental or deliberate ingestions?

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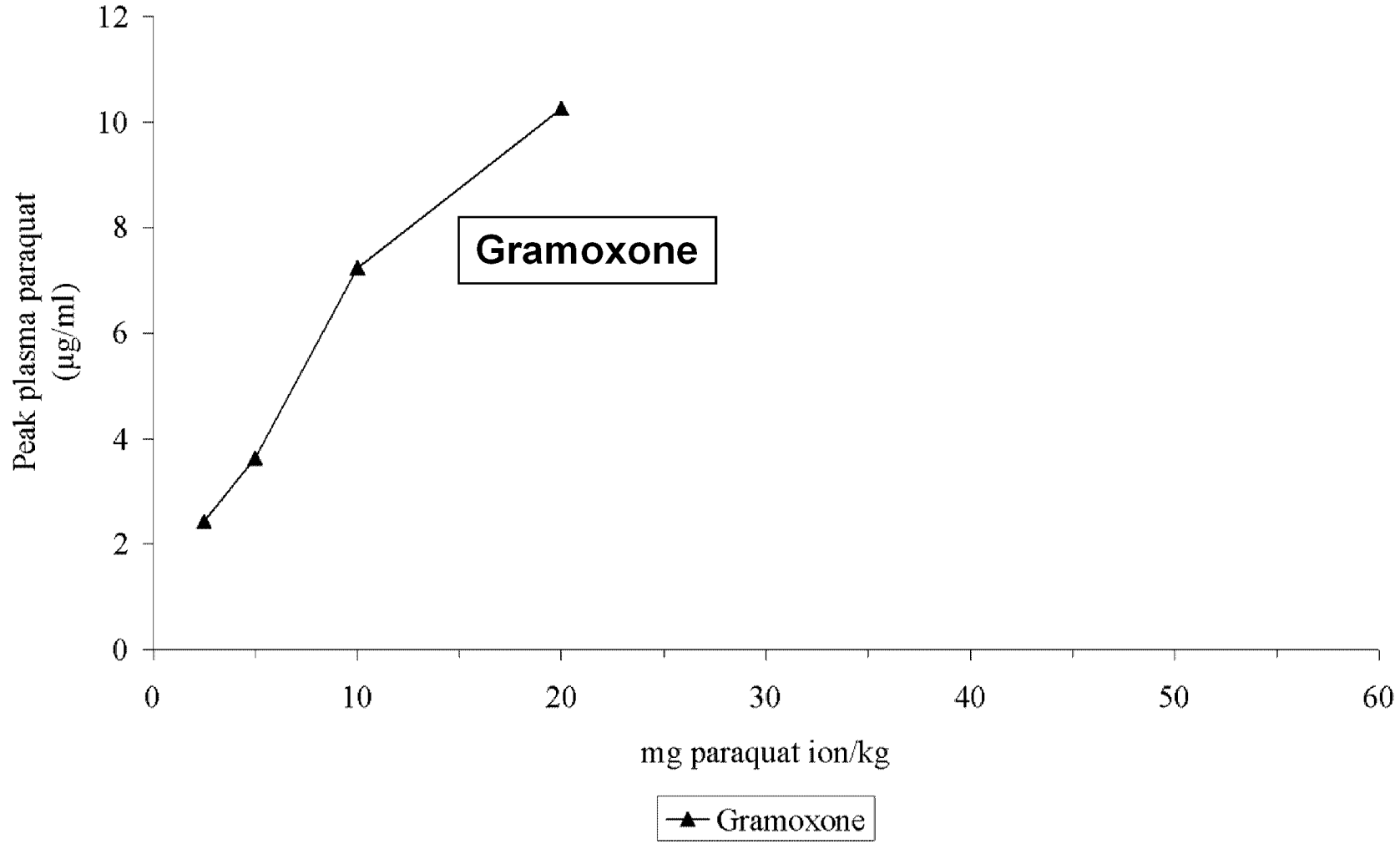
Toxicity of paraquat to the dog – Pre Inteon

- Background Gramoxone data based on 200g paraquat/l with built in wetters
- Widdop et al 1977 – mortality at 10mg paraquat/kg
- Zeneca data confirmed MLD in dog to be 12mg paraquat/kg for Gramoxone formulation (0.5g/l emetic)
- Increasing emetic level in Gramoxone offered only limited protection
- In US Gramoxone Max is a 360g paraquat/l which will be more toxic on a volume basis

- Inteon changed mechanism to acid triggered gelling, changed site of absorption to the stomach resulting in more productive emesis
- Inteon US formulation >10 fold safer in the dog

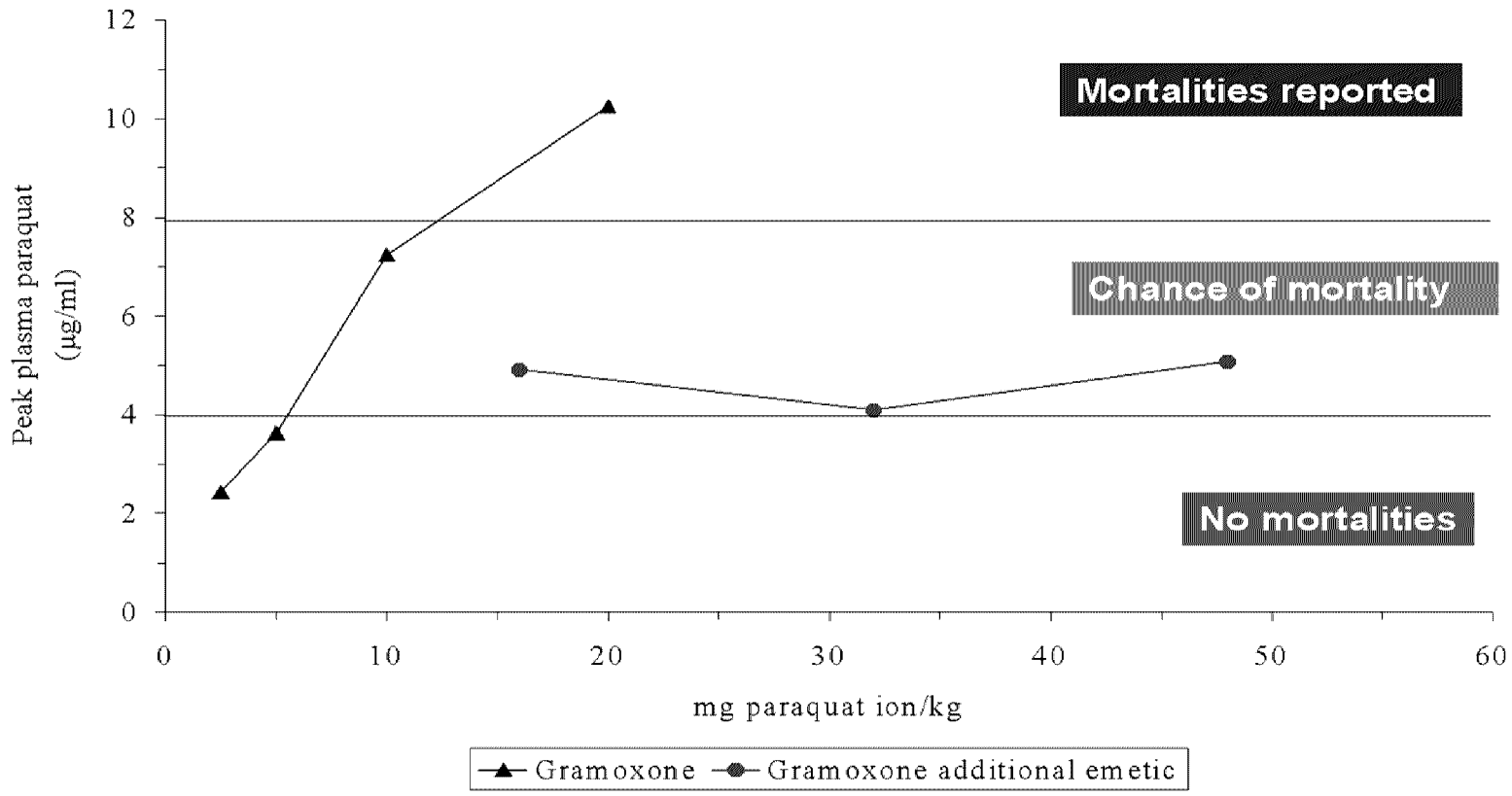
GRAMOXONE: Peak Plasma Paraquat Levels in Dogs (1987 Study)



Gramoxone: 1 out of 4 dogs died at 10 mg paraquat ion/kg

all 4 dogs were terminated day 7/8 at 20mg paraquat ion/kg

Gramoxone: influence of increased emetic on peak plasma paraquat levels



Controls peak plasma paraquat

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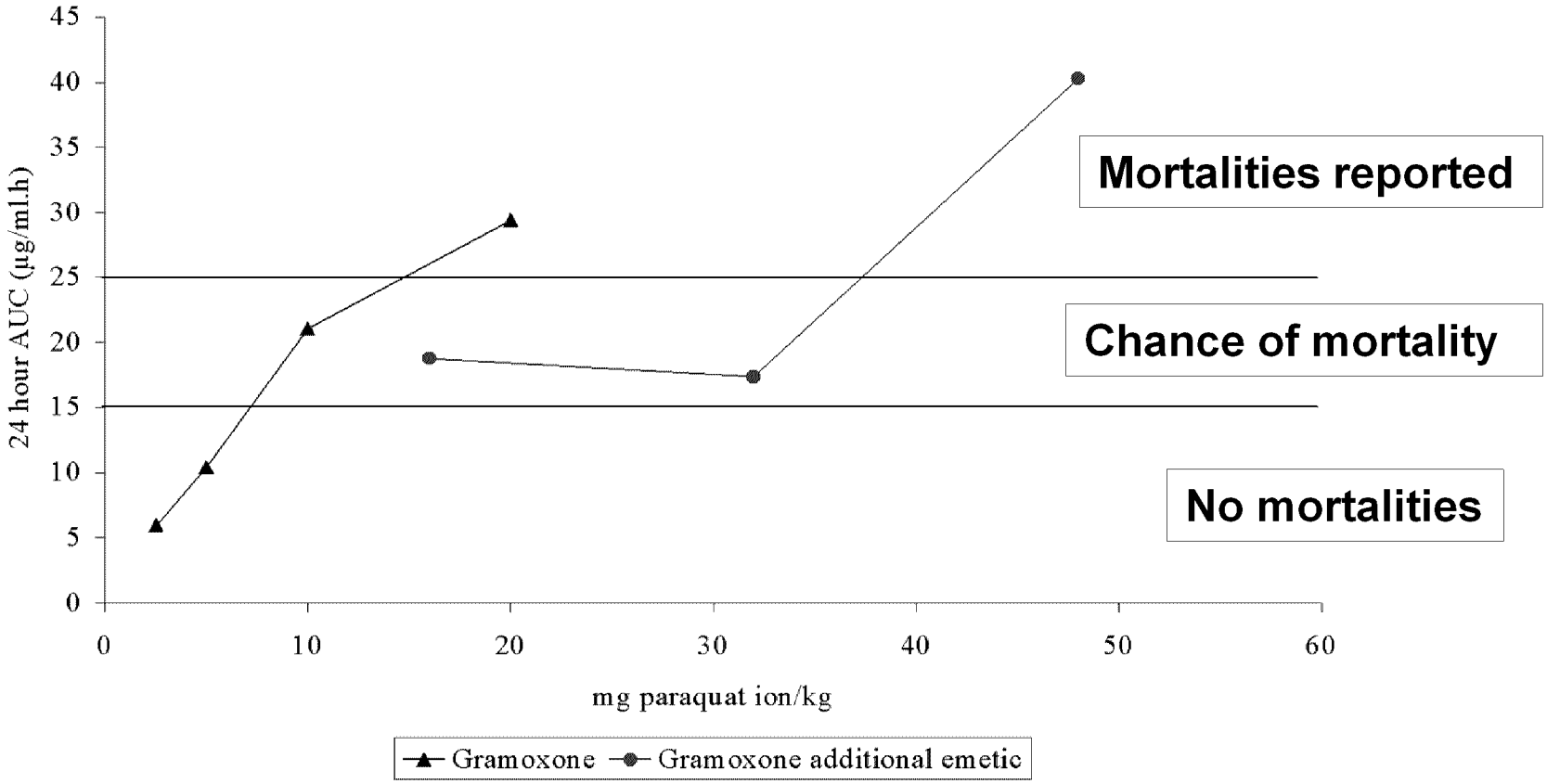
Gramoxone: Influence of increased emetic (2.4g/l) on plasma paraquat 24h AUC levels



Does not control total systemic absorption

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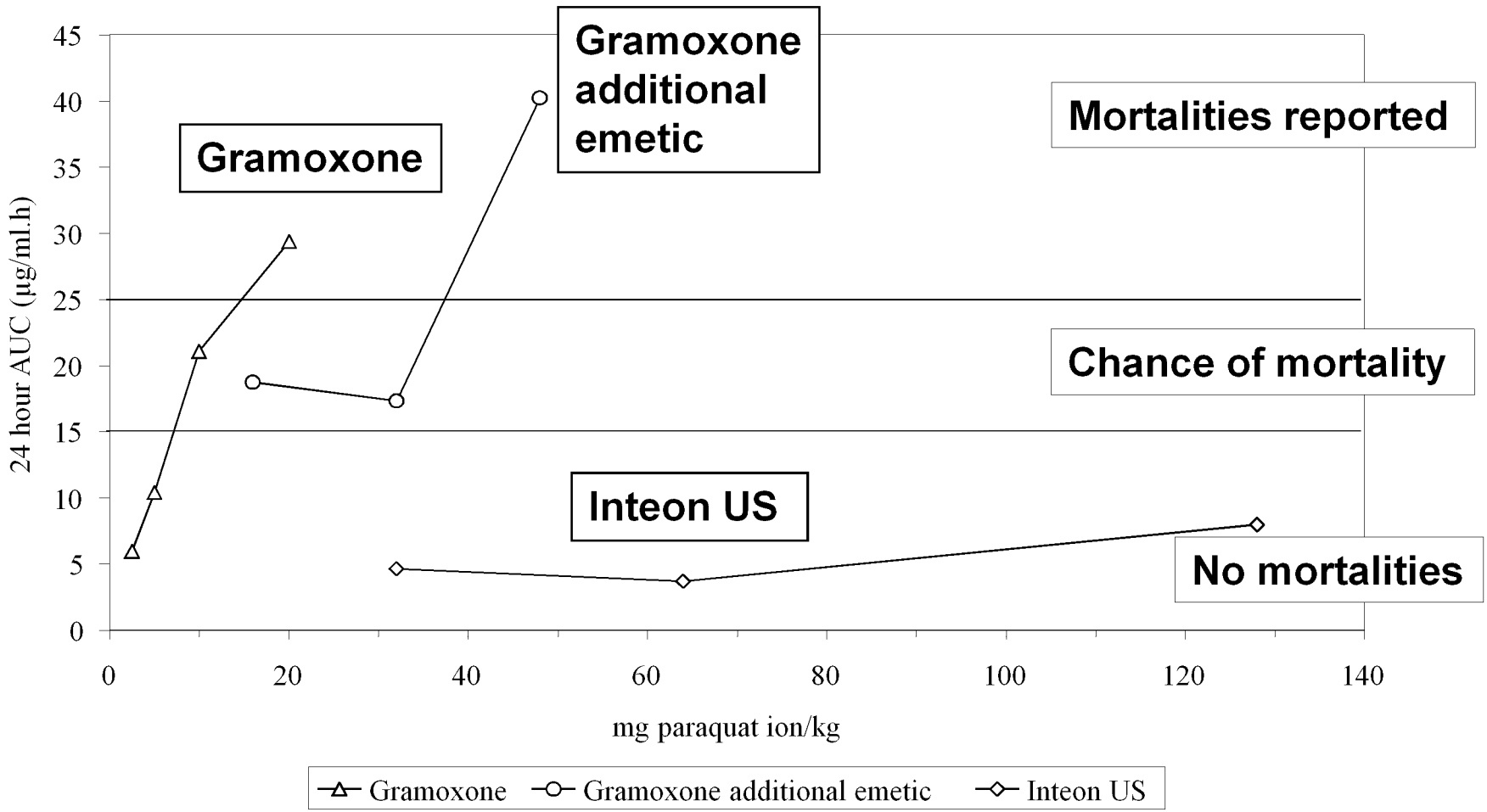
Comparison of 24h plasma paraquat AUC levels



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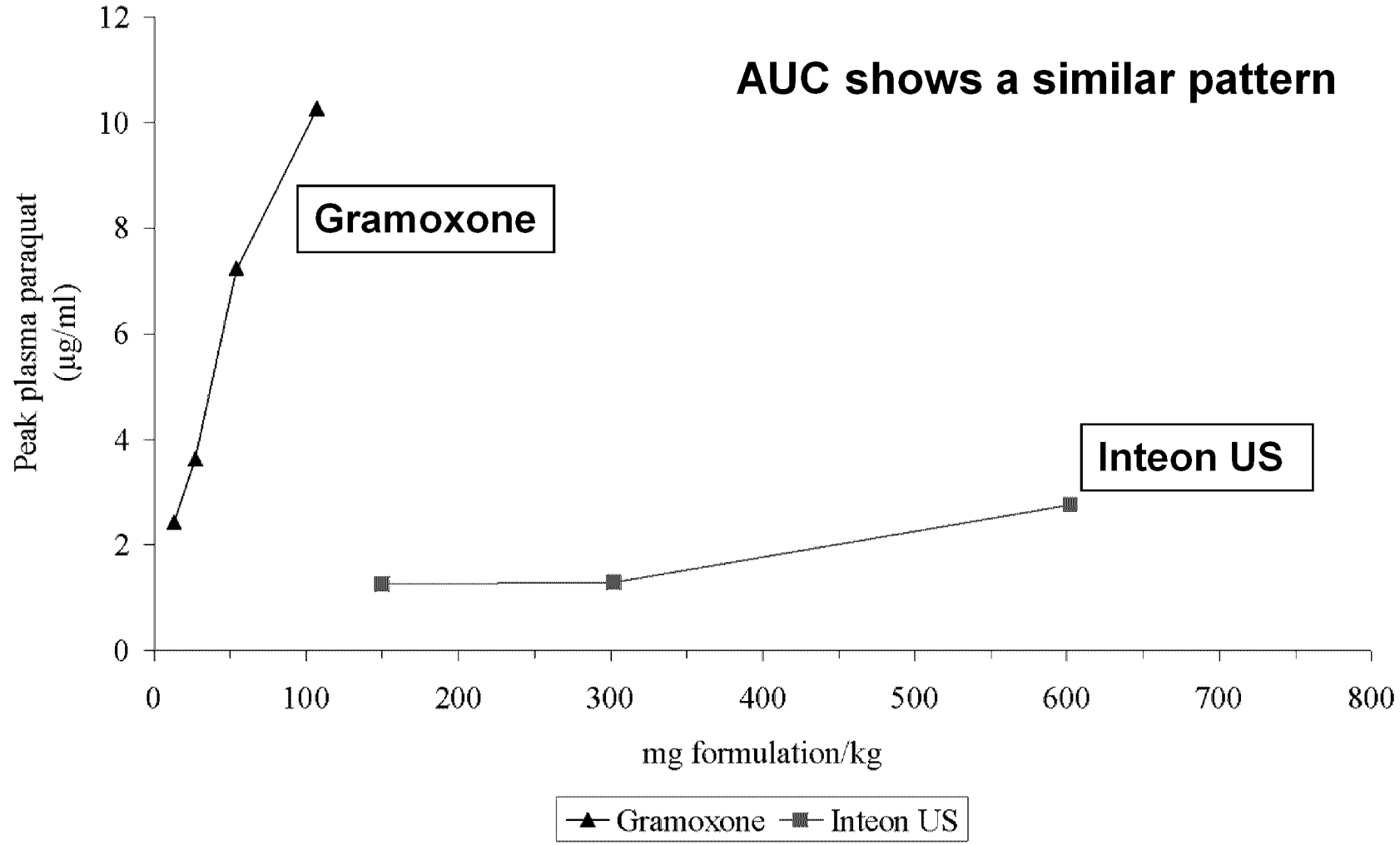
GRAMOXONE INTEON: Lower Peak Plasma Paraquat Levels in Dogs



Human ingests formulation

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Inteon US: Consideration of lung pathology from toxicokinetics in the dog

- All dogs tolerated the highest dose of 602mg A7813K well and there was no clinical evidence of toxicity
  - No pulmonary auscultation
  - No effect on clinical chemistry
  - Minimal bodyweight loss quickly recovered
- Small, discoloured areas (<1cm^2) were present in the left and right apical lung lobes of Male 2 at *post mortem* examination. Not seen in other dogs
- These were areas of minimal interstitial fibrosis, interstitial pneumonia, alveolar macrophage infiltration and pneumonocyte hypertrophy.
- Although considered to be treatment related, not life threatening and not progressive.

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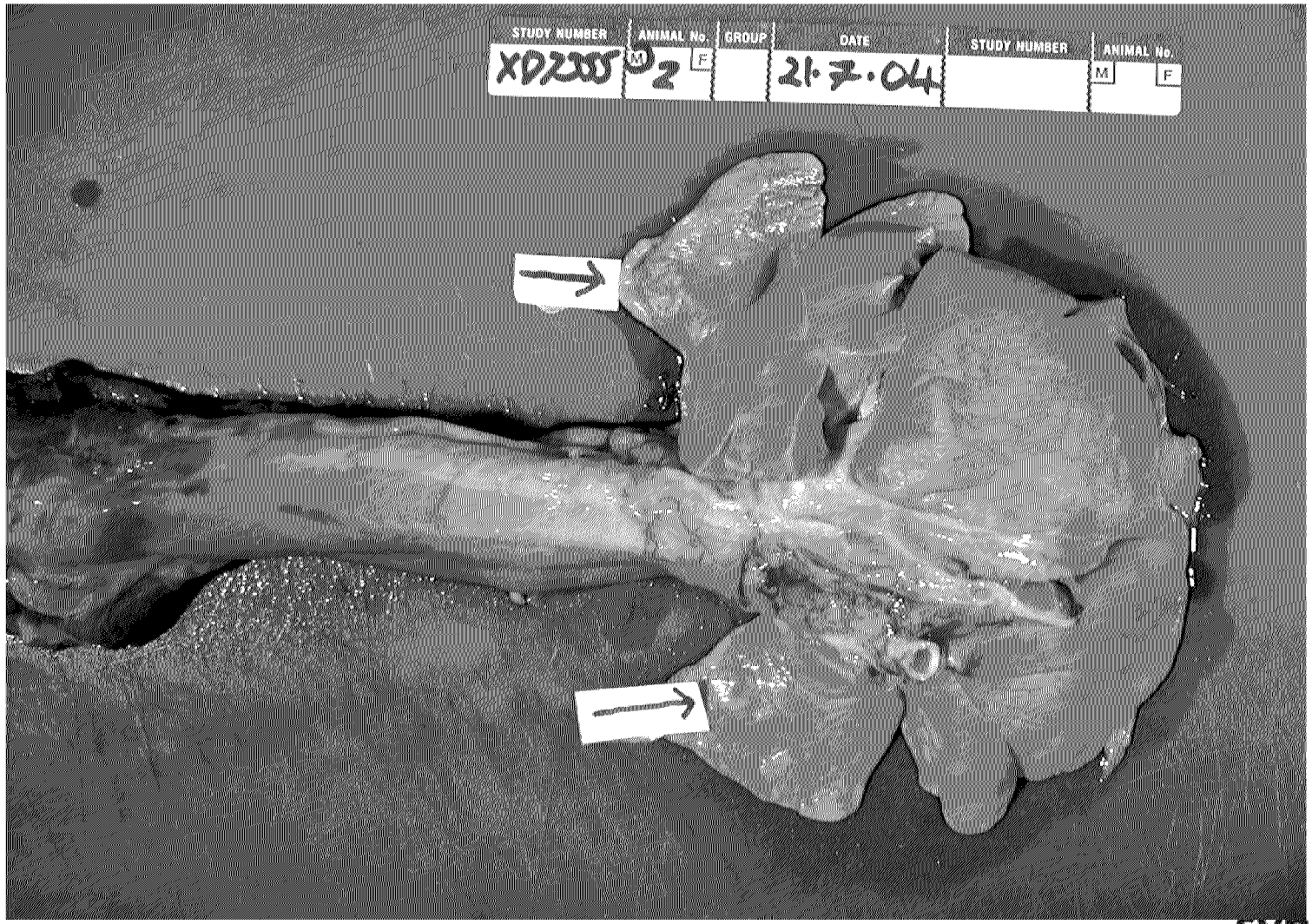
XD7355 Dog 2. Lung: Several small discrete areas of discoloration are present (denoted by arrows). The remainder of the lung appears normal.



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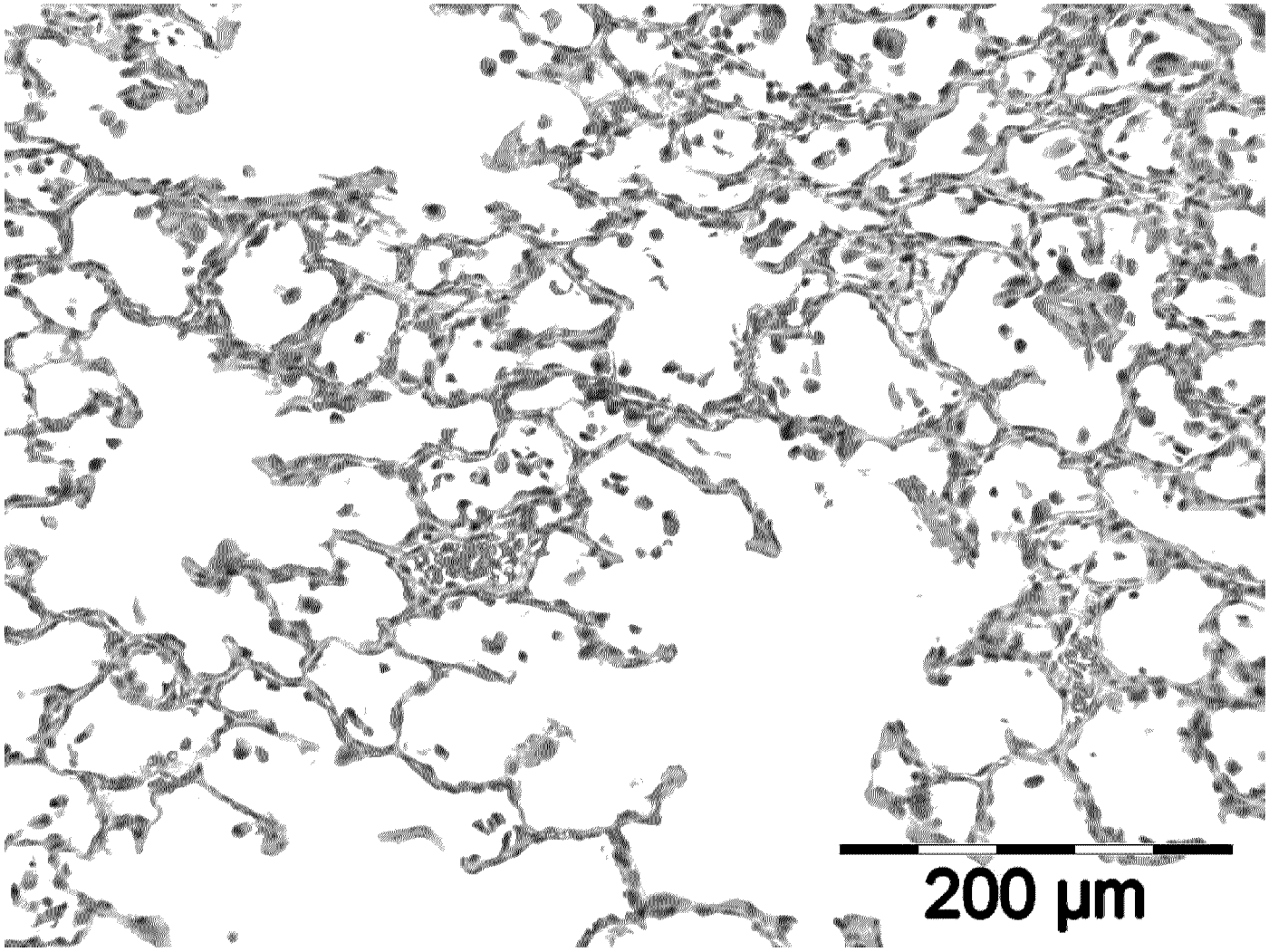
XD7201 Dog 3. Lung: Focal area showing thickened alveolar septae (septal fibrosis). Note the absence of progressive changes (alveolar edema, acute inflammation, fibroplasia).



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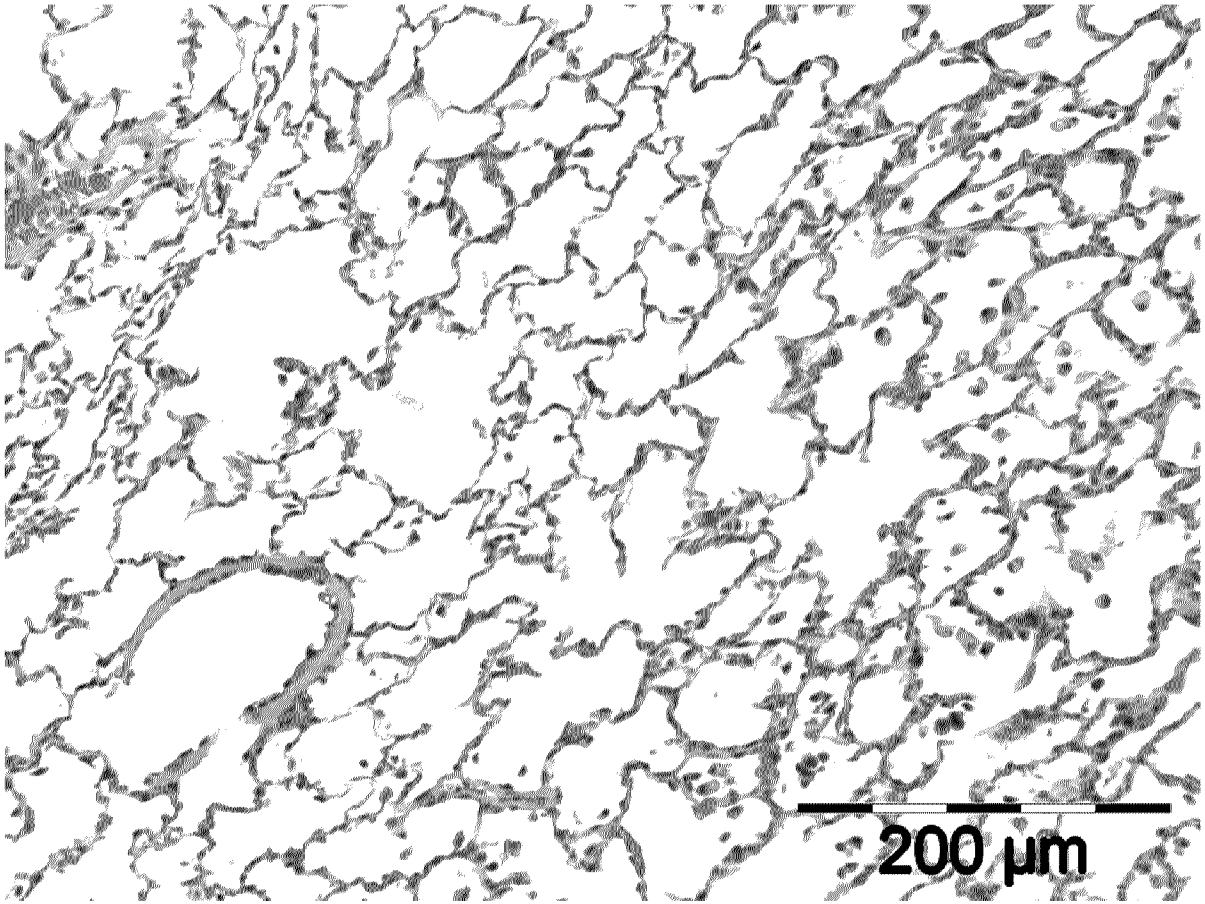
***XD7201 Dog 3. Lung: Junction between affected and normal appearing alveoli. Note the absence of progressive alveolar changes*.**



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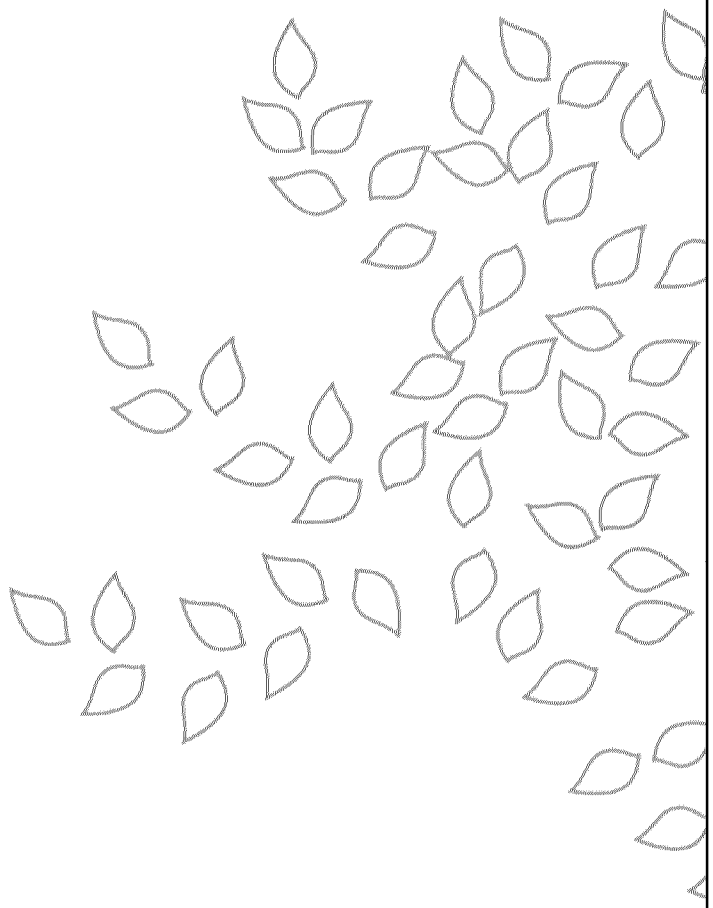
Critical end point for Human

- Basis of safening should be survival with no progressive lung lesions
- Observations in the dog at 602mg of Inteon US formulation /kg.
  - Nor mortality at this dose
  - Minimal lung lesion, nonprogressive, only seen in 1 of 3 animals
- Conclude 602mg/kg is the appropriate dose for risk assessment

Relevancy of Dog Data to Predict Human Safety

Comparison of Human and Dog GI Tract



Human: Dog – Comparison of GI Anatomy and Physiology

	Characteristic	Human	Dog
Relevant Similarities	**Chamber**	**Single/glandular**	**Single/glandular**
	Capacity	**1-1.6 L**	**~2 L**
	pH fasted	**1.4 – 2.1**	**1.5**
	Gastric Mucosa	**Predominantly "Proper Gastric" (see diagrams)**	
	Emptying rates	1-2 hrs	1-2 hrs
	Proportional GI lengths (%): Small	80	85
	Cecum	3	2
	Colon	17	13
	Vomiting	Initiated by local irritation and/or similar neural reflex pathways to/from CNS	
Potentially Relevant Differences	Total GI Transit Time	8 – 72 hrs	6 – 8 hrs
	Small Intestine Transit time	3-4 hrs	>4 to <8 hrs

From: Kararli, TT (1995). Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm & Drug Disp. 16: 351-380.

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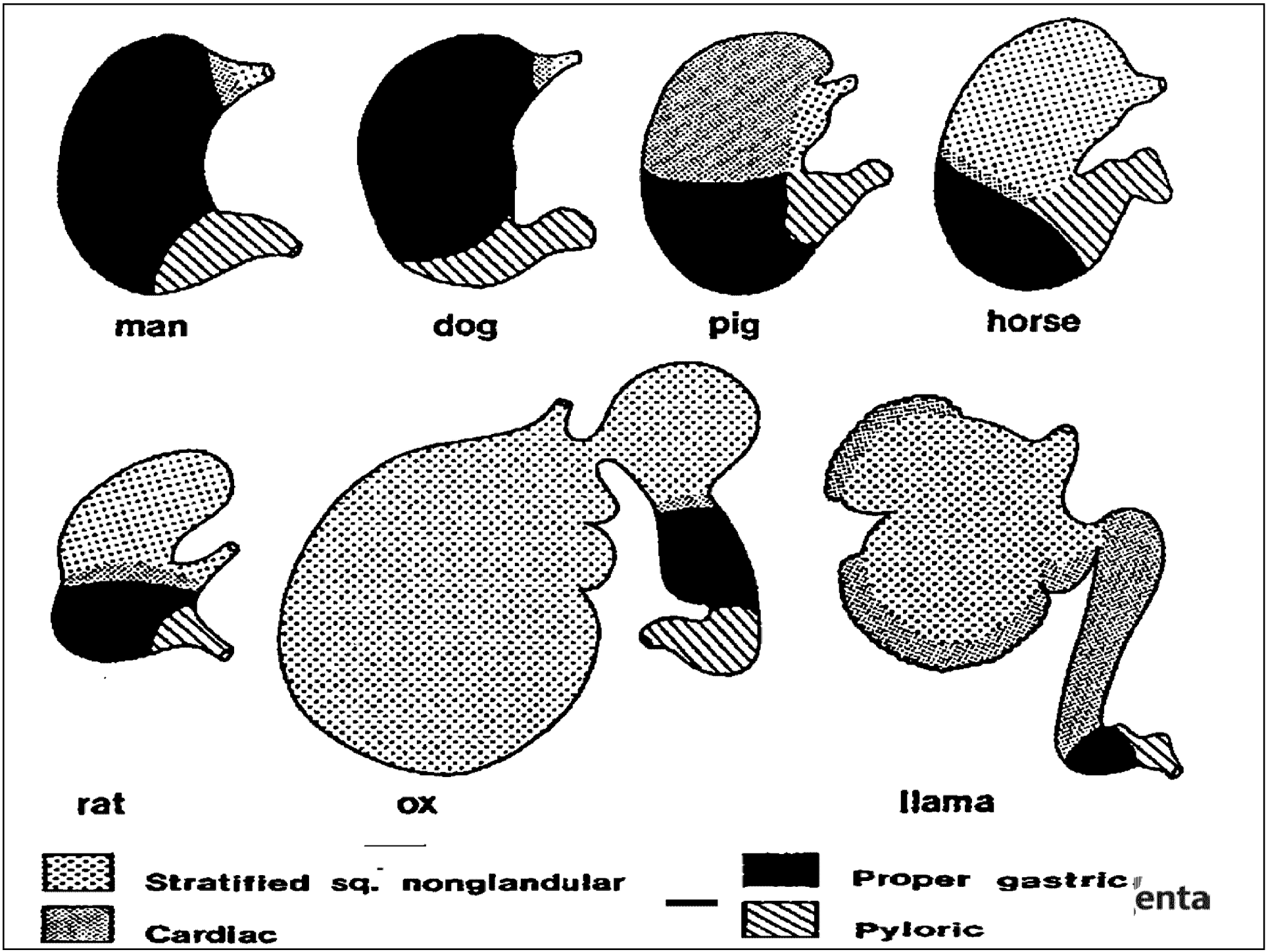

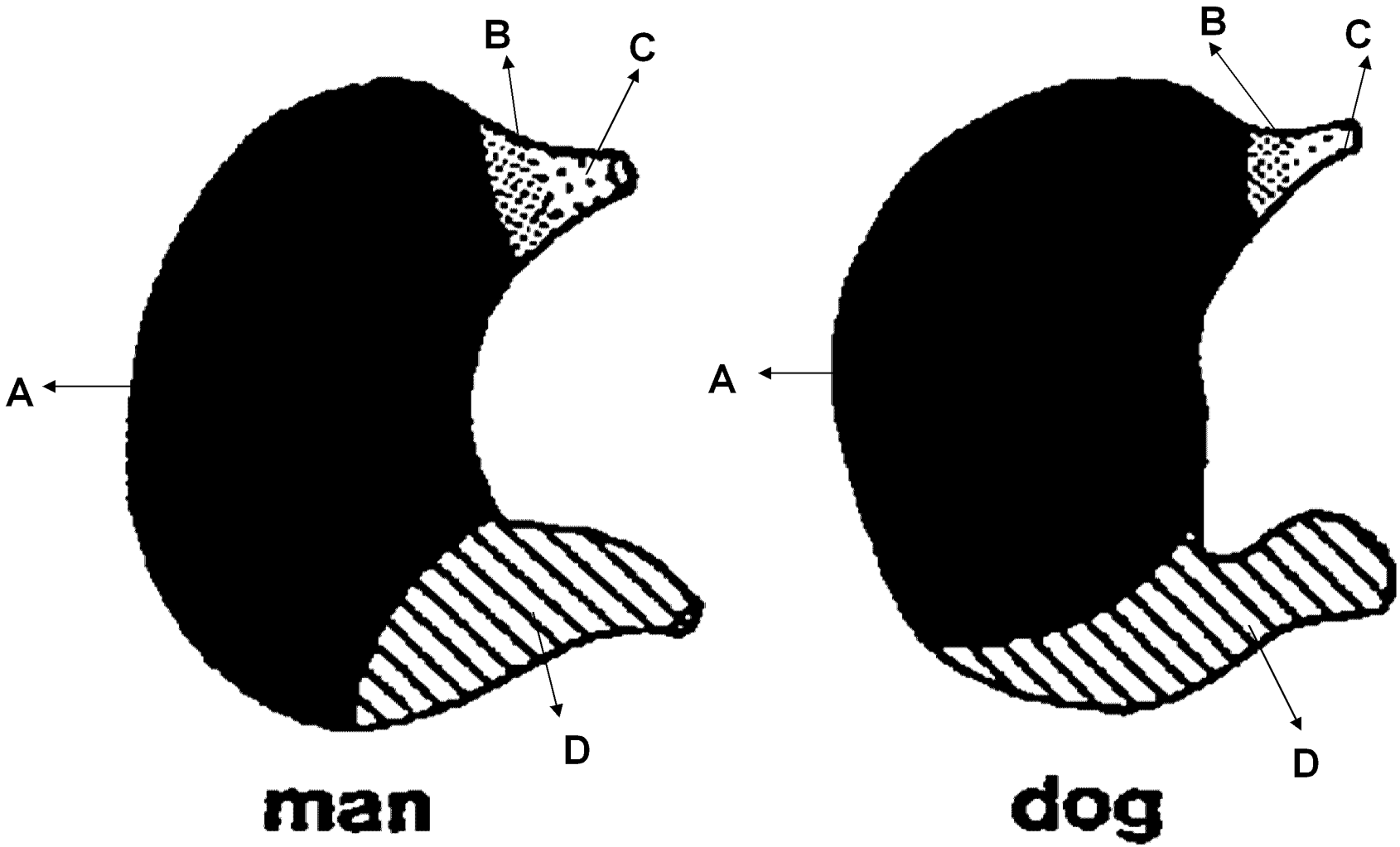

Variations in types and distribution of gastric mucosa

A = Proper gastric **B = Cardiac**

C = Stratified squamous non-glandular **D = Pyloric**

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Human:Dog- Irrelevant Differences in GI Anatomy and Physiology

Upper GI (stomach and small intestine tract) Microflora

- **Numbers**
  - **Human: 0-5 x 10/gram wet weight**
  - **Dog: 4-7 x 10/gram per wet weight**
- **Types**
  - **Human: predominantly bacteroides and bifidobacteria**
  - **Dogs: predominantly other flora, e.g. *E. coli*, streptococci, *C. perfringens*, lactobacili**

Paraquat is not affected by gut flora

Summary: Dog is a Valid Model for Improving the Safety of Paraquat Following Ingestion

- The dog is physiologically and anatomically a valid model for testing the improvements in this formulation
- More than an order of magnitude reduction in absorption is demonstrated in dogs for Inteon
- More than an order of magnitude reduction in acute oral toxicity in dogs for Inteon
- Formulation change will save human lives

Two questions posed by EPA

1. Is the paraquat Inteon formulation safer for human than the paraquat non-Inteon formulation?

- ✓ Clear evidence in the dog
- ✓ Dog model is relevant to human

2. **Is the paraquat Inteon formulation safe for humans at the high exposures achieved after accidental or deliberate ingestions?**

