Gramoxone Inteon Improvement in Oral Toxicity Experimental Evidence

Michael Clapp, PhD

Two questions posed by EPA in Discussion Piece on Paraquat Inteon, 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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Formulation Terms Used

Gramoxone	200 g/l	Non Inteon	Global, not reg. in US
Gramoxone US	360 g/l	Non Inteon	US Voluntary cancellation request
Inteon	200 g/l	Inteon	Global, not reg. in US
Inteon US	240 g/l	Inteon	Registered in US

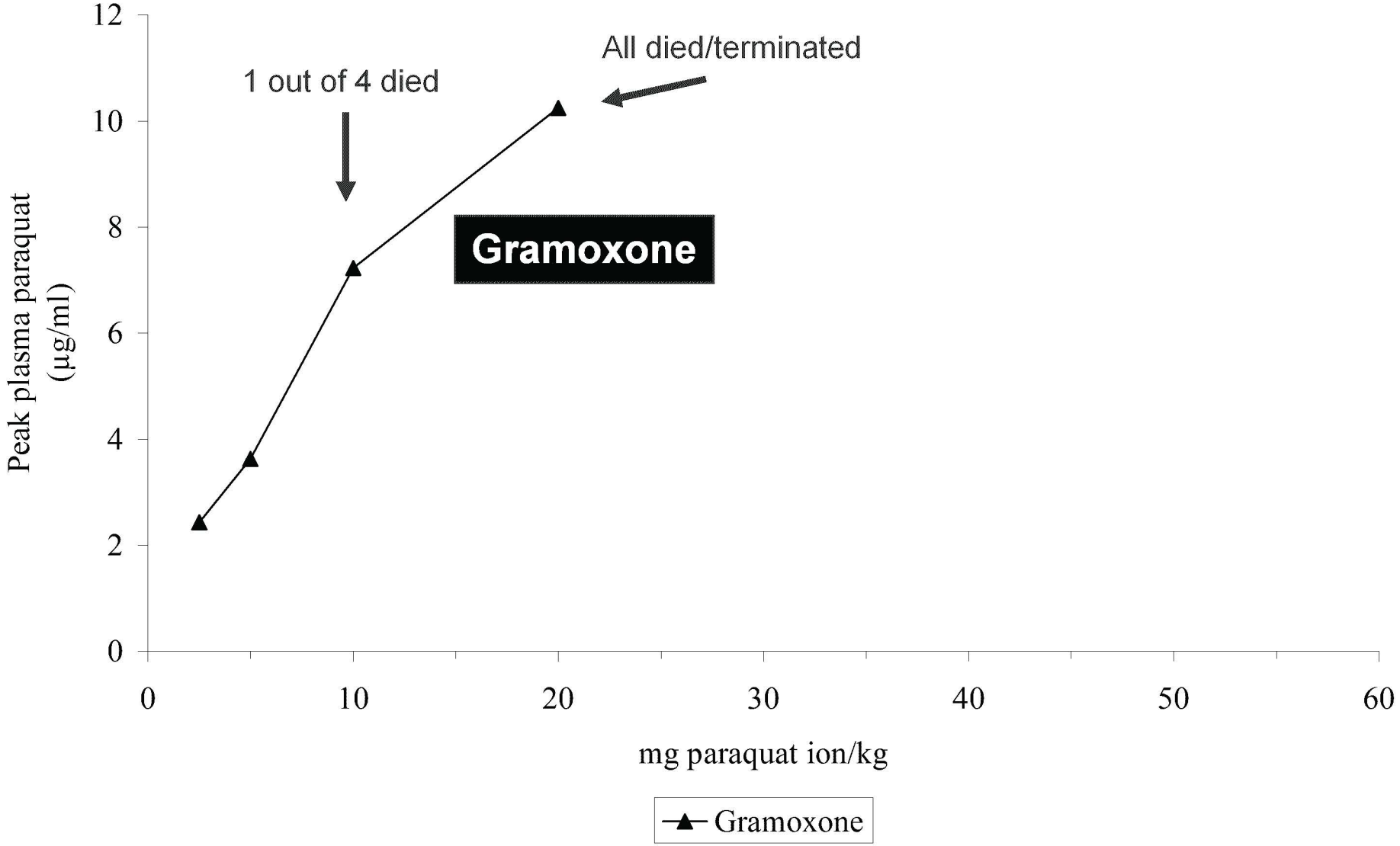
Clear Evidence in the Dog - Background

- Gramoxone data based on 200g paraquat/l with built in wetters

- Widdop et al 1977 – mortality at 10mg paraquat/kg

- Syngenta data (1987) confirmed median lethal dose (MLD) in dog to be 12mg paraquat/kg for Gramoxone formulation (0.5g/l emetic)

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

Clear Evidence in the Dog - Background

- These data provide the rationale for not testing Gramoxone at doses greater than 8 mg/kg (sub-lethal)

- Higher doses of Gramoxone (e.g., 16, 32, 64, and 128 mg/kg) doses would have resulted in mortality

Clear Evidence of Inteon US Safening in the Dog

- All dogs survived Inteon US doses at 32, 64, and 128 mg/kg

- The peak paraquat plasma concentration following dosing with Inteon US at 128 mg/kg was similar to that following dosing with Gramoxone at 8 mg/kg.

