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PARAQUAT HEALTH SCIENCE TEAM
ACTION MINUTES - WINDSOR MEETING
25-26 May 2011

Paraquat Health Science Team (PHST): PA Botham, LL Smith, NC Sturgess, KZ Travis, AR Cook, L Navarro, K Mewes, JD Sullivan, A Nadel, DJ Berry

Attendance by telephone: CB Breckenridge, RA Brown

Apologies: J McFarland

Extended Health Science Team attendees:

External: CL Berry, JC Wolf (EPL), MT Butt (Tox Path Specialists), JS Wolff (Fulbright & Jaworski)

Syngenta: D Minnema (by phone), P Hertl, C Campbell

Apologies: PL Nicotera

Non – attendance: D DiMonte

Case ID: 220300429

SYNG-PQ-31426104

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25th -26th May 2011

ITEM	AGENDA ITEM	ACTION
1.	Introduction and opening remarks, meeting structure No minute/ actions recorded.	
2.	Paraquat brain kinetics • Summary of new data for mouse, rat & existing publications on NHP, incl. recent Bartlett publication • Brain binding' study objectives \\usgrrd1.nafta.syngenta.org\pdresearchteam\Meetings\Windsor_May_25_2011\Final_Slides\Windsor_kinetic_slides_Wed_am_May_2011.pptx In the mouse, PQ is rapidly excreted in the urine and faeces; a small amount is retained in the brain with t½ of ~ 21 days. The pattern of PQ excretion is similar in the rat with a t½ in the brain of ~ 28 days. In the NHP (squirrel monkey), the PQ concentration in the brain (following s.c. dosing at half the lethal dose) was steady 2-8 weeks after the final dose, suggesting a longer t½ (> 6 weeks) than in rodents. α-synuclein was increased but there was no loss of neuronal cells. Bartlett (2011) reported a rapid loss of PQ in the brains of macaques' dosed i.v. with C¹¹ over 90 mins. Further work is required to establish where and how PQ is held in the brain and whether it is biologically available and active. It is important to consider the doses used in studies and accumulation in the brain in relation to the whole PQ body burden and concentrations in the kidney and lung, in particular wrt occupational use of the product.	
3.	Summary of new findings from current stereology and pathology studies (higher doses, alternative stains, different pathology time points): Study Design and Neurochemistry \\usgrrd1.nafta.syngenta.org\pdresearchteam\Meetings\Windsor_May_25_2011\Final_Slides\Mininema_Study_Design_Neurochem_Windsor_May_25_2011.pptx All metabolites were decreased for the MPTP group, although the decrease compared to the controls was less than has been observed previously. As observed in previous studies, there was no evidence of effects in the PQ treated groups. Pathology feedback from Mark Butt \\usgrrd1.nafta.syngenta.org\pdresearchteam\Meetings\Windsor_May_25_2011\Final_Slides\Mark_Butt_Pathology_Windsor_May_25_2011.pptx	

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	All slides were read “blinded”; the only effects seen were in the MPTP group and the finding clearly charted the processes associated with neuronal death. Owing to the inclusion of earlier pathology time points, MB was now confident that we have not missed any effects due to PQ administration. The likelihood of finding vacuoles under EM (possible evidence of autophagy) was considered extremely unlikely. Stereology feedback from Jeff Wolf \\usgrrd1.nafta.syngenta.org\pdresearchteam\Meetings\Windsor_May_25_2011\Final Slides\Jeff Wolf Stereology Windsor May 25 2011.ppt <i>Fluorescent stain data are still being compiled and will be available in 6 weeks; a decision will be taken at that time whether to read the chromogenic data at the 2 lower dose levels. The following comments are therefore provisional.</i> Decreases in TH+ve neurons were observed in the MPTP group; the decreases were not as large as has been seen in previous studies. The different response to MPTP in separate studies highlights the importance of always having a +ve control. There were no statistically significant effects in any of the PQ treated groups; the sensitivity of our protocol suggests that if PQ was eliciting a toxic neuronal response it would have been detected. Fluorescent staining gives higher numbers of neurones – although this is more likely to represent the “true” number of neurones in the S.nigra, chromogenic staining will be the preferred method for assessing neuronal numbers in our future studies as this is the method used by the majority of researchers in this field.	
4.	DEFRA / Exponent study design & outcome summary, implications of current results \\usgrrd1.nafta.syngenta.org\pdresearchteam\Meetings\Windsor_May_25_2011\Final Slides\UK DEFRA Exponent study May 2011 v1 (2).pptx The design of this study (combined PQ and MB exposures) has no real relevance for normal use. There are suggestions that other routes of exposure (other than ip) may have been explored (eg intra-nasally), but information is limited. ACTION: KZT to establish what the study remit and protocol was. The numbers of TH+ve neurones reported are very low. Although a recent poster reported evidence of an effect at 16 months in mid and high dose males treated as neonates and as adults, we consider that inappropriate statistics have been employed. The ANOVA indicates there is no statistical evidence for an effect. ACTION: LLS to discuss with Abbi Li at the Xian conference in June 2011.	KZT LLS
5.	90-day mouse dietary study design / sign-off \\usgrrd1.nafta.syngenta.org\pdresearchteam\Meetings\Windsor_May_25_2011\Final Slides\SC Study Design (2).pptx This study is required to support our regulatory license to operate in Australia.	

