

Notes from a Brainstorm on Robustness of AWT held at CTL on 5th May 2004.

Present: John Doe, Phil Botham, Dave Vitolo, Martin Wilks, Dick Lewis, Brian Jones, Matt Jacobsen, Andy Griffiths, Jonathan Richards, Jon Heylings, Ros Sheldon, Barry Elliott and Mike Clapp.

1. **Robustness of biological conclusions on Gramoxone AWT and extrapolation from dog to human.**

Question/Comment	Action
<p>A. Influences on study design</p> <p>Capsule dosing.</p> <ul style="list-style-type: none"> • Would gel form a film as ingested? • <u>The hypothesis this technology is based on assumes that on contact with acid in the stomach there is a transformation of the flowable liquid product into a more viscous gel. This process slows the delivery of the product into the absorptive small bowel. The alginate is thought to physically entrap (but not chemically bind the paraquat). In addition, the bulking effect caused by the swelling of the alginate into a gel closes the pylorus. This is a well documented digestive reflex. It is also known that alginate coats the mucosa, providing a protective film against the damaging influence of gastric acid and other irritating chemicals present in the lumen of the stomach. The alginate will only be transformed once it has contact with gastric acid. There is no reason why it should alter within the capsule or form any type of film. The question is: could the delivery into the stomach of the AWT contained within a capsule in any way compromise the effect we anticipate in man following delivery via swallowing the neat concentrate product? Delivery of a bolus from a swallow may in fact coat and protect the oesophagus and buccal cavity giving rise to less of a corrosive effect compared with Gramoxone. Once the AWT reaches the stomach the gelling process should be similar. The stomach always has a basal acid secretion and inherent motility. It is not a hollow empty sac. It responds to engorgement from ingestion. As soon as the product is delivered from oesophagus or via disintegrated capsule the same process will operate.</u> • Would gel not form? • <u>The protonation of the alginate and formation of the acid triggered gel should occur whatever the method of introduction of the product into the human stomach. In the dog studies it is released as a bolus from a capsule into the gastric acid contents. In a swallow it is likewise delivered as a bolus from oesophagus to stomach. Gaviscon treatment for heartburn (which contains alginate gel) is in both liquid and tablet form. These would enter the stomach differently.</u> • Could gel underestimate exposure? • <u>This is thought to be unlikely. Several doses over a wide range of oral exposures have been given in the same size capsule. If the capsule itself had an effect to reduce the absorbed dose this would surely be more apparent at the low doses where the amount of capsule: product is high. The opposite occurs with systemic exposure staying the same over the range of increasing doses from one capsule.</u> • Gelatine interaction with formulation? – method of dosing. 	<p>JH with input from MFW on experience in human particularly buccal cavity and oesophageal effects</p> <p><i>Unlikely to form a gel until it reaches acid in the stomach, but regurgitation more likely to offer some protection</i></p>

- Mimicking the exact way in which a human would ingested the product is difficult in an animal study. We do not want to contaminate the mouth, causing any local irritation or run the risk of under-exposure if the animal spat a portion of the dose out. It is assumed that a human would gulp several swallow of the product. This would deliver several pulses of perhaps 20-30mls at a time into the oesophagus. The limitations of dosing via a gelatin capsule has been investigated. We compared this to direct injection via a dosing catheter in the dog. There were no differences in plasma kinetics between gavage and capsule (CTL/026118) dosing. Furthermore, we explored the disintegration time of the gelatin capsule in vitro. The capsule comprises two loosely fitting halves. The surfactants in the product melt this very quickly and the capsules have to be filled immediately prior to administration to prevent the gelatin from going very soft and risk spillage. Addition of a capsule-containing product to a stirred beaker of saline at 37 degrees and pH 2 showed very quick disintegration. In fact the capsule opens first as it is. It is not simple digestion by gastric acid. The capsule deforms and the motility of the stomach soon releases the contents as a bolus. Capsule fragments are sometimes seen in the first vomit.
- How confident are we that results obtained between 1987-1991 are still relevant in 2003/4? – Control data more than 12 years old.
- We have a high degree of confidence on this point .The strain of dog has not altered from the 1980s and we still use similar body weights and only males. The repeat dosing across 4 years showed excellent reproducibility with respect to paraquat absorption kinetics across different groups of naïve animals and those that had been re-used many times (CTL/026118). Many of the same personnel are also involved despite the 12-year gap between studies and the study protocol has not changed. Ethical constraints on studies involving paraquat and dogs were also a consideration when we decided not to test Gramoxone once more. However, it may be prudent to examine this again in a naïve group of dogs and generate a new contemporaneous Gramoxone control.

B. Physiological differences

Species differences Dog vs. Human

- Absorption kinetic differences
- The choice of the dog as our closest species to man that is practical and has the digestive attributes required, including a vomit reflex, is based on the following. The toxicokinetics for paraquat (and many drugs and chemicals) is very similar. It is the Pharma industry's species of choice for studying pharmacokinetics because of this. Dogs like humans are omnivores and intermittent feeders. The physiology of digestion is also very similar. The pH, gastric emptying and hormonal control of digestion and secretion make this the model of choice for the development of acid antisecretory drugs. The vomit reflex control centrally by the vomit centre in the brain, responding to changes in cAMP is the same in dog and man. Phosphodiesterase inhibitors, like our emetic agent PP796, work through this process.
- AUC similar, amount absorbed differences
- Data we have in man indicates that the plasma paraquat profile and AUC at a minimally toxic dose is similar between dog and man. Across species there are differences in the acute oral lethal dose e.g. (in mg ion per kg) 100mg/kg in rat, 200mg/kg in mouse, 12mg/kg in dog and 25-50mg/kg man. However, toxicokinetic studies in animals have attributed these differences to the differences in the amount of paraquat absorbed from the gastrointestinal tract. Analysis of the 0-24h AUC across these species shows similar paraquat systemic exposure at a peri-lethal oral dose (CTL/R/1250).
- Lung uptake and weight differences?
- There are species differences with respect to pulmonary effects of paraquat uptake kinetics as assessed in vitro. For example, the rabbit lung does not readily accumulate PQ following oral dosing. However, dog and man can both develop the characteristic oedema and fibrotic changes associated with high acute oral paraquat ingestion. We are making the assumption that the prevention of absorption using AWT technology will reduce the pulmonary changes in man as it does in the dog.
- Dog absorbs more than humans
- It is known that higher mammals absorb a greater proportion of a single oral dose than rodents. In house studies in the dog suggest that this species absorbs as much as 40-50% of a dose. Rats eliminate much more of the dose in faeces and systemic exposure is closer to 10% of an oral dose. Evidence that we have from human poisoning, where the volume of product could be reasonably accurately predicted and the ensuing toxicity is known, suggests that man probably absorbs a smaller proportion of an oral dose than the dog but a higher proportion than rodents.

JH with input from MJLC on lung uptake and weight

JH

JH with input from MJLC and MFW with respect to human data.

- Are we only inhibiting specific absorption in the dog?
- This is very unlikely. Paraquat is predominantly absorbed via a passive diffusional process from the small intestine. Paraquat absorption from the stomach is very slow in all species we have studied. Alginate, emetic and purgative are each acting independently, interacting on the digestive process affecting gastric emptying, vomiting and purgation, respectively. All these responses occur in dogs and man and all three actions have been proven at the dose levels we are using in humans.
- Influence of length of small intestines?
- The dog gastrointestinal anatomy and physiology is similar to man. However, one notable difference is the overall length of the small and large intestines as expressed per kg. These regions are shorter and the dog can pass an oral dose relatively quickly. However, the AWT technology is predominantly focused on the interactions within the stomach in order to prevent the oral dose from reaching the intestine. The stomach size, volume and pH is not too dissimilar between dog and man. Therefore, much more important considerations are whether there are differences in stomach emptying and emesis.