- ✓ **Species differences in toxicity**
- ✓ **Extrapolation from human ingestions**

Species differences in acute oral toxicity

Species	MLD paraquat ion mg/kg	Inteon US MLD mg formulation/kg
Rat	~100	310
Dog	~12	602 (Equivalent to 128mg paraquat ion/kg)
Human* Pond (1990)	50 - 80	X2 ? 470 **X5 ? 1177** X10 ? 2350

Toxicity in human is unknown but 5 fold safening giving a figure of 1177mg formulation/kg used for subsequent modelling

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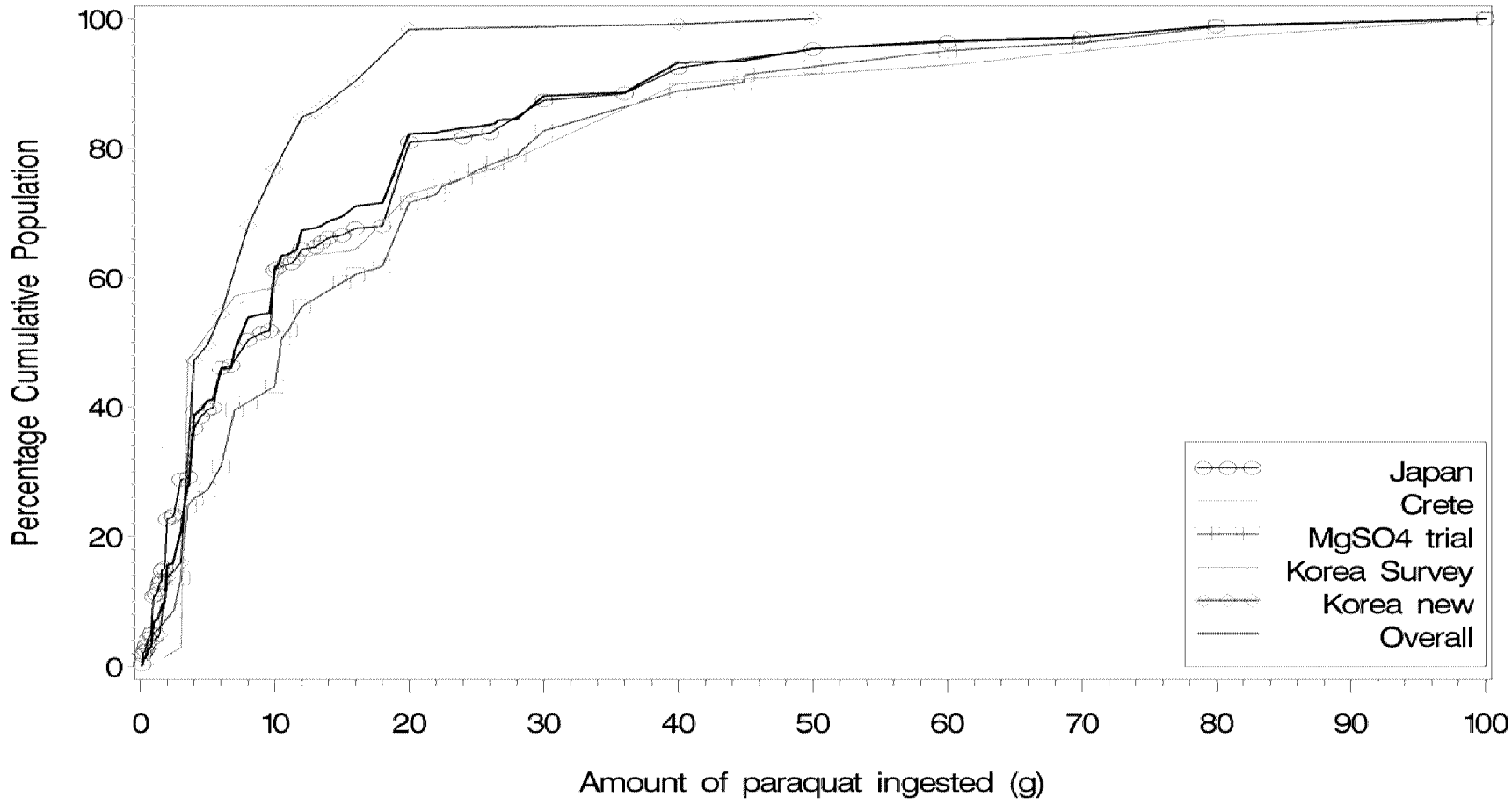
Other

1) Add intentional and accidental ingestion slide

Distribution of intake from human ingestions for 5 sub-populations totalling 563 cases.



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Estimated human intake and survival

- Pond 1990 estimated the MLD of Gramoxone to be 15 – 25mls (3 - 5g paraquat ion)
  - Equates to ~50 - 80mgkg for 60kg human
- From the previous distribution of intake of those deliberately ingesting paraquat formulations
  - Approximately 50% consumed less than 50mls (~10g paraquat ion)
  - Overall survival from this population was ~25%
  - For some subpopulations this figure is as low as 25mls of paraquat formulation (5g paraquat ion) and they showed improved survival.

Possible impact of Inteon US formulation

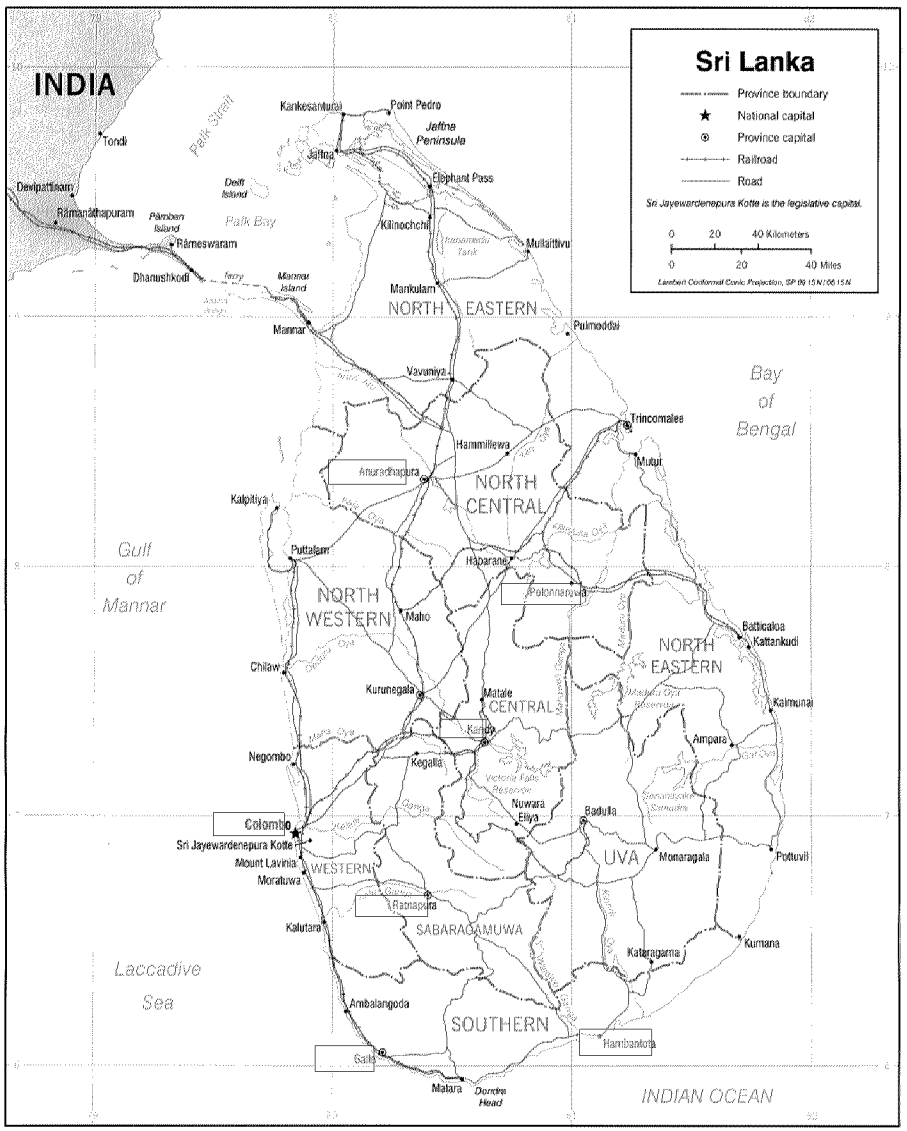
- If Inteon US showed 5 fold safening in human
  - Estimated human MLD 1177mg/kg
  - Assuming SG of Inteon US formulation is 1.13 and
  - Average human bodyweight of 60kg
  - Then volume to consume MLD is 1177/1.13 x 60 = 62.5mls
  - Based on the dose response from the human data and intake distributions then this would equate to a survival rate of 59%
  - A very significant improvement in human survival.

Sri Lanka Observational Monitoring Survey

Michael Clapp, PhD

Sri Lanka Observational Monitoring Survey

- 9 hospitals involved
- ~ 350 Gramoxone cases (June '03 – Aug '04)
- Introduced Inteon Sept '04, later discovered formulation problem
- Closed to new cases 26 Jan '06, at that time 224 confirmed INTEON ingestions (predominantly intentional)
- Criteria for survival: patient alive 3 months after release from hospital - end April 2006
- Independent Experts to meet 30th May 06.
- Summary of the findings expected June 2006.

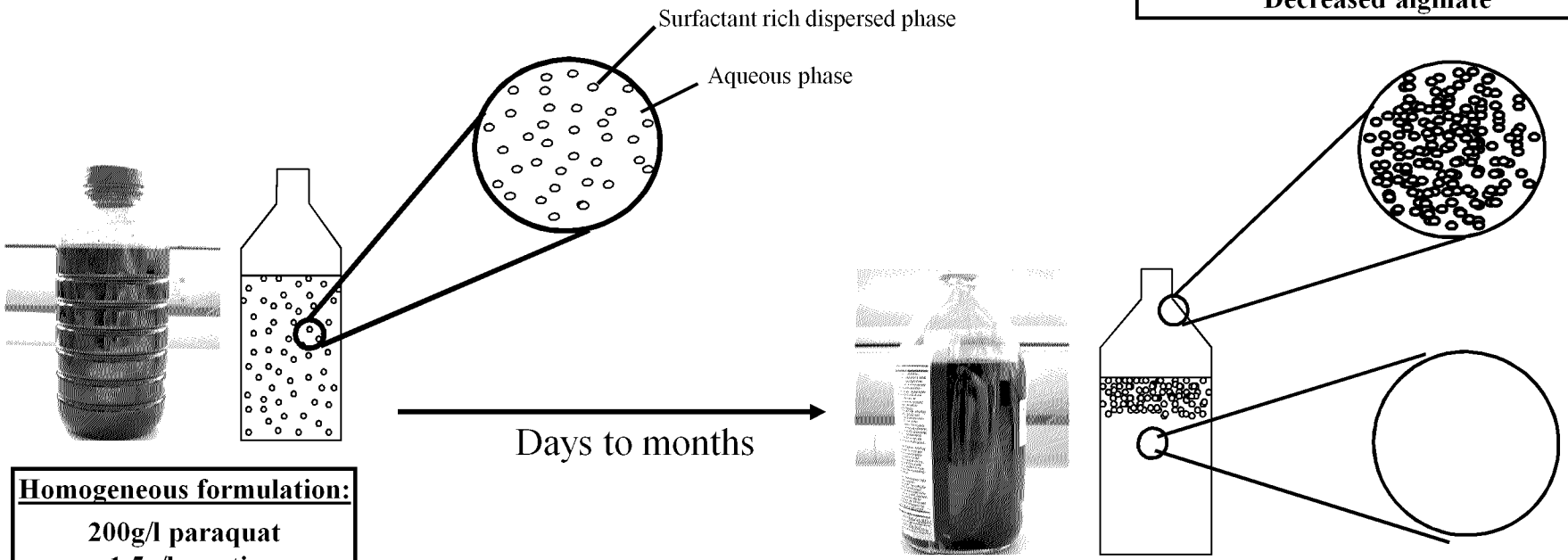


Base 802734AI (C00127) 3-01

Inteon Sri Lankan formulation: Illustration of formulation separation

Top:

Decreased Paraquat loading
Increased Emetic loading
Greatly increased Surfactant loading
Decreased alginate



Homogeneous formulation:

200g/l paraquat
1.5g/l emetic
9g/l alginate
~100g/l surfactants

Bottom:

Increased Paraquat loading
Decreased Emetic loading
No Surfactant
Slightly increased alginate

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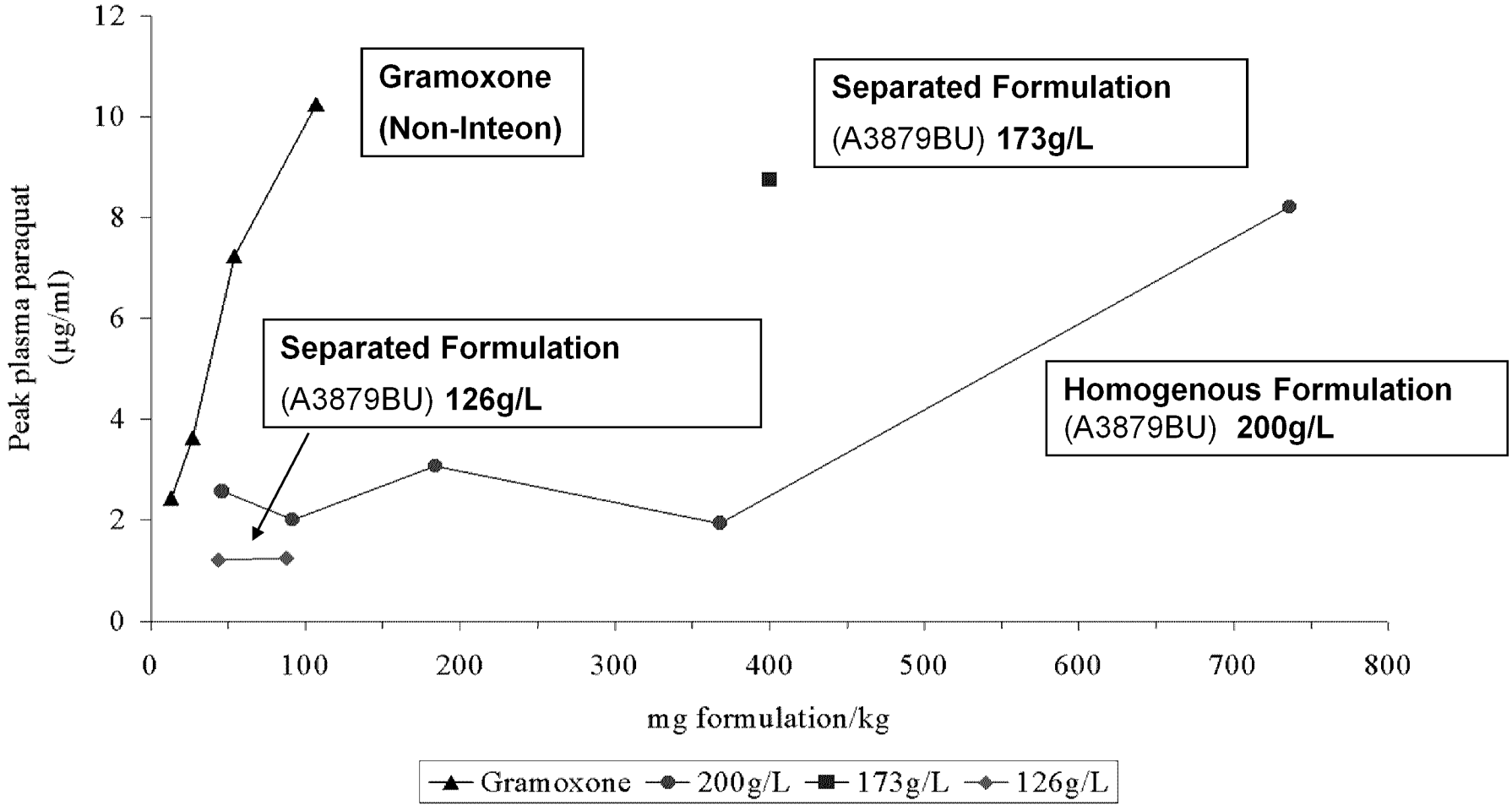
- The analytical profile of bottom phase is very similar to US formulation

- NB – no visual difference between homogeneous and separated formulation (recall demonstration), even in clear packs – and sales packs are essentially opaque (recall demonstration)

Inteon formulation in Sri Lanka: Lower Peak Plasma Paraquat Levels in Dogs



Separated Inteon formulation shows reduced safening but safer than Gramoxone

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Challenges in the Interpretation of the Observational Monitoring date

- Separated Inteon formulation in Sri Lanka [A3879BU]
  - ✓ It is safer than Gramoxone
  - ✓ Not as safe as homogenous Sri Lankan Inteon
  - ✓ Not as safe as the US Inteon formulation which does not separate (approximately half)
- Study conduct and data collection
  - ✓ Quality control checks on the hospital records, analysis (plasma and urine) and follow up survival data ongoing.
  - ✓ Estimating ingested volumes
  - ✓ Variable circumstances

Summary

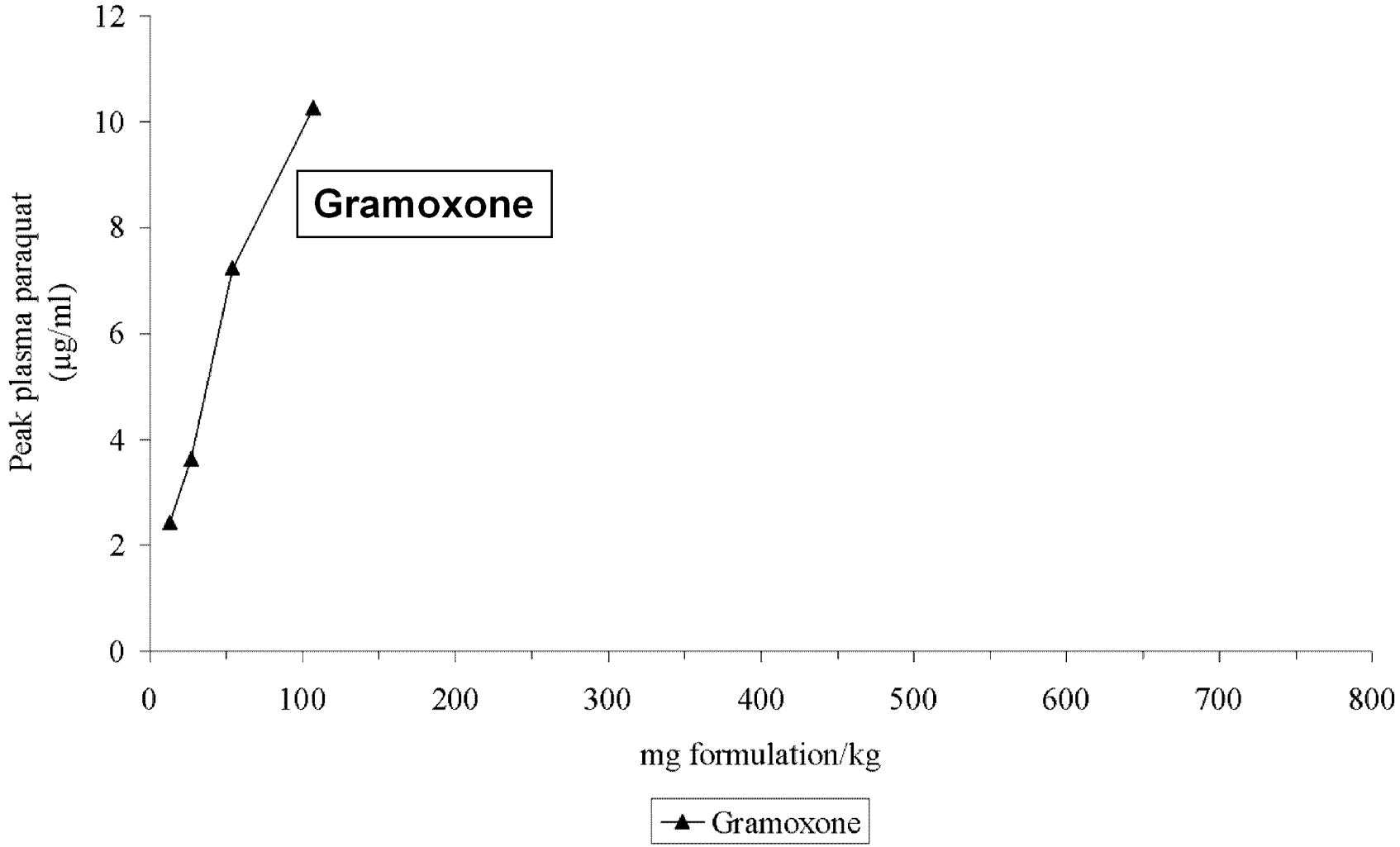
- **Dog is a valid and a useful model to predict human outcome**
- **We expect even greater safety with the US formulation compared with current formulation in Sri Lanka**
  - ✓ The US formulation showed > 10X safening in dogs
  - ✓ US formulation does not separate
- **Changing to INTEON formulations will save lives in the US and Internationally**

Reserve slides

Paraquat lung lesion

- An initial effusive stage, (characterised by acute inflammation, widespread pulmonary oedema and hyaline membrane formation),
- Followed by a chronic phase, (characterised by widespread alveolar fibrosis).
  - alveolar fibrosis is a progression of the acute effusive stage
  - rather than a progressive fibrosing disease *per se*.
- In these dogs no evidence to suggest that the lesions would progress
  - adjacent lung tissue appeared normal
  - affected areas were small foci and showed no evidence of significant active fibrosis.
  - the kidney, a sensitive target organ in the dog, showed no histological evidence
- Lung lesion observed in those with highest exposure, others NAD.

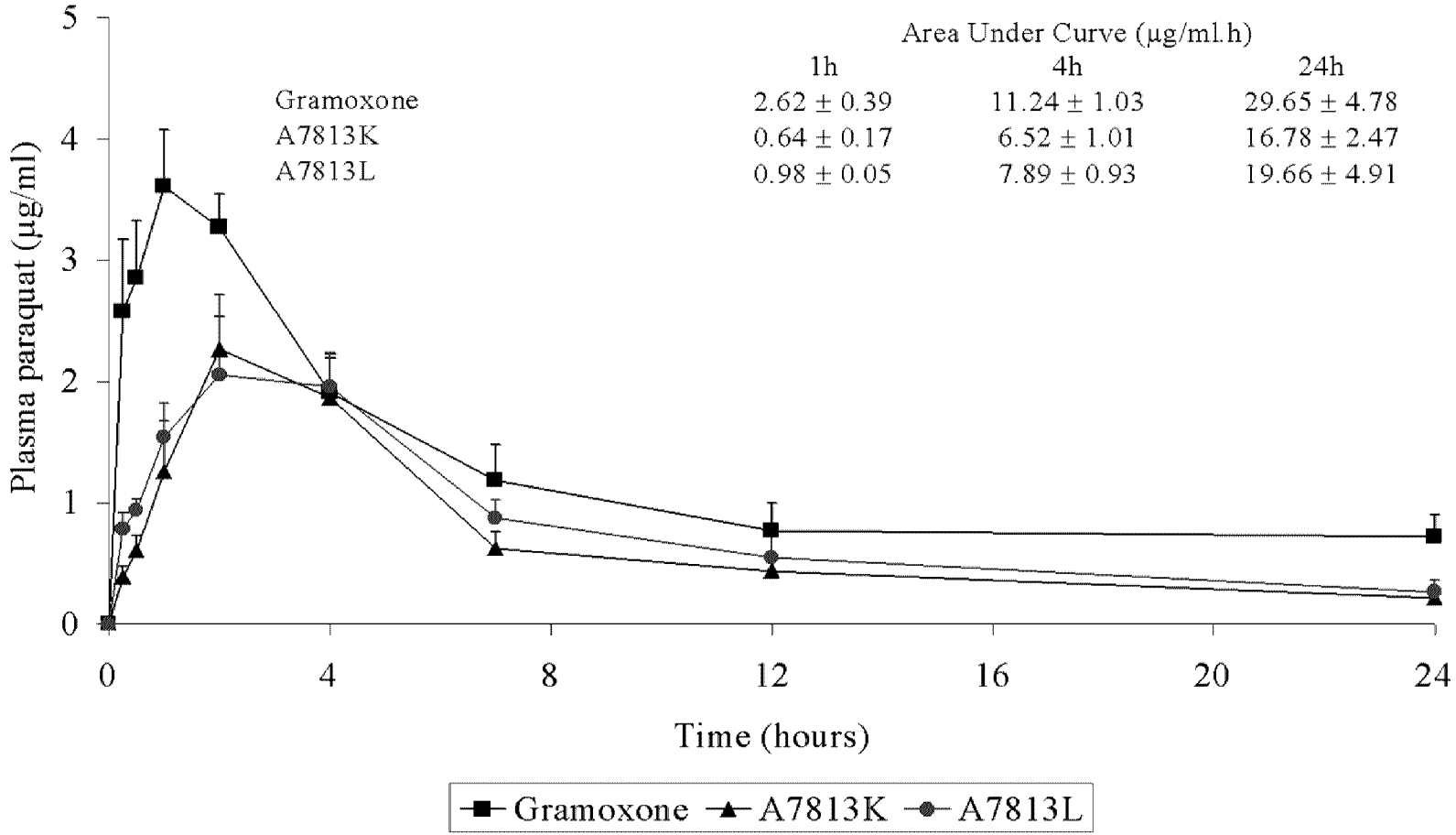
GRAMOXONE: Peak Plasma Paraquat Levels in Dogs



Gramoxone: 1 out of 4 dogs died at 55 mg formulation/kg

all 4 dogs were terminated day 7/8 at 110mg formulation/kg.

Plasma Paraquat following an oral dose of 40 mg paraquat ion/kg the rabbit



Reduced paraquat absorption in rabbit (non vomiting species) due to gelling

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A7813K: Toxicokinetics in the dog

- Kinetic profile across doses of 150 to 602mg Inteon US formulation/kg showed a flat dose response for both peak plasma paraquat and 24hr AUC values. This is equivalent to a paraquat ion dose of 32 to 128mg/kg.
- Gramoxone acute oral MLD in dog is 66mg formulation/kg (12mg paraquat ion/kg)
- No deaths occurred with Inteon US formulation at 602mg/kg
- The minimal pathology observed in one dog together with a higher peak plasma concentration would suggest a higher dose 1204mg Inteon US/kg (256mg paraquat ion/kg) would give rise to significant toxicity in the dog.
- Hence it is concluded that A7813K shows > 10 fold reduction in toxicity although this maybe be greater.