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	Proposed that the bottom dose would be 1.2mg ion/kg/day (equivalent to ~10ppm); this dose is ~ x2 the existing NOEL for AOEL setting, and is equivalent to 3 weekly ip 1.6mg/kg doses x 3 weeks as used by DiMonte (2001). The top dose of 6mg ion/kg/day (equivalent to 50ppm) is ~ half the MTD and x10 the dog 90 day NOEL. This dose would cover possible exceedences of the AOEL. No middle dose is considered necessary. Separate sub-groups will be included for pathological assessment at various time intervals over the 90 days of dosing. ACTION: CBB to agree details with MB. The study will have 95% power to detect a 20% decrease in neurones by stereology. This will require 20 animals per sex per group. An MPTP group of 10/sex will be used to demonstrate the ability of the design to detect changes. The animals will be the same age as the main study mice at termination, being dosed towards the end of the 90 days. ACTION: NCS to review literature for MPTP effects in old(er) mice – or else to recommend if a preliminary study is require to set an appropriate MPTP dose. Stereology will use chromogenic stains (only). The study will be scheduled for a mid August start. A “recovery” study will be conducted, if required, once we have the results of this study.	CBB NCS
6.	Inter-lab study objectives, timelines and status \\usgrrd1.nafta.syngenta.org\pdresearchteam\Meetings\Windsor_May_25_2011\Final Slides\Multilab Study Design.pptx This study is required to establish independent validation of our findings and to test our current hypothesis of no stereology effects at 10 and 25mg/kg. All labs will use the same source of mice, the same compounds and stereology readings will be “blinded”. Labs will prepare and read their own slides, and then all slides will be sent to a separate lab (proposed Dr Nyengaard, Aarhus University, Denmark). Discussions with Dr Nyengaard are ongoing. PQ dose levels are still to be agreed with co-workers. There are likely to be 2 MPTP control groups, one to the Syngenta protocol and the other to the more toxic regime used by Przedborski et al. The extent of any pathology has yet to be discussed (e.g. should we just look for glial activation?). ACTION: LLS to write to D DiMonte asking him to confirm he does not wish to participate in this study. There will be a face-face meeting 11/12 August to agree protocol and contractual issues. Proposed attendance: LLS, CBB, NCS, MB, JW, DM, PAB and possibly Dr Nyengaard. ACTION: PAB to appoint a Project Manager for the study.	LLS PAB
7.	Epidemiology publications update Item not discussed owing to time constraints	

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8.	Conclusions arising from data • There is no definitive epidemiological evidence that PQ in normal occupational use adversely affects human health • Our animal studies are well conducted to GLP standards; the studies use larger numbers of animals compared to other workers; our studies are read “blinded”; our results are reproducible. They show no evidence for an adverse effect of PQ as measured by neurochemistry or pathology and, although the data are incomplete, it is considered likely that this will extend to stereology. Clear adverse effects are observed with MPTP using all three parameters. • In the absence of evidence for the difference in response to PQ in this study compared to published studies, no definitive conclusion can yet be made on the potential for PQ to cause neuronal cell loss in the S. nigra. • However, this study is the most thorough investigation of the effect of PQ on the S.nigra and the complete absence of any pathologically-detectable adverse effect on neuronal cells is compelling • Nevertheless, a multi-lab study is needed to investigate possible methodological reasons for the discrepancy between the outcome of the recent SYT studies, and that of earlier studies conducted by SYT and those in the published literature • A 90 day dietary study in the mouse will be conducted mainly to fulfil our obligations to the Australian regulatory authority but also to ensure that sub-chronic dosing by a more relevant dosing route for human risk assessment does not elicit adverse changes in the S.nigra.	
9.	Alpha-synuclein presentation Item postponed. ACTION: LLS to invite PLN and DDM to discuss relevance of α synuclein to PD. Also to consider inviting another external expert to participate in the discussion.	LLS
10.	NHP (squirrel monkey) paraquat data, reminder of study design and outcome Discussed under Agenda item 2	
11.	External communications strategy - Scientific publications ACTION: CBB, NCS and LLS to prepare a “storyboard” for a publication strategy. It was proposed we would publish papers detailing ○ Pathology from both studies	CBB, NCS, LLS

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	○ Stereology from both studies ○ Risk assessment paper dealing with in vivo animal studies, kinetics and epidemiology. - External Presentation LLS will present data at the Society of Neurotoxicity meeting in Xian in June 2011. Tina Levine (EPA), Carol Burns (Dow) and Abbi Li (Exponent) will be present. Abbi Li is presenting on risk assessments with PQ and chlorpyrifos as case studies. ACTION: PAB to present in vivo study results at the September 2011 BTS meeting ACTION: LN to review existing Q+As and consider if these need updating to reflect our emerging data, and also to prepare for possible Qs arising out of the Xian and BTS presentations.	PAB LN
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Minutes compiled by DJ Berry

31 May 2011

Circulation:

PA Botham	L Navarro	C Campbell
LL Smith	K Mewes	RA Brown
NC Sturgess	P Hertl	J McFarland
KZ Travis	JD Sullivan	JS Wolff (Fulbright and Jaworski)
AR Cook	A Nadel	

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