CONFIDENTIAL AND PRIVILEGED

Paraquat Health Science Team

Action Minutes from Marlow Meeting 20 & 21 April 2009

The Compleat Angler, Marlow UK

Guest Speakers: Joan Abbott, David Brooks, Jeff Wolf

Expert Advisors on Epidemiology: Jack Mandel, Hans-Olav Adami, Phil Cole, Dimitrios Trichopoulos

Health Science Team: LL Smith, CB Breckenridge, MF Wilks, PA Botham, NC Sturgess, KZ Travis, AR Cook, J McFarland, DJ Berry, K Mewes

Extended Health Science Team: Health Science Team plus CL Berry, PL Nicotera , D DI Monte, C Campbell, J Tomenson

Syngenta Legal Team – A Nadel, J Wolff (Fulbright & Jaworski) , J Sullivan (JS on 21 April only)

Dial-In Participants: D Kim, D Minnema, M Tisdel, M Butts, C Campbell

MONDAY 20 April	AGENDA ITEM	Participants	Minute/Actions
	Meeting format and Objectives	Extended Health Science & Legal Teams	To review the current and existing scientific information available and to consider what future studies may be required.
	Blood Brain Barrier – Prof Joan Abbott *Professor of Neuroscience* Blood-Brain Barrier Group Pharmaceutical Science Research Division School of Biomedical & Health Sciences King's College London	Extended Health Science & Legal Teams + all invited attendees	Comments arising from presentation: • There are multiple transporter systems in mice, and these are likely to be similar across species. It is probable that only 50% of potential transporter systems have been identified to date. • There is a need to identify if efflux and influx of compounds across barriers is similar in rodents and humans • Abbott may be willing to participate in collaborative projects with Syngenta.
	PET Imaging – Prof D Brooks	Extended Health Science & Legal Teams + all invited attendees	***Requested PDF of presentation*** Comments arising from presentation: • Clinical signs in humans are only evident once there is a 30-40% loss of dopaminergic neurones. • Antivestibular drugs (anti-giddiness) pills can lead to PD-type clinical signs. • MPTP induced PD shows a different MRI pattern compared to idiopathic PD. • Pattern of PET images observed by Barlett et al in monkey brains dosed with MPTP would depend on dose and duration. E.g. a small, long term insult by toxins will likely lead to a loss of lateral cells. • PQ dose used in the Michigan PQ monkey study was low (possibly due to systemic toxicity). • Agreed we do not need to pursue further collaboration with Prof Brooks at this stage.
	PQ study updates	Extended Health Science & Legal Teams	Slides not available. Comments from Prof DiMonte: • In mice, PQ exposures show 25% reduction in dopaminergic neurones and up regulation of α-synuclein. It is believed the up regulation is a response to the insult, and is not necessarily associated with the dying neurones. • Preliminary results from squirrel monkey; studies o monkeys 8-12 weeks old, n=4 o 1-6mg/kg MPTP results in reduced TH 1 week and 1 month after dosing. o At 5mg/kg s/c PQ, monkeys died due to lung toxicity after 2nd/ 3rd dose. At 2.5mg/kg, animals tolerated the dose for 6 weekly injections at which time they were sacrificed. NO clinical signs of toxicity. No difference in numbers of dopaminergic neurones was observed. . o In PQ treated animals there was up regulation of α-synuclein. o Dose regime of 6 weekly injection of 2.5mg/kg PQ. Four weeks later monkeys given MPTP. Prior dosing with PQ appeared to protect the brain neurones from MPTP. o Preliminary assessment of results – primates are more sensitive to the systemic toxic effects of PQ (lung toxicity) and it may not be possible to give a large enough dose for PQ to have an effect in the brain. o DDM would be willing to share striatial material with Syngenta for PQ concentration analysis. • ACTION - KZT to estimate quantity of tissue required for analysis. • ACTION - DDM to conduct stereology on monkey brains. • ACTION – DDM to send to Syngenta the lung pathology autopsy report. • ACTION - consider adopting same protocol in mice with lower PQ doses.
	Mechanistic studies - Tolerability study - Neurochemistry - Stereology	Extended Health Science & Legal Teams Wolf Dial In Participants	ACTION – DM to resolve with analysts which neurochemical methods to use, ECD or MS. Irrespective of which method is selected, the importance is to look at relative changes with different exposure regimes. DDM suggested that oxidation of samples can be prevented if samples are placed in acid/ice. ACTION – MB to consider using Caspase3 + TUNEL + Nissl staining to confirm apoptosis. Cell death usually occurs 2 days after MPTP dosing.