Physiological differences human vs. dog

- Gender and age differences
- There are no known sex differences relating to paraquat toxicity in any species, including man. It is unlikely that age will have a significant bearing on toxicity in its own right, certainly in experimental animals. However, in man where the kidney function may be impaired in the more elderly individuals, this may exacerbate the toxicity.
- Weight differences in human population
- This is an important point. Conventionally in pharmacokinetics we refer to the healthy 70kg male. Many cases of paraquat poisoning involve populations where the average body weight may be significantly below this e.g. Sri Lanka.
- Genetic variability of dog
- Genetic variability may be important in relation to carcinogenesis or other end point where strain effects have been observed. However, paraquat absorption is a passive diffusional transport process not a complex liver induction phenomenon. Pharmacokinetics guidelines do not stipulate a particular dog strain.
- Drug interactions in human population e.g. antidepressants?
- Paraquat-drug interaction in suicide attempts will be presumably the same with AWT as it has been for 40 years with Gramoxone. It is unlikely that the regions where human ingestion is common that individuals will have access or be

treated with expensive controlled prescription medicines.

- Potential for chronic effects due to paraquat
- MJLC to add. JRH comment: No chronic effects have been seen in dogs even in our previous colonies that have had many oral doses. If animals survive the acute phase they make a complete recovery. This does not discount an underlying small level of lung fibrosis that cannot be seen clinically, in life.
- Role of magnesium in dog vs. human
- Magnesium sulphate or Epsom salts is an effective osmotic purgative in dogs and man. We have only observed fluid faeces at high doses in a few animals with AWT, presumably because it has been vomited out. In previous projects where the intrinsic protection and emesis productivity was less than we have seen with AWT, the purgative action was more prevalent. This is still core to the safening since when we reduced MgSO₄ from 300 to 200 to 100g/l the safening in dogs dropped in a dose related fashion. The mass of purgative in AWT at the high volume exposure end is known to be a purgative dose in man. Dogs and humans both have osmoreceptors in the duodenum.

Response to emetic

- Vomiting – do humans vomit as effectively
- Humans have a highly regulated gastrointestinal physiology. Our digestive system is highly sensitive to a variety of potentially ingested toxins. We are particularly sensitive to topical irritants of the gastric mucosa, many bacterial and viral toxins and food/drink that have a high salinity. Vomiting can be initiated centrally or locally. Local irritation e.g. alcohol, paraquat etc is a slow inefficient emetic stimulus. Centrally acting emesis via the hypothalamus is highly efficient in all higher mammals. The vomit centre once triggered causes a complete closure of the pyloric sphincter, gastric muscle contraction from pylorus upwards through fundus. Following relaxation of the oesophageal sphincter the pressure effect expels the gastric contents very effectively. There is no reason anatomically or physiologically why human vomiting should be less effective than dogs
- Species difference in response to PP796?
- We are in a unique position with the emetic agent PP796 in that it has been examined in many species including pigs, primates, dogs and man. The ED₅₀ values to produce effective vomiting within 30 min are very similar across all species, including man.
- Productiveness of vomiting
- See response to first point above. The “productivity” of emesis depends on dose of emetic and physical constitution of the gastric contents. Assuming the product gels and stays in the stomach and humans receive a dose of PP796 that causes

prompt emesis, coupled with closure of the pylorus, human vomiting should be as productive as the dog.

- Size of dog stomach relative to human (volume related for effective response?)
- Human stomach capacity is variable between individuals but acid output and physiological control is geared to this capacity. Our adult male dogs are a more homogenous population. The AWT technology relies on the presence of acid and the ability for the formulation components to alter motility and cause vomiting. This is independent of the actual organs size.
- Limited human data for emeticised material – MFW.
- MFW, JRH comment: During the development of PP796 there is evidence in volunteers and several hundred human patients of the effective dose to cause emesis in 30 minutes (Bayliss PFC, ICI Pharmaceuticals Report PH20992B, 1973). Emeticised Gramoxone (in a small number of poisonings) did increase the incidence of emesis compared with unemeticised product but the time to emesis was too late for a liquid product. Hence the productivity of the vomiting in the current product is low and there was only a very small improvement in survival. Patients are likely to vomit more with the current Gramoxone product than AWT since although the dose is relatively low compared with AWT, it will empty quickly into the small intestine. Once beyond the pylorus it is difficult to vomit out the residual PP796, as well as the paraquat.
- In addition, ICI increased the level of PP796 to 2g/l in France in 1985. Assuming the same volume of the French product has continued to be consumed there is no evidence of adverse clinical signs in patients as a result of emetic overdose.
- Aspiration of vomit
 - Influence of gastric lavage
 - Paraquat lesion vs. aspiration pneumonia
 - Choking
 - MFW to add. JRH comment: Fortunately, paraquat itself does not cause sedation unlike some other drug overdoses. Sedation is associated with aspiration related complications.
- Over dose on emetic – physical damage due to vomiting
- MFW to add. JRH comment: As we have seen in the dog, prompt pharmacological-induced emesis empties the stomach effectively. Delayed irritant-induced emesis is more prolonged and may lead to mucosal damage. The alginate is used to treat acid induced gastric and oesophageal irritation by protecting the mucosa. There is as much alginate in a lethal dose of AWT as a single Gaviscon oral dose.
- Potential for any situation where AWT could be less safe than Gramoxone
- This would be most surprising!

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| <ul style="list-style-type: none">○ Position when vomiting – aspiration○ <u>MFW to add. JRH comment: Many humans adopt the crouching position during emesis not too different from a dog.</u> | |
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C. Robustness of modelling

Safening factor

- 5 fold safening factor predicted – not seen in dog (Gramoxone emeticised vs. non emeticised)
- Gramoxone containing 5 times the level of emetic than the current product produces a 5-fold safening effect in the dog (CTL/R/1250). This is because the time to emesis is brought forward from 1hr plus to around 12-15mins. This very early time to emesis captures some, but not all, of the ingested dose. Thus increasing the emetic in dogs and primates gives effective emesis and a 5X improvement in safening (CTL/R/390). The TRC in at that time thought this an insufficient safety factor to approve increasing the emetic alone since many individuals drink more than 5 lethal doses (ICI Agrochemicals TRC review report PJ9/WG3/88/0IC) . Emeticised Gramoxone at the current level of 0.5g/l PP796 does increase the incidence of emesis in dogs and man but the timing is too late to be effective for a liquid composition that empties from the stomach rapidly.
- If we tested in dog what would be the predicted kinetics for Gramoxone with and without 0.5g/l emetic.
- The kinetics are unaffected in the dog at 8mg/kg paraquat ion since 0.5g/l PP796 delivers a sub-effective emetic dose. We have compared many paraquat formulations containing various levels of emetic from zero through to 2.4g/l in the dog. The high dose of 2.5g/l PP796 provides a 5X safening in the dog. You can dose animals with 48mg/kg PQ ion with minimal toxicity (MLD for standard Gramoxone = 12mg/kg). This is reviewed in CTL/R/1250. The ED50 for effective emesis is around 0.5mg PP796/kg in all mammals tested, including man (CTL/R/390).
- What response would we predict in human by increasing level of emetic to 1.5g/l
- A measurable improvement in survival since the time to emesis would be earlier so more toxicant would be removed prior to clinical intervention. The concentration of 1.5g/l was arrived at by a trade off with the gelling agent in Magnoxone. A full 2.4g/l would be needed to see the benefits of increasing the emetic alone.
- Sensitivity of human analysis curve – need to improve explanation of analysis done.
- MJLC
- Role of surfactant on model (different for different formulations – oral absorption)
- Comparisons with Gramoxone W (surfactant containing) and Gramoxone S (no surfactants) and Preglox with and without wetters showed similar MLD values (IRI contract studies) in the dog. There are small but measurable differences in

JH with input from MJLC on extrapolation to human data

<u>absorption in the rat with the surfactant containing material</u> <u>increasing the absorption in vitro and in vivo. Gramoxone and</u> <u>AWT both contain surfactants.</u>	
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D. Confounding factors

Food in the stomach.

- Inhibit gelling process?
- Food in the stomach inhibits gastric emptying and increases acid output. Both of these features are good for the process of acid triggered gelling and slowing of emptying by bulking. Most suicides do not occur following normal food intake, alcohol is more likely. There are no warnings on the use of alginate in Gaviscon etc relating to its ineffectiveness following food.
- Stimulation of acid release and influence on stomach pH/
ethnic /cultural differences / sex differences
- There are only minor differences in acid output across populations. Males generally have higher acidity than females but both have sufficient to cause gelling.
- Influence of antacids?
- Unlikely that individuals in developing countries will be taking proton pump inhibitor drugs like Losec and Nexium or even antacids like Aludrox. This is more likely in Westerns society where a small percentage may have their acid output regulated. However, even with such therapy acid secretion is only suppressed back to normal levels.
- Absorption of emetic?
- The emetic agent is quite lipophilic (unlike the highly polar paraquat molecule). It is absorbed very rapidly and effectively from the upper gastrointestinal tract. In cases where emetic dissolution and absorption may be slowed, e.g. when the stomach is full, this will also slow the delivery of paraquat to the absorptive small intestine.

Influence of alcohol

- Stomach emptying, gelling, pH
- Ethanol can inhibit gastric emptying. This has been shown in human volunteers who ingested various amounts of alcohol and plasma drug concentrations were measured. Moore et al in Gastroenterology 81,(6):1072-5, 1981 showed in human volunteers that the more acidic a wine is then the more emptying is retarded. Ethanol should not affect total acidity though may dilute the contents depending on volume. The gel changes physical form on protonation. It is not known whether high concentrations of ethanol would actually stop the gel from forming or break the gel down.
- Acute / chronic alcohol status
- Liver function impairment may make an individual more susceptible to paraquat toxicity but this would equally apply to the current product.
- Gastritis
- Mucosal permeability would be higher where there are gastric

JH, MJLC to summarise data on influence of food on emetic uptake

JH, and MJLC

<p><u>erosions or ulceration. The alginate may actually be useful to protect a more leaky epithelial barrier.</u></p> <ul style="list-style-type: none"> • Influence of alcohol on emetic vs. paraquat absorption. • <u>Several drugs have been studied in relation to the ability of alcohol to affect their bioavailability. Mattila et al in Acta Pharmacol. Toxicol. 50(5):370-3, 1982 showed that whisky had little or no effect on drug absorption in human volunteers. It may be useful to re-examine the poisoning cases where we have detailed clinical notes to investigate whether mortality rates differ significantly where there is either an known association with alcohol intake and/or chronic liver effects attributed to alcoholism.</u> 	
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2. Robustness of formulation chemistry.

Jonathan Richards presented current position and points raised were discussed. The following questions or comments were posed.

Question/Comment	Action
How robust is the formulation to be used in Sri Lanka?	JR
What is the potential for inter-formulation differences?	JR
How much variability have we tested in the dog model?	JR
Question about storage - if consider peak plasma paraquat level in dog a key parameter rather than 24 hour AUC, then consider repeating 64mg paraquat ion/kg dose – with stored material since few data points. Not required because even if real effect significant safening still present).	
Have we degraded formulation in accelerated storage trial beyond where it would be after usual storage?	JR
What do we understand about the effect of heat on alginate?	JR
Need to test something that has failed QC checks if we are to gain true insight into robustness of biological model. This should be considered further	JR/AG/JH/MJLC
Phase separation issue	
• Could formulation be worst than Gramoxone?	JR
• Have we lost protective effect? If so what were the circumstances?	JR
• Bulk of material would retain safening effect (paraquat, alginate, emetic)	JR
• Surfactant layer – would be dilute paraquat but still have alginate and emetic but be rich in surfactants.	JR
• Ensure analysis is available to support this.	JR
• Agreed that US formulation covers more concentrated (bulk)	JR

of material therefore no need for further testing.	
Sri Lanka manufacture material Sri Lanka trial – need to ensure samples of all batches of formulations are held to understand what individuals were exposed to if required. For 11th June meeting to brief LLS, head of development – need proposal for screening SL batches of test material.	JR MJLC/JH

The plan following discussion with those with actions above is:

- 1) Draft contributions to MJLC by 22nd May 2004**
- 2) Circulate draft for peer review on 28th May (JR, MFW, JED, PAB, JH and MJLC)**
- 3) Return comments to MJLC end 1st June.**
- 4) Pre circulate report to LLS and those attending 11th June meeting on 4th June.**

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