- Greater than 10X improvement in oral toxicity in dog with Inteon and Inteon US

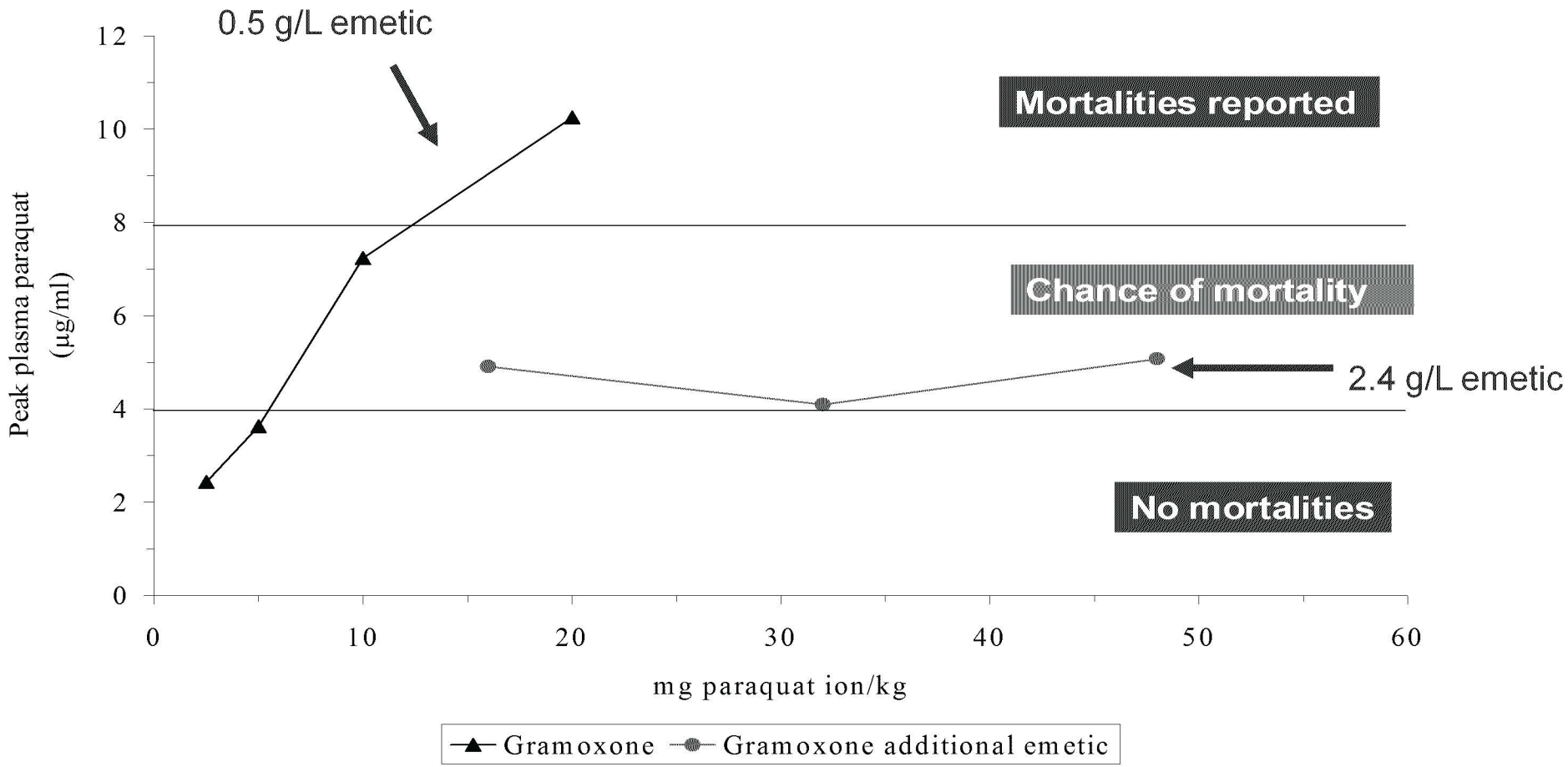
Is Improved Inteon Safety Due to Increasing Emetic?

- Increasing emetic level in Gramoxone offered only limited protection and posed its own clinical risk issues

- 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

Gramoxone: influence of increased emetic on peak plasma paraquat levels in dog



Controls peak plasma paraquat

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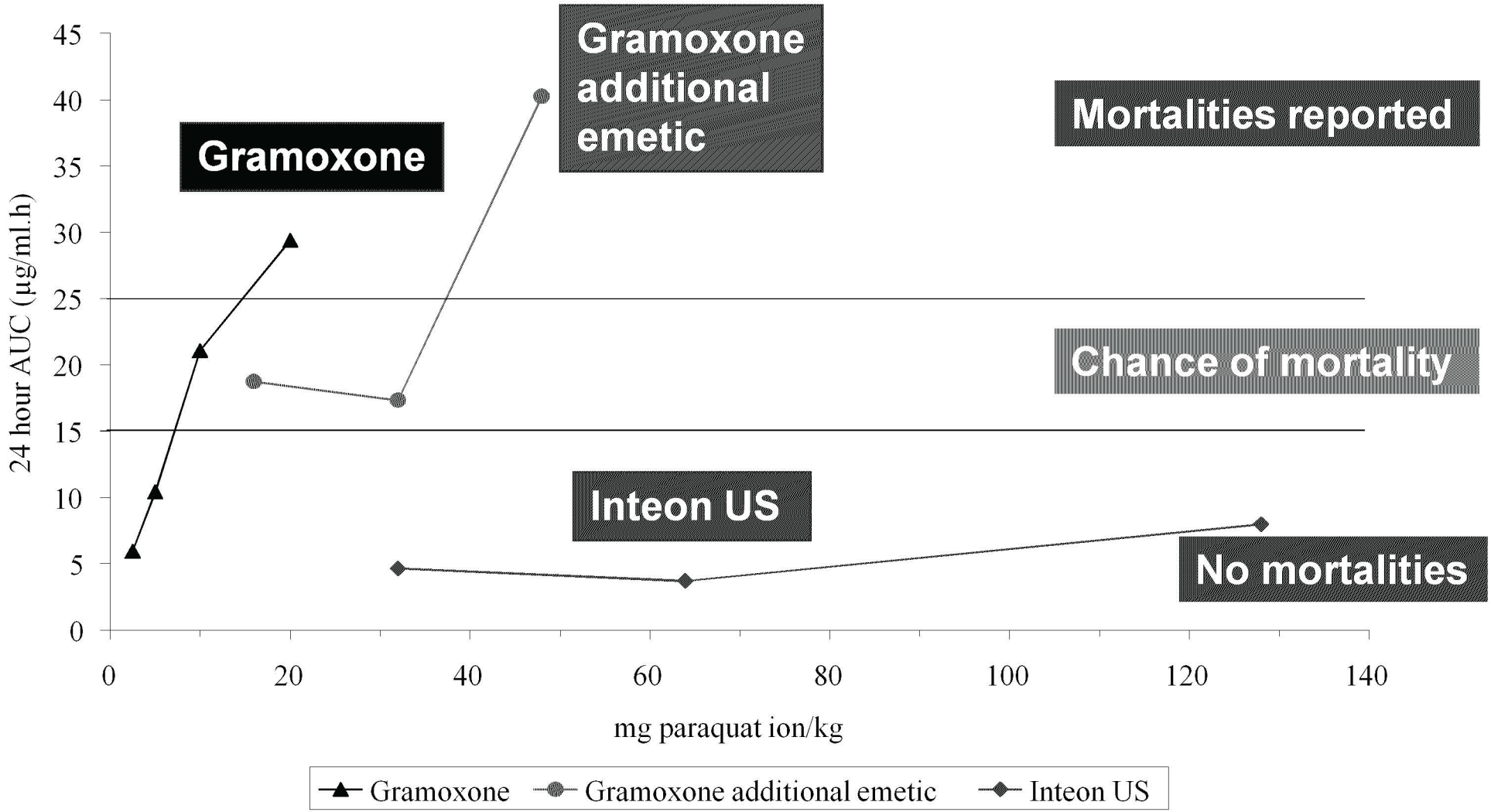
Comparison of 24h plasma paraquat AUC levels in dog



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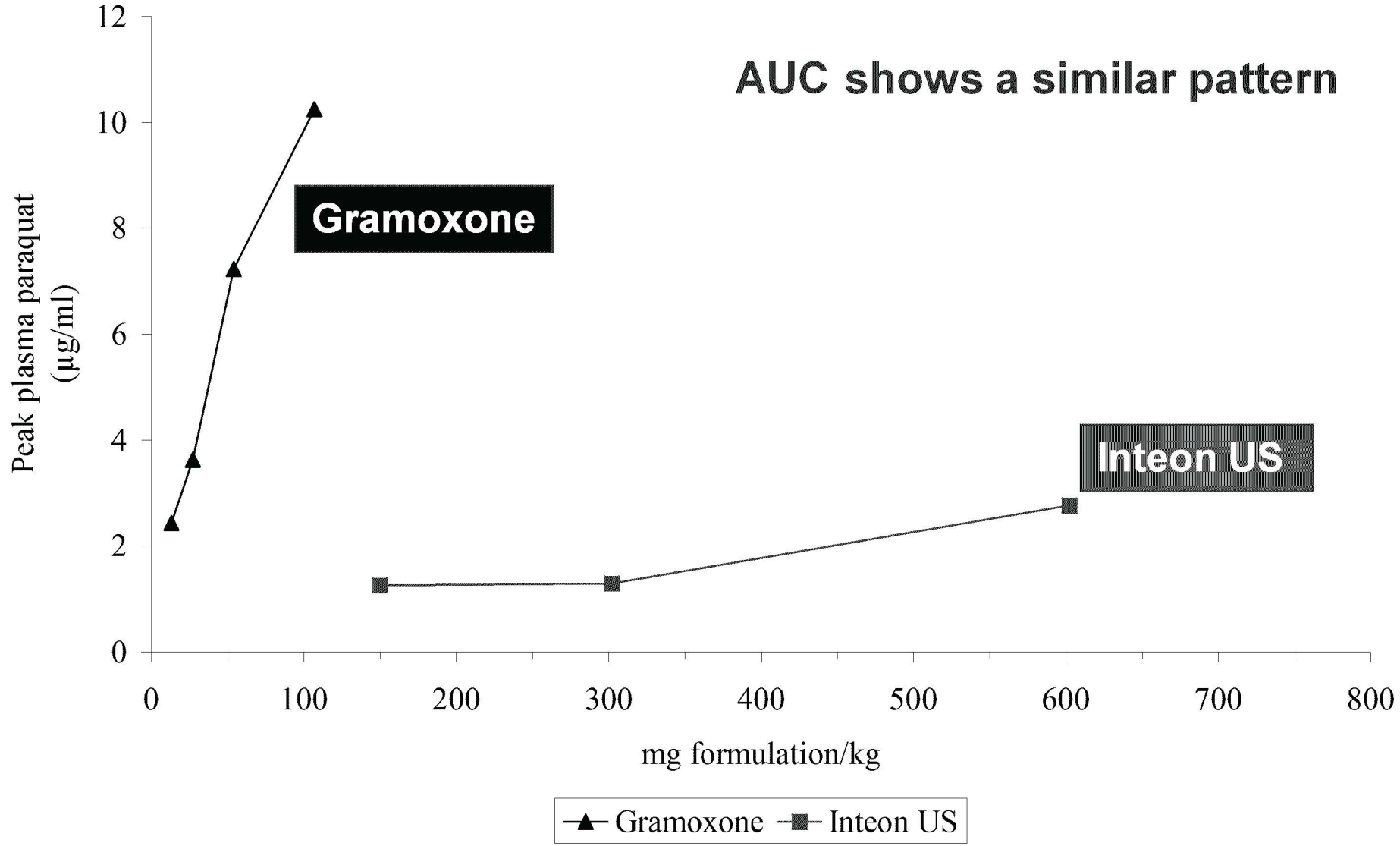
GRAMOXONE INTEON: Lower Peak Plasma Paraquat Levels in Dog



Human ingests formulation

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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?**

- ✓ **Proper endpoint for assessment**
- ✓ **Species differences in toxicity**
- ✓ **Extrapolation from human ingestions**

Critical end point for human is survival

- Basis of safening should be survival with no progressive lung lesions

- Observations in the dog at 602mg of Inteon US /kg
  - No mortality at this dose
  - Minimal lung lesion, nonprogressive, only seen in 1 of 3 animals

- Conclude 602mg/kg Inteon US is the appropriate dose for risk assessment while for Gramoxone the range is 37 to 66 mg/kg depending upon the formulation

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

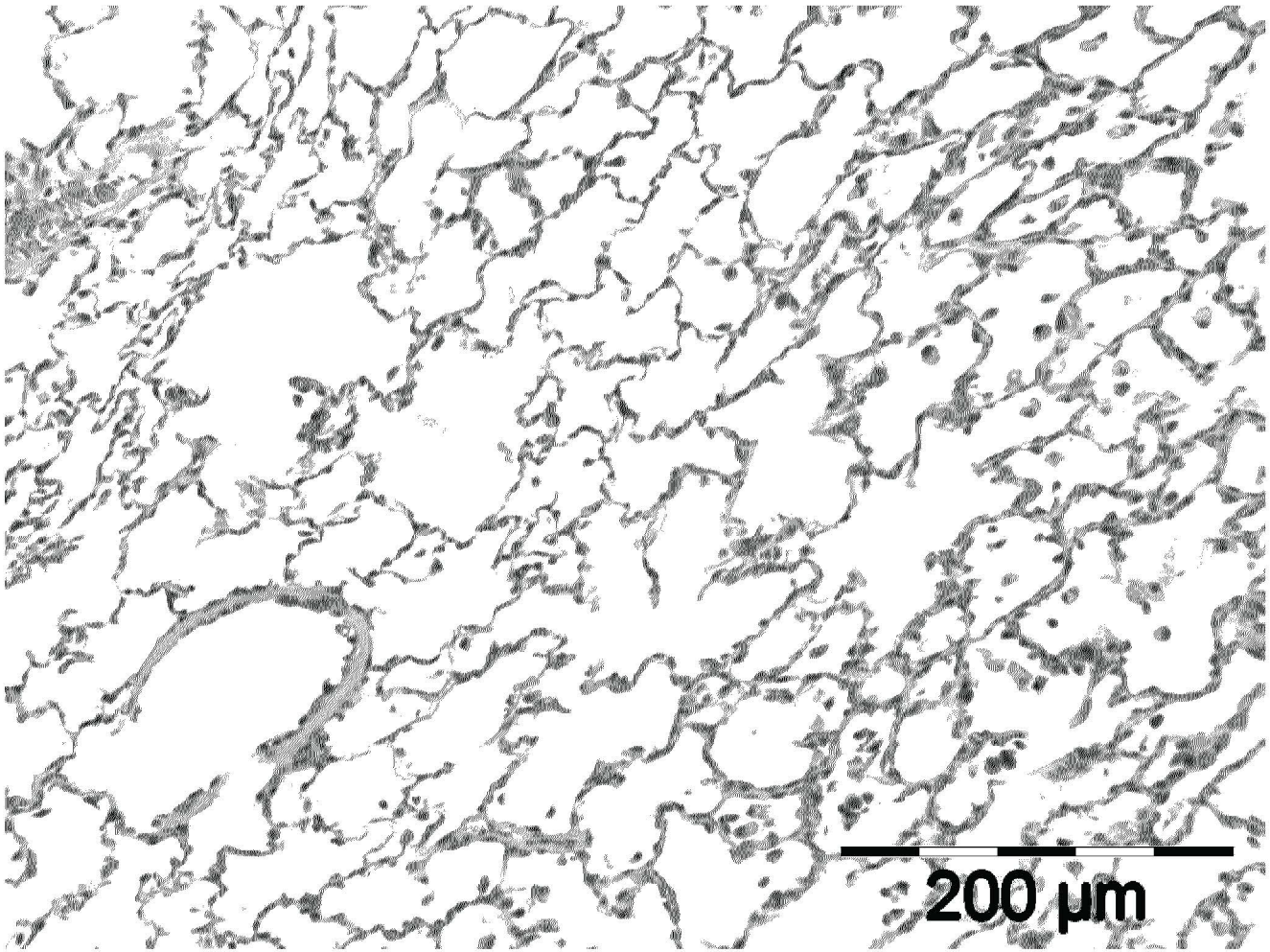
- All dogs tolerated well highest dose of 602mg of Inteon US/kg bw and there was no clinical evidence of toxicity
  - No pulmonary auscultation
  - No effect on clinical chemistry
  - Minimal bodyweight loss quickly recovered

- 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.**

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



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Relevance of Dog Data to Predict Human Safety

- Comparison of Dog and Human 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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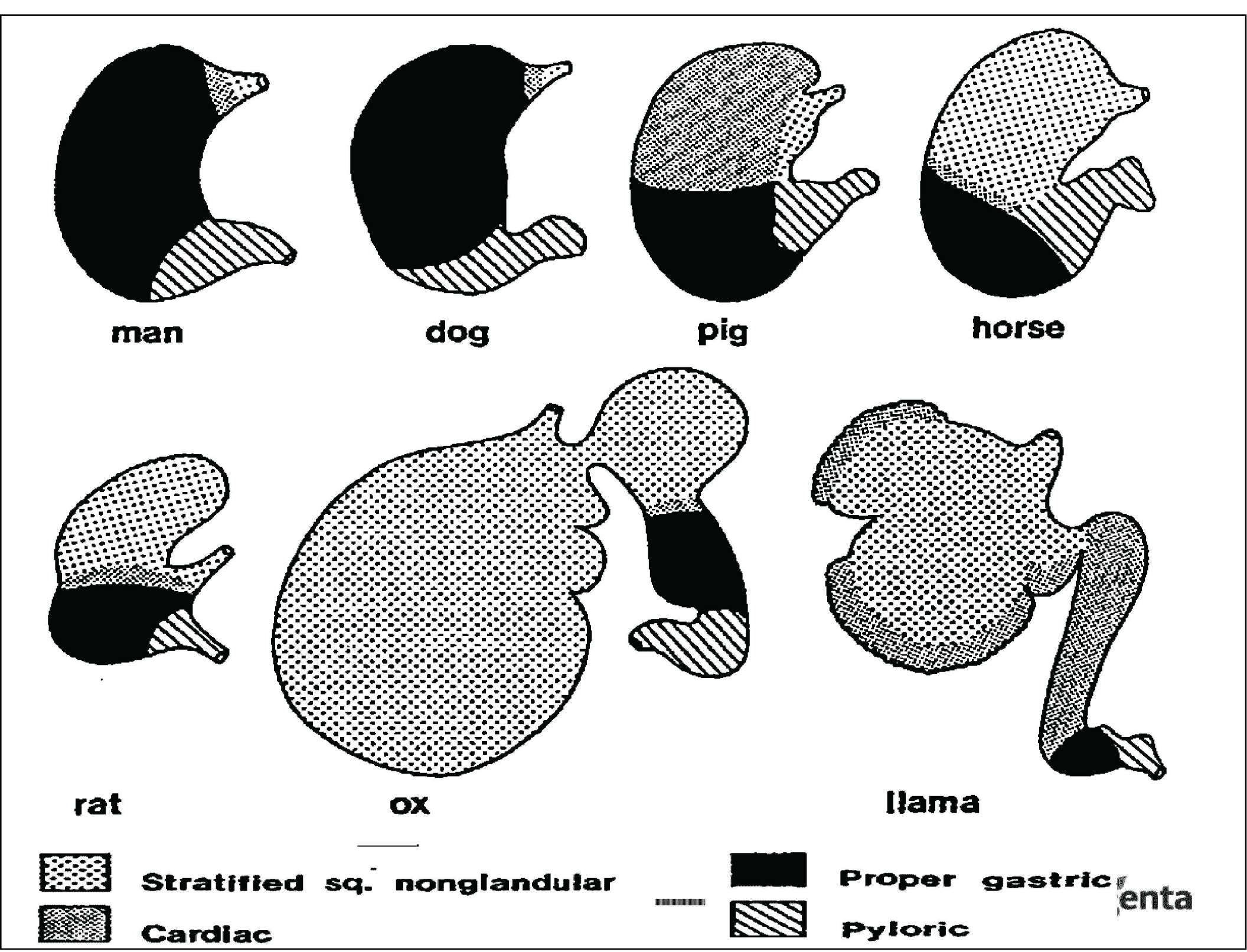

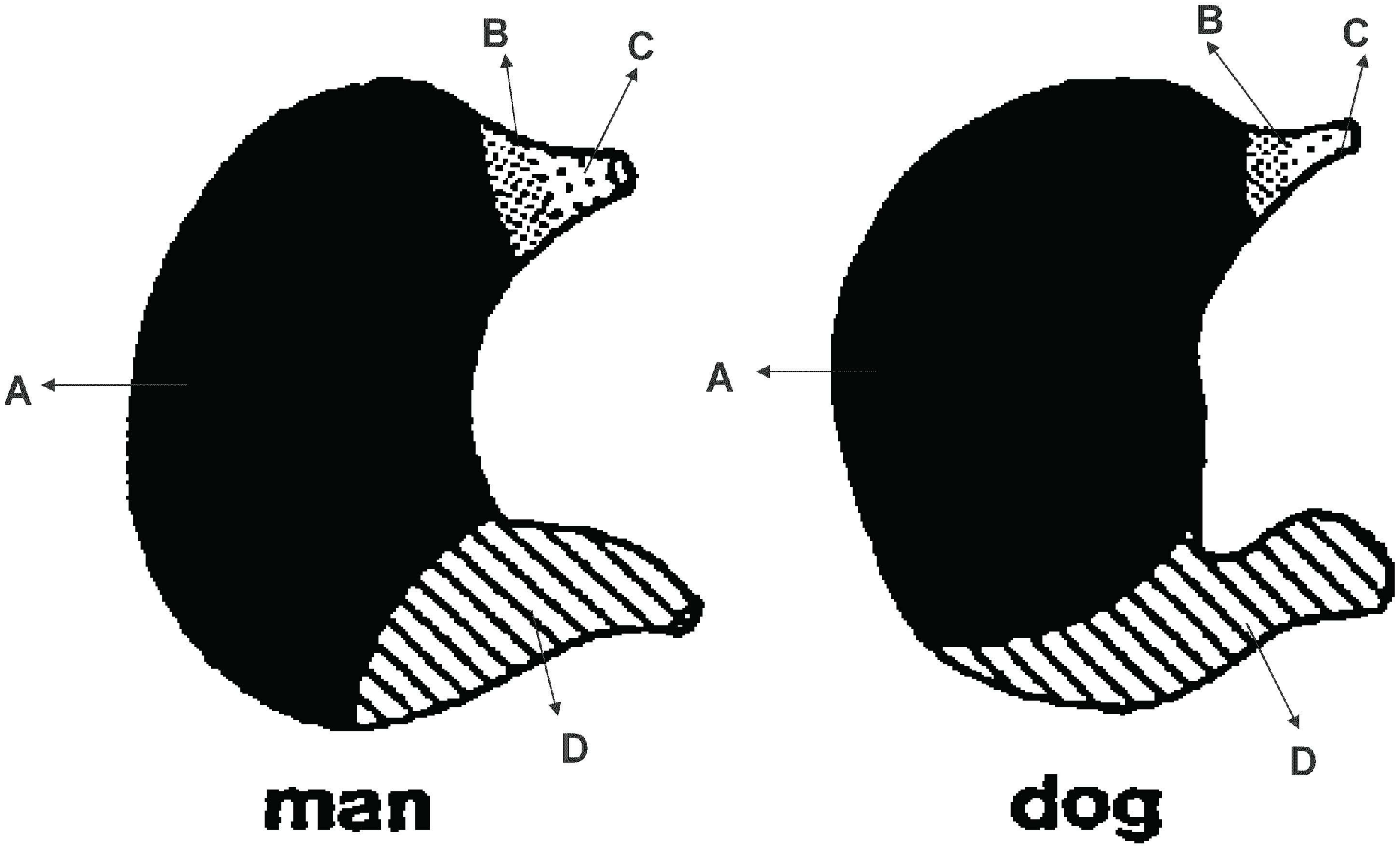

Variations in types and distribution of gastric mucosa

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
- > 10x reduction in absorption is demonstrated in dogs for Inteon
- > 10x reduction in acute oral toxicity in dogs for Inteon
- Formulation change will save human lives

Relevance of Dog Data to Amount Ingested by Humans

- ✓ Species Differences
- ✓ Human Incidence
- ✓ Extrapolation from human ingestions

Species differences in acute oral toxicity

Species	Median Lethal Dose (MLD) paraquat ion mg/kg	Inteon US mg formulation/kg
Rat	~100	MLD 310
Dog	~12	Non-lethal at 602 (128mg paraquat ion/kg)
Human	50 – 80 (15-25 ml Gramox.) (Pond,1990)	

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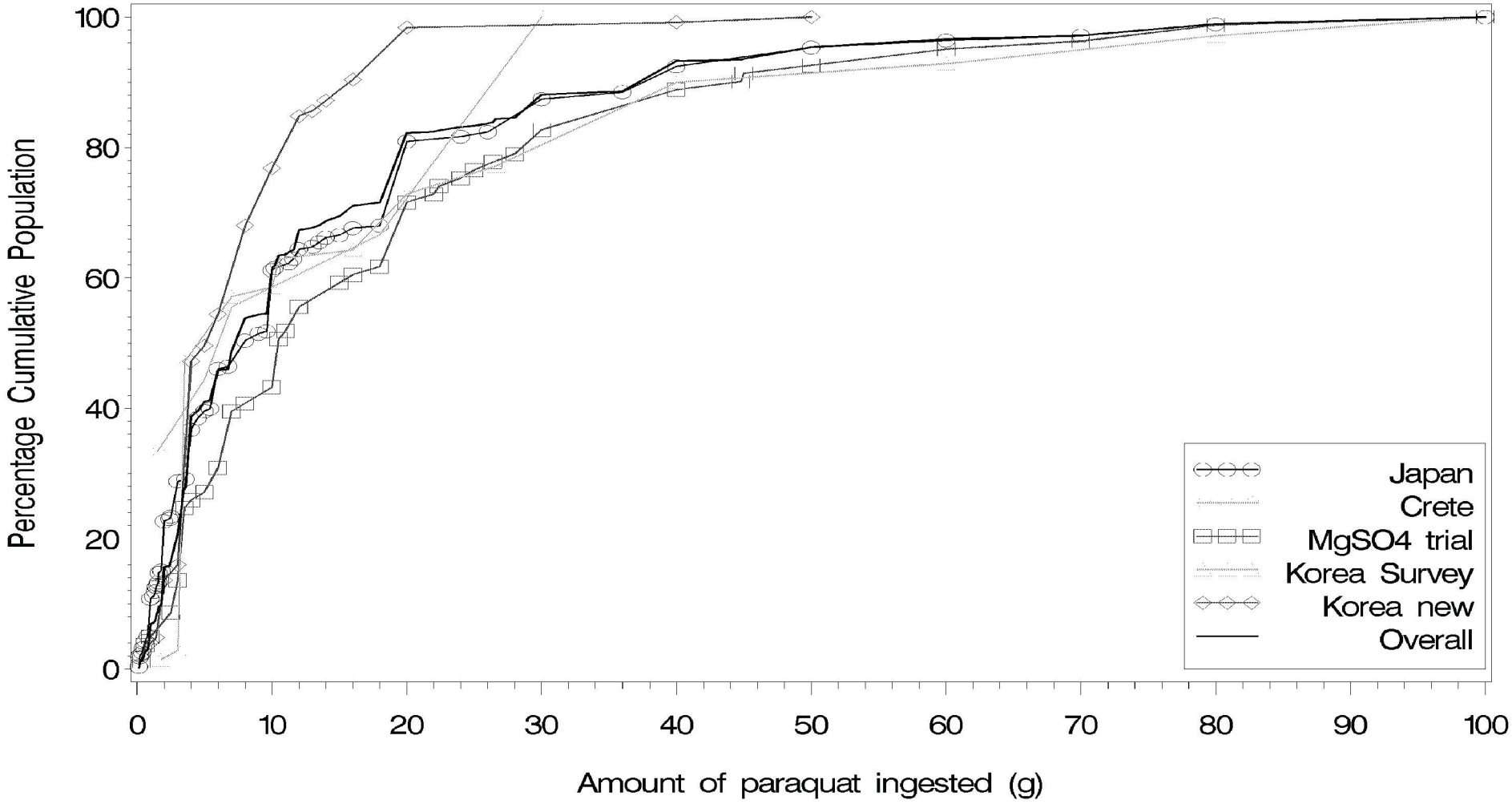
Intentional and accidental ingestion

- **Ingestion incidents in US**
  - ✓ On average – 2 to 4 fatalities per year in the US (Actual, not theoretical)
  - ✓ Accidental and Intentional
  - ✓ Volumes ingested (accidental versus intentional)
  - ✓ Vomiting occurs rapidly
  - ✓ No incidents with INTEON since introduction in fall '05
- **Rest of world**
  - ✓ Mexico ~ 20 – 45 per year
  - ✓ Rest of World – significantly greater in some countries
- **The Inteon formulations are safer and will significantly improve survival worldwide**

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



10g is approximately 50mls Gramoxone

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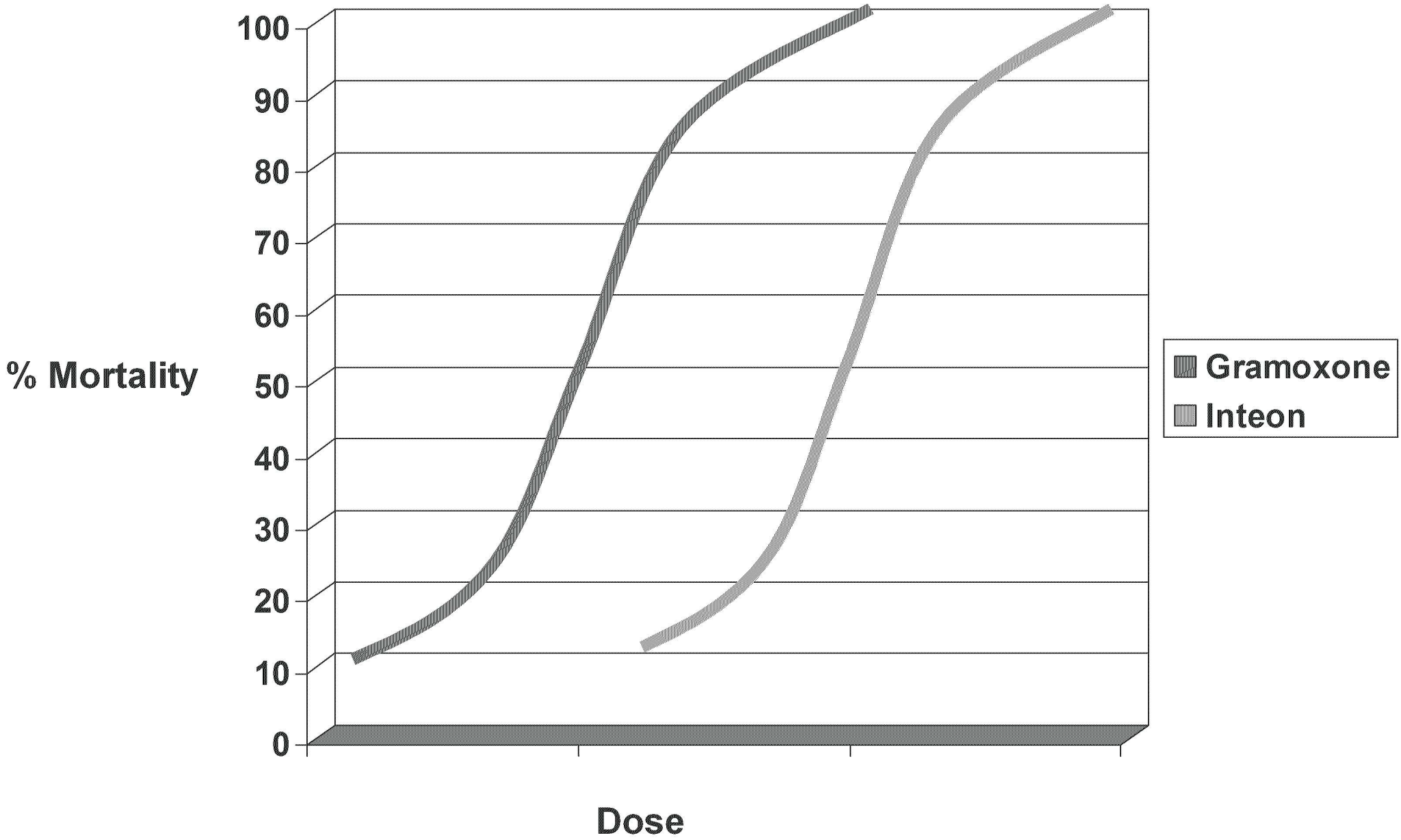
Estimated human intake and survival

