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CONFIDENTIAL

Gramoxone AWT: What additional studies are needed on the lead 200g/l development candidate (A3879BU) in 2004?

1.Substantiate the health claims we are making on reduced oral toxicity.

We have demonstrated that the MLD of Gramoxone AWT (A3879BU) >10X in the dog (May-Sept 2003) using a certified lab scale batch. This was the same batch that was used in the Handler's Tox package in 2003. All studies were conducted to GLP.

The first certified technical scale batches of Gramoxone AWT will be produced in Q1 04 as part of the scale up process. It is recommended that HA test one of these scaled up batches early in 2004 in the dog to determine whether the manufacture process has had any impact on the performance of AWT, in terms of oral toxicity. It is proposed to evaluate this at 2 dose levels, probably 32 and 64mg paraquat ion/kg. The acute toxicity and paraquat and emetic absorption will be compared with the recently completed XD7201 study.

The larger scale batch for Sri Lanka (circa. 350K litres) is expected to be ready by the end of Q2 04, ahead of the Epidemiology start date of Q4 04. It is important that Health Assessment also evaluate this batch in the dog, again at 2 dose levels, to ensure that we have the relevant acute oral tox data on the material in the field.

2.Substantiate the health claims we are making on improved dermal and eye irritation.

There is a considerable database on the skin and eye irritation of paraquat formulations. An examination of the skin irritation studies during the 1980s and 1990s demonstrated that all these Gramoxone and PQ/DQ mixture concentrates would be classified in the EU as either R38 (irritant) or R34 (corrosive). Indeed, in our quest to develop suitable formulations for France in

the 1990s, the vast majority were corrosive to skin.

1987

Gramoxone Plus and R Bix (France) – Both corrosive to skin R34

1993

Gramoxone YF7697A (Global) – Moderate/Severe skin irritant R38

1994

YF7632B (Germany) – Moderate/Severe skin irritant R38

1997/8

30 PQ and PQ/DQ Mixtures – 26 Corrosive, 4 Mod/Severe R38

We know that the major route of entry into the skin for small polar molecules, like PQ, is via hair follicles. This makes the rabbit Draize model particularly sensitive to PQ dermal irritation, since the rabbit has a highly follicle-dense skin and a very permeable stratum corneum compared to other animals, and particularly compared to man. The alginate wall technology (AWT) provides a membrane coating effect on the skin that markedly reduces the penetration of PQ into the surface layers of the epidermis. All formulations containing AWT have reduced skin penetration properties in vitro. This includes something of the order of 50 different formulations over the last 3 years. The lead AWT formulation, Gramoxone delivers more than 5 times the amount of PQ through resected mouse skin compared with the lead AWT formulation,A3879BU, over a 4h period of exposure.

It was this effect, in a modification of the SIFT in vitro model, that gave us the confidence to test these AWT concentrates in vivo in 2001. A total of seven AWT formulations with wide ranging surfactant systems, and including PQ/DQ mixtures, have now been tested in the standard Draize rabbit in vivo skin irritation model.

2001

YF12023 (parent of A3879BU) – Not Classified (EU)

2002

A3879BU, A3879BV, A3879BW, and A3879BX - All Not Classified (EU)

2003

A128281 and A9409N (French AWTs) - Both Not Classified (EU)

All 7 would not have triggered an EU skin irritant irritant label. We are not proposing that they are non-irritant since there is always some mild oedema and erythema and stewardship measures would not be reduced as a result of these tests. However, the degree of skin irritation is much improved with AWT, particularly compared to the formulations tested in the 1980s and 1990s that were classified as corrosive to skin. Thus, we have exposed a total of 21 rabbits to AWT formulations with no individual animal triggering an R38 level of irritation.

The questions of robustness and influence of surfactants have caused variability in the oral tox data in early AWT systems but the behaviour in the acid (triggered gelling) environment of the stomach is completely difference from the filming effects as a concentrate dries down on the skin. Our hypothesis for skin protection relates to slow diffusion and possible plugging of hair follicles by the precipitating alginate gel polymer.

The barrier-forming effects of alginate polymer may also be beneficial to the eye. Others within HA are better qualified to comment on the improved eye irritation effects observed with the AWT formulations, but the basic thermodynamics of reduced PQ diffusion would occur wherever the polymer and PQ are in high concentration.

Further Regulatory Work

There are considerable risks in re-testing the lead A3879BU formulation in the skin and eye. If a spurious observation occurred in one animal this would have serious consequences in the overall AWT development process. Normally, we would run one GLP test. Batch testing of the same composition would be setting a new precedent. Presumably, any repeat would require a new composition and certified batch. Changing the composition would invalidate the other regulatory tests and non-safety tests already conducted. There were concerns over homogeneity in earlier storage batches of AWT, but all CTL tests used fully homogenized formulation and it has been shown that simple inversion of the container is all that is needed to achieve homogeneity. There are also project licence/ethical issues around re-testing the same formulation. I also assume that any new adverse effect would be referable.

Another argument against further testing of A3879BU, if the Handler's Tox robustness is still in question, is the overall benefit that we are claiming for AWT. The reduction in acute oral toxicity was the original project specification of Prometheus. The dermal and eye benefits are somewhat secondary to the overall objective.

If we did not re-test A3879BU, one option is to re-examine the Cis-3-Hexanol stenched version (A3879BV). This formulation was more irritant to the eye in the regulatory tests. Repeating an eye only test could not worsen the position but how could one stand-alone test replace another already generated? The other issues around repeating GLP animal tests still apply.