CONFIDENTIAL AND PRIVILEGED

TUESDAY 21 April		Participants	
	Kinetics update - Single ip dosing - In vivo micro imaging update Objectives and study outline for 28 day kinetics study	Extended Health Science & Legal Teams Wolf	Comments arising from presentation: • Following dosing of mice with MPTP, most of the MPP+ present in the brain will efflux within 8 hours. The retention of PQ in mouse brain appears to be longer than with MPP+. • Microimaging work at Charles River (Tranet) will cost $150,000. ACTION – KZT to consider the need for further investigation.
	Future studies	Extended Health Science & Legal Teams Wolf	Comments arising from presentation: • KO mice study: TNFα, R1 or R2 KOs and interferon δKO. • LPS study – need results from other studies before the protocol can be finalised. May not be required, depending on results from the TNFα investigations. • Species/ strain – studies not to start until we have further information from other studies : note, DMM has not seen wide variations in different mouse strains with MPTP, however, PLN has seen large differences with PQ in in vitro systems. Syngenta has data showing that mouse is more sensitive than rat to PQ exposure ACTION – consider publishing paper (or poster) – but we require further kinetic data in the rat to support hypotheses. ACTION – KZT to plan in rat kinetics work. • Need to investigate potential mechanistic differences between mice and primate, and, for example, if compounds such as LPS play a defining role in the expression of effects. • Other areas for potential investigation: ○ Other factors which have been referred to in external studies as potentially influencing effects (e.g. maneb) ○ Factors that may decrease as well as increase risk ACTION – consider if and when we should conduct kinetics studies in non-human primates. ACTION – consider putting PQ through Abbott's neutral amino acid transport system (utilising glial/ neuronal cell mixed cultures)
	Epidemiology studies - Widnes - Risk Factors paper - Case control study Ritz et al, 2009; Costello et al, 2009	Expert Advisors on Epidemiology, Extended Health Science & Legal Teams	**Mortality study at Widnes:** • Our Expert Advisors on Epidemiology (EAE) and Prof Coggan to review protocol, to be prepared by J Tomenson and agreed to by Syngenta. Coggan has agreed to be thanked in any subsequent publication for his advice and consultation. It was agreed we would not seek further authorship from a 3rd party. • The study will be implemented as an update of the Paddle paper, looking at all causes of death as per Paddle, plus PD. • ACTION – Support to be sought to proceed with the study on this basis. JS to brief Aruffo, PAB to brief Doe. • **Survivor cohort at Widnes:** ○ ACTION - JT to prepare a document identifying limitations in conducting a survivor study. AN to gather comments from our EAE on the merits of conducting a survivor cohort study at Widnes. AN/JS to advise how to proceed, following consultation with LLS and MFW. [we have already determined that it would be inappropriate to involve Coggan as this may create a conflict with his other professional activities.]. • **Risk Factor Paper:** • The EAE have agreed to progress this and have identified a post-Doc from the Karolinska Institute who will prepare data for this. • ACTION – Mandel to discuss practicalities with AN (and consult with CBB). • **Case Control Study:** • Syngenta seeks to optimize its ability to start a study following outcome from the Ag Health survey. • EAE has commented that this study will be difficult to initiate, complicated to execute and unlikely to add value to our understanding of the problem compared to a case-control study already being pursued as part of the Aghealth survey. It may be difficult to find a suitable study investigator because of these circumstances and consequently the benefit of a further study appears extremely dubious. When all the comments from the EAE have been submitted the decision on whether to conduct a further study will be made. • **Costello Paper;** • ACTION – Mandel to provide an objective assessment from the team by 1 May 2009. Syngenta stated that we would use their review to support our positions without direct reference to the identification of the contributors. • **Ritz Paper:** • The EAE need more time to consider their response to this paper. • **Ag Health Study:** • ACTION – CBB/MFW to arrange a meeting to consider the strengths and weaknesses of conducting the nested control study within the cohort.

Circulation: