Formatted: Width: 8.5", Height: 11", Header distance from edge: 0.49", Footer distance from edge: 0.49"

Formatted Table

Minutes and Actions from the Paraquat/Parkinson's Disease Task Team Meeting at CTL on 18th October 2001

Formatted Table

Present	Mike Clapp	Nick Sturgess
	Georg Krinke	Nicola Wellis
	Ted Lock	Martin Wilks
	Chris Sheard	

1. Remit of Team — NCS

NCS presented the remit that had been discussed and agreed for this HAT team. The team will report to the Paraquat Steering Group, which is chaired by Lewis Smith. Quarterly updates will be presented to the PLT by MJLC.

The remit of the team was stated:

"To evaluate recent developments in the understanding of Parkinson's disease, to evaluate the models for Parkinson's disease and to establish the relevance of the data for Paraquat"

2. Why the business has become alerted ?alerted? — MJLC

MJLC presented a summary of the areas of concern regarding a suggested link between paraquat (PQ) and Parkinson's disease (PD)

A range of studies (principally in mice) have emerged and continue to emerge, that claim to demonstrate that PQ, when co-administered with the dithiocarbamate fungicide maneb, can cause site-specific effects in the substantia nigra – the area of the brain that is affected in classic PD. Some of the more recent studies postulate that mice exposed as neonatesally have shown an increased susceptibility to PD-like symptoms

It is known that PQ can give rise to non-specific brain lesions in suicide cases but even in such patients (who are concurrently suffering irreversible multi-organ failure) there is no evidence of functional neurotoxicity. Diquat is known to cause brain stem infarcts but, again, only following high, suicidal doses.

The decreasing pattern of dopamine levels in the nigrostriatal brain region production, with advancing age, is well described and a model was presented showing the theoretical potential for toxicity to predispose individuals to PD at an early age.

On a "weight of evidence" basis, the Advisory Committee on Pesticides has advised the PSD that there is no basis to link PQ to neurotoxicity. The ACP has, however, recommended an epidemiological study to look at the possibility of a link between pesticides and PD.

3. Summary of recentof recent developments in the literature; new developments on the cause of PD — NCS

The molecular structure of PQ has been compared unfavourably to the structure of MPTP; a compounddrug that is known to cause a PD-like syndrome.

From a series of studies that have emerged investigating a putative link between pesticides and PD (and, more specifically, between PQ and PD), NCS focussed on the work of Dr Cory-Slechta's group in Rochester, NY and on publications by DiMonte *et al* and Eriksson *et al* (see presentation).

"NCS # 3.ppt"

NCS also referenced a *New Scientist* article that implicates a soil bacterium, *Nocardia asteroides*asteroides as a potential cause of PD. It was noted that the pattern of incidence of, and exposure to *N. asteroides*asteroides, maymight offer a better 'fit' with the frequency and incidence of PD than does PQ/maneb exposure.

4. Background to CTL experimental approach including experimental work with MPTP — NCS

The initial strategy of the PQ/PD research group at CTL was to seek to reproduce the experiments carried outresults obtained by the Rochester group in the C57Bl6 mouse (paper by Brooks *et al*, 1999). CTL has concerns that the use by the Rochester group of fluorogold breached the blood-brain barrier and allowed PQ to enter the brain.

"NCS # 4.doc"

NCS reviewed work done to date, explaining that it has not proved possible to duplicate the positive control effects obtained by the Rochester group using MPTP. Work is ongoing to address this issue in order that the MPTP mouse model is established in CTL. The work is principally centred on using the appropriate dosing regimen.

5. Current and proposed experimental studies — NCS

During the period of activity in CTL, Thiruchelvam *et al* (2000-2001) [also of the Rochester group] have published studies showing that co-administration of PQ and maneb leads to neuronal cell loss in the substantia nigra. In addition, the Rochester group is looking into the developmental sensitivity of mice to PQ.

Current and future CTL work which is planned/proposedwork that is planned/proposed includes:

Validation of the MPTP mouse model
Further work is required to establish the MPTP mouse model in CTL. Focus on areas where the CTL approach may be different from the Rochester approach:

- age of animals
- suitability of pathology techniques including morphometric analysis
- establish a more sensitive marker for effects on dopamine (such as HPLC analysis of brain regions for dopamine and its metabolites)(!)

Multiple dosing of PQ — role of kidney toxicity ?toxicity?
A study design is in place to evaluate the role of kidney toxicity in accumulation of PQ in brain due to reduced renal clearance.