If the repeat regulatory work is needed to give additional confidence that AWT will always perform better than current PQ products in the Handler's Tox tests, another piece of information that may be useful relates to future work we will be undertaking in 2004. A number of new AWT formulations are being developed for different regions. These will go through the normal regulatory testing process. If these also perform better than the in-country standard they are aimed at replacing, then this adds further weight to the robustness of the AWT system.

Further Investigative Work

An alternative (low risk) option that may be useful would be to examine shorter exposure times using the concentrate in both the in vitro (SIFT) and following review of the risks, in vivo in more rabbit studies. I circulated this proposal separately on September 10th 2003 –attached at the end.

Briefly, if we tested dilutions of A3879BU alongside Gramoxone at 1:100, 1:50 and 1:10 we could find the point at which the barrier properties (e.g. TEWL in vitro), and erythema/oedema (rabbit in vivo) started to approach the levels that indicated an irritant response. Use of an exposure as short as 10min should minimize risks associated with such an evaluation. The advantage of TEWL is it could be used both in vitro and in vivo and can detect changes prior to visual effects. The in vitro experiments would have the added advantage of providing kinetic and mass balance data as well as the barrier effects.

Dr Jon Heylings
October 3rd 2003

September 10th 2003
From: Jon R Heylings
To: Diane Castle; Mike Clapp
Cc: Phil Botham

Diane/Mike

Following our brief discussion a couple of weeks ago, I have been considering how we could generate new information on the diluted Gramoxone AWT with minimal risk to our regulatory position.

If you recall, we discussed some potential approaches over lunch and I thought it may be useful to capture some of this ahead of a more detailed debate within the relevant project team. There are also other experts in CTL that we can involve in this thinking as well as the relevant Basel folks, like Ian/Martin etc for their views.

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We know that the hairy species like rabbit are more sensitive to skin irritants than man and particularly so when the irritant is low molecular weight and polar, like PQ, since it can get through the barrier via polar routes such as hair follicles. Back in 2000, we first identified that the alginate formulations caused less perturbation of the skin barrier than Gramoxone, using a modification of the in vitro SIFT method. Less PQ penetrated through the skin and we therefore decided to investigate this in vivo. The better alginate formulations in the SIFT gave less erythema and oedema in vivo and this is the basis of our current reduced irritation position with AWT 200g/l concentrates.

Although the transepidermal water loss (TEWL) and electrical resistance (ER) of the skin barrier are the main end points of the SIFT for neat industrial chemicals, we demonstrated that the 4h PQ absorbed dose was a better indicator for the in vitro screen for paraquat formulations. This is possibly due to the nature of the irritant response with bipyridyls which cause a different and more prolonged type of effect compared to typical neat surfactant type

delipidisation of the stratum corneum. Our efforts at this time were focused on the labelling implications of the concentrated product so little was done on dilutions.

The attached table shows the PQ, TEWL and ER values for Gramoxone and 7 AWT concentrate formulations. The clearest differences between Gramoxone and the AWTs are in the PQ absorbed. However, the TEWL values for the AWTs are all well below Gramoxone and much lower than a standard surfactant irritant, SLS. the positive control in this test. Our first and still best candidate YF12026 is a parent of A3879BU and still holds pole position with the lowest PQ absorption, one of the lowest TEWL and highest ERs of the series.

SIFT T-Gel May 03.ppt

My approach would be to utilise one element of the SIFT method, namely TEWL to re-visit the effects of dilution on this end point both in vitro and in vivo. The method is non invasive and we have the equipment and technology to do this in animals as well as in our skin chambers. Indeed a number of years ago I looked at some irritant lambda cyhalothrin formulations in the rabbit in vivo, measuring the increase in TEWL on the application site, in addition to conventional scoring. This was very straightforward since water loss is very sensitive and occurs before reddening etc. It also returns to baseline as the repair phase kicks in. The evaporimeter probe is held for a few seconds on the shaved site and the computer does the rest. During development of the SIFT we moved to the in vitro approach and found that similar increases in water loss could be seen with resected skin.

The point I am getting to is a 2 stage approach to dilution testing using TEWL as the indicator of minor perturbation of the skin barrier.

1. Examine Gramoxone and AWT in parallel in the SIFT as neat concentrate, 1:10, 1:50 and 1:100 dilutions. Following topical exposure, we would measure TEWL, ER and PQ flux (4h) in the normal way. Review the data and risks for going in vivo. (We actually could do with these data for completeness, since dilutions of the actual lead product have not been through the SIFT).

2. Examine Gramoxone and AWT in parallel in the rabbit in vivo (as per regulatory Draize approach) measuring the TEWL and visual observations in the normal way, following a 10 min and subsequently a 4h application (if assessed risks low). TEWL would be measured at the shaved site before and at regular intervals after exposure, for say 24h. I would design this the other way round starting with the 1:100 and working up with an agreed stop/go at each point. A precuationary approach could be built into the protocol e.g. termination of the experiment if a particular score was likely to be observed (e.g. avoiding PII scores that would be difficult to live with).

If the in vivo TEWL changes are significantly lower with AWT form compared to Gramoxone for dilutions, in particular 1:100, then we have a new benefit, without the use of erythema and oedema. Indeed, a short exposure to concentrate and TEWL measurement may also provide more ammunition for the benefit we already have in the bag.

TEWL is well accepted by dermatologists as a marker for barrier function in normal and diseased skin. We also have several CTL publications on the use of this technology with the SIFT, as part of our current EU validation of the model as an alternative for skin irritation assessment.

This is off-the-top-of-the-head, but we need to kick the thinking off somewhere and challenge the ideas to see if we can come up with a useful low risk study on the AWT dilution.

I welcome your thoughts.

Jon

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