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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<td><strong>Gramoxone</strong></td>
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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: 1 out of 4 dogs died at 10 mg paraquat ion/kg. All dogs were terminated day 7/8 at 20 mg 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

- **0.5 g/L emetic**: Mortalities reported
- **2.4 g/L emetic**: No mortalities

Graph showing the relationship between mg paraquat ion/kg and peak plasma paraquat (µg/ml).

Controls peak plasma paraquat

- **Gramoxone**
- **Gramoxone additional emetic**
Comparison of 24h plasma paraquat AUC levels in dog

- **Gramoxone**
- **Gramoxone additional emetic**
- **Inteon US**

**Mortalities reported**

**Chance of mortality**

**No mortalities**

---

24 hour AUC (µg/ml.h)

- Gramoxone
- Gramoxone additional emetic
- Inteon US

mg paraquat ion/kg
GRAMOXONE INTEON: Lower Peak Plasma Paraquat Levels in Dog

AUC shows a similar pattern

Gramoxone

Inteon US

Peak plasma paraquat (µg/ml)

mg formulation/kg

Gramoxone

Inteon US

Human ingests formulation
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 (<1cm²) 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.
Relevance of Dog Data to Predict Human Safety

- Comparison of Dog and Human GI Tract
## Human: Dog – Comparison of GI Anatomy and Physiology

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<tr>
<th>Characteristic</th>
<th>Human</th>
<th>Dog</th>
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<tr>
<td>Chamber</td>
<td>Single/glandular</td>
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<tr>
<td>Capacity</td>
<td>1-1.6 L</td>
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<td>85</td>
</tr>
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<td>2</td>
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<tr>
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Variations in types and distribution of gastric mucosa

A = Proper gastric  
B = Cardiac  
C = Stratified squamous non-glandular  
D = Pyloric
## Human: Dog – Comparison of GI Anatomy and Physiology

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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 10^9/gram wet weight
  - Dog: 4-7 x 10^9/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

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

% Mortality

Dose

Gramoxone
Inteon
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
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.
Comments:
You're not going to put the Sri Lanka study results on the slides??
Inteon Sri Lankan formulation: Illustration of formulation separation

Homogeneous formulation:
- 200g/l paraquat
- 1.5g/l emetic
- 9g/l alginate
- ~100g/l surfactants

Surfactant rich dispersed phase
Aqueous phase

Days to months

Top:
- Decreased Paraquat loading
- Increased Emetic loading
- Greatly increased Surfactant loading
- Decreased alginate

Bottom:
- Increased Paraquat loading
- Decreased Emetic loading
- No Surfactant
- Slightly increased alginate
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

- **Gramoxone (Non-Inteon)**
- **Separated Formulation (A3879BU) 173g/L**
- **Separated Formulation (A3879BU) 126g/L**
- **Homogenous Formulation (A3879BU) 200g/L**

Separation Inteon formulation shows reduced safening but safer than Gramoxone
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