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

![Graph showing the influence of Gramoxone on plasma paraquat AUC levels with and without an additional emetic.](image)

- **Mortalities reported**
- **Chance of mortality**
- **No mortalities**

**Does not control total systemic absorption**
Comparison of 24h plasma paraquat AUC levels

- **Gramoxone**: Additional emetic
- **Inteon US**: No mortalities

**Mortalities reported**

**Chance of mortality**

24 hour AUC (µg/ml.h) vs. mg paraquat ion/kg
GRAMOXONE INTEON: Lower Peak Plasma Paraquat Levels in Dogs

AUC shows a similar pattern

Peak plasma paraquat (µg/ml)

mg formulation/kg

Gramoxone

Inteon US

Human ingests formulation
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 (<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.
XD7355 Dog 2. Lung: Several small discrete areas of discoloration are present (denoted by arrows). The remainder of the lung appears normal.
XD7201 Dog 3. Lung: Focal area showing thickened alveolar septae (septal fibrosis). Note the absence of progressive changes (alveolar edema, acute inflammation, fibroplasia).
XD7201 Dog 3. Lung: Junction between affected and normal appearing alveoli. Note the absence of progressive alveolar changes.
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

<table>
<thead>
<tr>
<th>Relevant Similarities</th>
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<th>Human</th>
<th>Dog</th>
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<tbody>
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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/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

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<th>Species</th>
<th>MLD paraquat ion mg/kg</th>
<th>Inteon US MLD mg formulation/kg</th>
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<tr>
<td>Rat</td>
<td>~100</td>
<td>310</td>
</tr>
<tr>
<td>Dog</td>
<td>~12</td>
<td>602 (Equivalent to 128mg paraquat ion/kg)</td>
</tr>
<tr>
<td>Human* Pond (1990)</td>
<td>50 - 80</td>
<td>X2  ? 470</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X5  ? 1177</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X10 ? 2350</td>
</tr>
</tbody>
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Toxicity in human is unknown but 5 fold safening giving a figure of 1177mg formulation/kg used for subsequent modelling.
Other

1) Add intentional and accidental ingestion slide
Distribution of intake from human ingestions for 5 sub-populations totalling 563 cases.
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.
Inteon Sri Lankan formulation: Illustration of formulation separation

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

Days to months

Surfactant rich dispersed phase
Aqueous phase

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
• 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
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: 1 out of 4 dogs died at 55 mg formulation/kg.
All 4 dogs were terminated day 7/8 at 110 mg formulation/kg.
Plasma Paraquat following an oral dose of 40 mg paraquat ion/kg in the rabbit

Area Under Curve (µg/ml.h)

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Gramoxone</th>
<th>A7813K</th>
<th>A7813L</th>
</tr>
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<tbody>
<tr>
<td>1h</td>
<td>2.62 ± 0.39</td>
<td>0.64 ± 0.17</td>
<td>0.98 ± 0.05</td>
</tr>
<tr>
<td>4h</td>
<td>11.24 ± 1.03</td>
<td>6.52 ± 1.01</td>
<td>7.89 ± 0.93</td>
</tr>
<tr>
<td>24h</td>
<td>29.65 ± 4.78</td>
<td>16.78 ± 2.47</td>
<td>19.66 ± 4.91</td>
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Reduced paraquat absorption in rabbit (non vomiting species) due to gelling
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.