- Pond 1990 estimated the MLD of Gramoxone to be 15 – 25mls (3 - 5g paraquat ion)
  - Equates to ~50 - 80mg/kg for 60kg human
  - Equates to ~50% survival

- From the previous distribution of intake of those deliberately ingesting paraquat formulations
  - Median intake is approximately 50mls (~10g paraquat ion)
  - Overall survival from this population was ~25%

Survival will be improved



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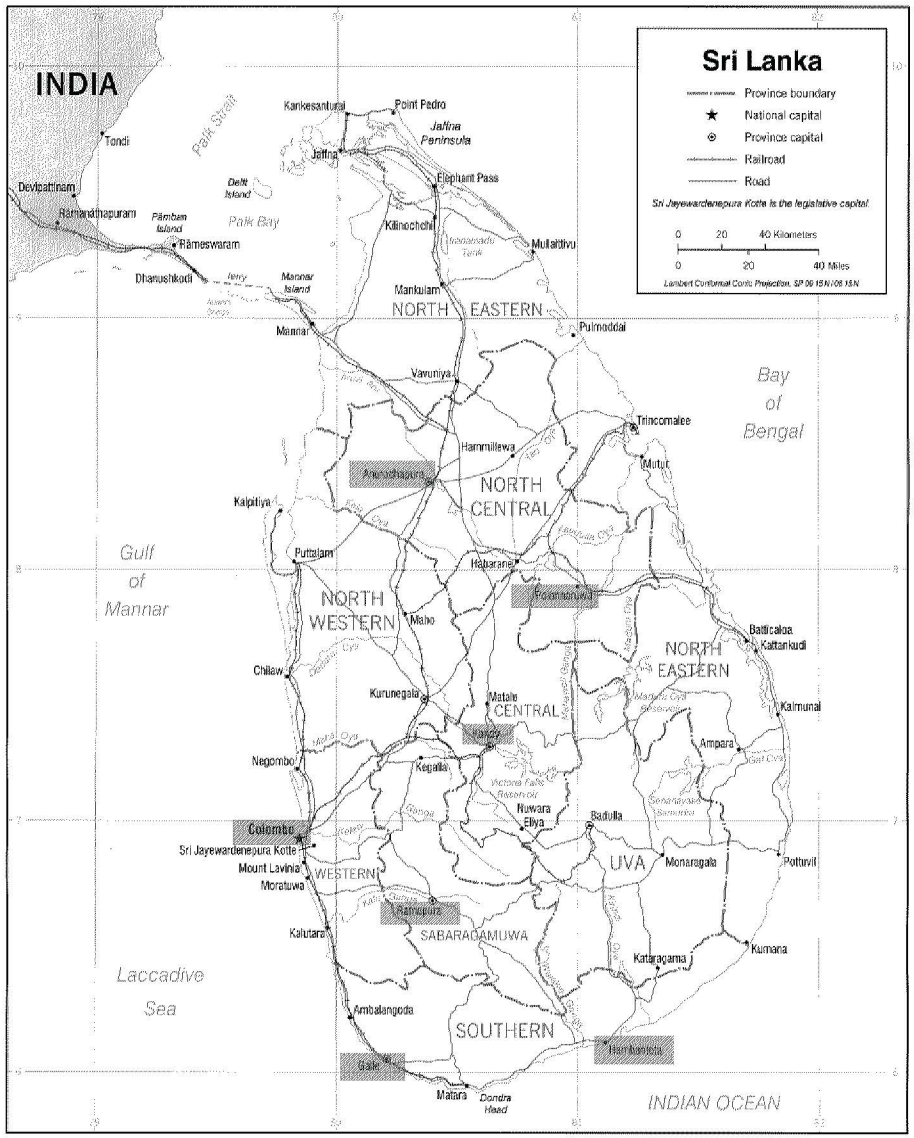
SUMMARY

- Studies Show > 10X Improvement in Oral Toxicity in Dog
- Dog Model is Relevant to Human
- Formulation Will Save Human Lives in US and Globally

Sri Lanka Observational Monitoring Survey

Sri Lanka Observational Monitoring Survey

- 9 hospitals involved
- ~ 350 Gramoxone cases (06/03 – 08/04)
- Introduced Inteon 09/04, later discovered formulation separation problem
- Closed to new cases 01/26/06, at that time 224 confirmed INTEON ingestions (predominantly intentional)
- Criteria for survival: patient alive 3 mo. after release from hospital - end 04/06
- Independent experts meet May 30, 2006
- Summary of findings expected 06/06.



Base 802734AI (C00127) 3-01

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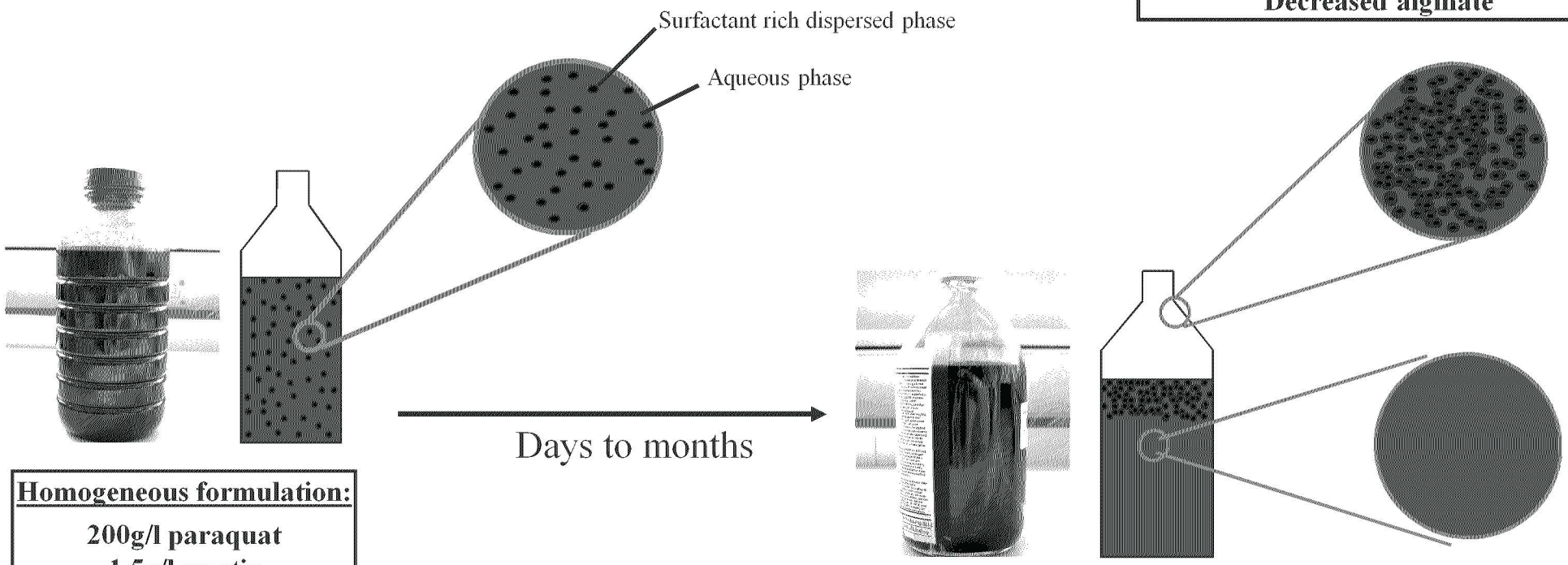
Comments:

You're not going to put the Sri Lanka study results on the slides??

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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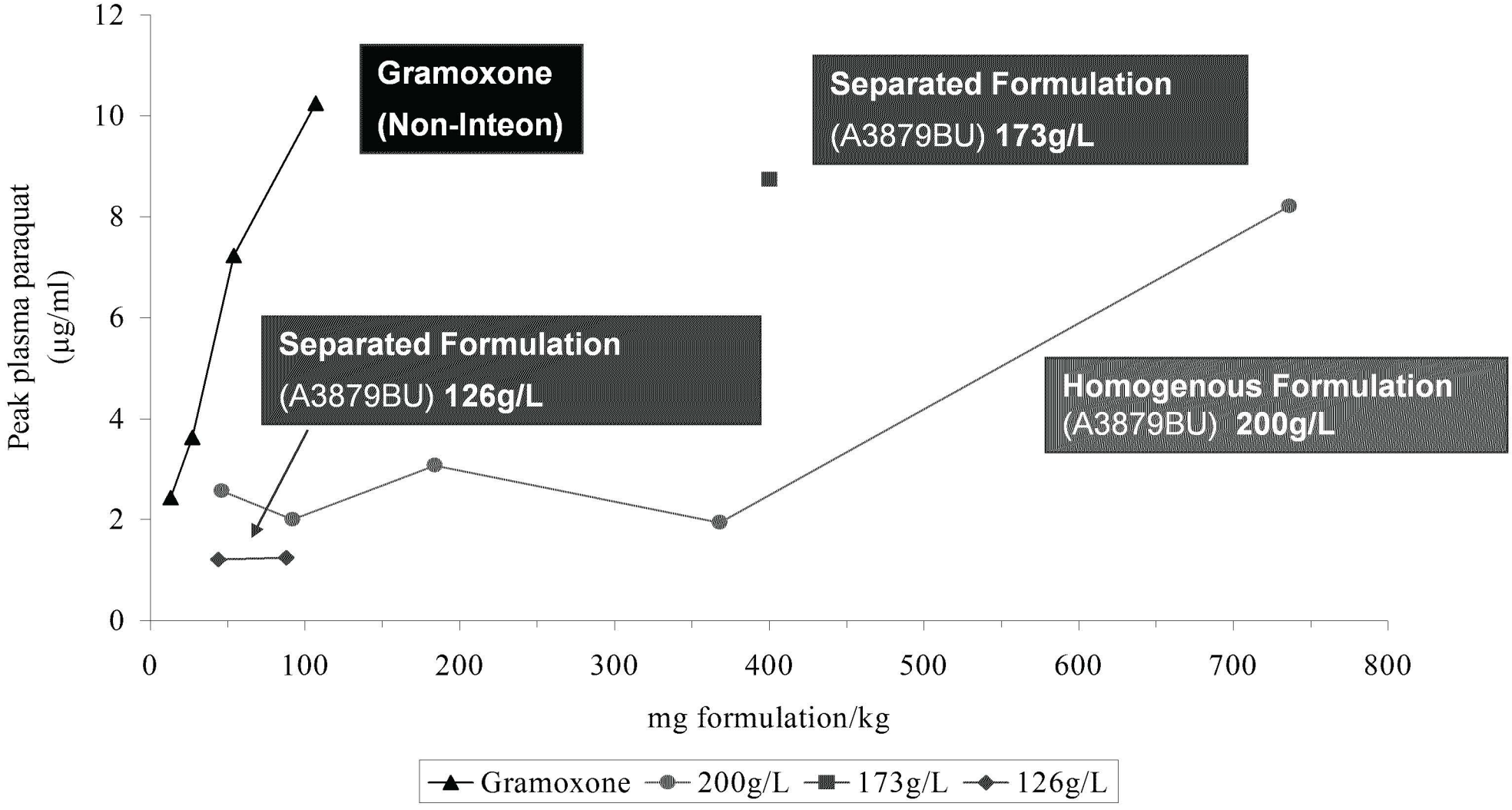
Speaker Notes:

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: 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 data

- **Separated Inteon formulation in Sri Lanka [A3879BU]**
  - ✓ It is safer than Gramoxone
  - ✓ Not as safe as homogenous Sri Lankan Inteon form.
  - ✓ Not as safe as the US Inteon formulation

- **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

Conclusions

- **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**