In vitro studies were proposed in order to understand the nature of any interaction of PQ with the dopamine re-uptake transporter, and other possible transport mechanisms that have been reported to account for accumulation of PQ into the brain. These studies included:studies — Formatted

- inhibition of dopamine re-uptake
- inhibition by valine of PQ uptake mechanism
- accumulation of PQ in synaptosomes

Toxicokinetics studies were proposed in order to examine the nature of the kinetics of PQ – accumulation in the brain following different routes of exposure. These included:

- develop toxicokinetics data in the mouse, including at different ages
- brain levels of PQ after different routes of exposure
- assess PQ accumulation after repeat exposure

"NCS # 5.doc"

Action: EAL to supply a copy of the manuscriptpaper for publication (but never published) by David Ray (MRC Tox Unit) on the repeat of Eriksson's work on mouse neonatal exposure to PQ, for incorporation into the PQ literature database. **EAL**

6. Review of Experimental Methods in Pathology — NTW/GK

NTW explained the basis of the histopathological techniques that have been used to investigate effects on the substantia nigra and striatum (SN). This included a description of the fluorojade and fluorogold staining techniques and of the critical differences between them.

Fluorojade is applied to prepared sections of tissue and is a stain that is taken up by damaged neurones. Fluorogold is injected into the live animal and becomes associated with damaged cells.

The data produced by the Rochester group, and the possibility of weaknesses in that group's scientific presentation werepossibility of weaknesses in that group's scientific presentation was discussed. For example, CTL experience of assessing damage in the SN reveals that counts of affected neurones are variable. Where the data from treated animals are presented as a percentage of control (as are the Rochester data) such variation is much less likely to be obvious.

Other possible weaknesses in the Rochester data include the fact that the density of staining across a measurement field was integrated, yet the significance of the readings obtained has been attributed only to the neuronal terminal bodies.

GK presented the results of a study done by Novartis that highlighted further some of the weaknesses of the Rochester work. From his experience as a journal referee, GK commented that on the basis of frailties in the pathological approaches taken in the published studies, he would very likely have rejected the papers by the Rochester group in the absence of further analysis. He also commented that the techniques involved in this type of work are exacting, and that if Syngenta wished to replicate andor challenge the PQMPTP results obtained at Rochester, there may be time-saving and significant advantage in taking the work to a laboratory where these techniques have already been established,:

there are laboratories in California that have significant experience of the MPTP model.

MPTP has no effect on the SN in albino strains of mice. Though it does have an effect in pigmented mice, the mouseice isare not the best model. Man is the most sensitive with cynemologus monkeys probably next.

NTW suggested that the team should reflect on the potential weaknesses of the Rochester results and on the results obtained to date in CTL with a view to developing a 'gold-standard' pathological procedure (with supportive behavioural observation techniques [NCS] and neuro-chemical assays [EALJ) for the identification and quantification of the lesion in the SN.

Even if PQ were shown to cause some degree of lesion in the SN, GK mused whether it might be shown to be reversible on cessation of exposure; dopaminergic neurones are not myelinated and may therefore be capable of regeneration following transient insult.

Action: NTW/GK will provide written summary of the limitations of the pathological analysis (and their subsequent findings) carried out by the Rochester group and described in their 1999 and 2001 publications (Brooks *et. al.*, 1999; Thiruchelvam *et. al.*, 2000). In order to assist in the establishment of the MPTP mouse model in CTL, GK would also provide additional technical information on the MPTP dosing regimen (based on his previous experience), and advice re relating to the technical complexity of the pathological evaluation of MPTP exposed brains. will prepare a statement on the technical complexity of the pathological evaluation of neuronal deficit due to MPTP (and, by implication, PQ/maneb). **NTW/GK**

Formatted
Formatted
Formatted
Formatted

7. Developmental Studies; Potential Effects on Pre/Post-natal Development — MJLC

As noted earlier in the meeting, some of the more recent Rochester work postulates that neonatal exposure of mice exposed neonatally to PQ shows an increased susceptibility to PD-like symptoms. The group also claims to have shown that mice exposed as neonatesally have an increased sensitivity following exposure to PQ in later life. Future work planned by the Rochester may group includes a study involving prenatal exposure of which mice will be exposed to PQ development-ally (i.e. during pregnancy).

Existing regulatory studies (developmental toxicity in mouse and rat, and multi-generation studies in the rat) conclude that there are no developmental alerts for paraquat.

Devtox.ppt

NCS, MJLC, NTW and Sandra Allen had met to discuss the options available to address the challenge whichchallenge, which the Rochester proposals represent. A set of actions wereSets of actions were agreed which are listed in the attached document.

8. Update on Epidemiology — MW

MW reported that there had been no significant progress in this area. It was agreed that MW would talk to LLS to establish whether an approach to Prof. Adonis had been made yet.

9. Research Budget Allocation — MJLC

Budget has been secured for research in this area:

2001 1 man year
2002 3 man years plus $50k for external consultancy
(note: this allocation is still under review)

Components of the research planned include:

- validation of MPTP mouse model
- pharmacokinetics of PQ in the mouse
- developmental changes in the mouse
- epidemiology
- exposure data

10. Maintenance of Literature Database **CMS**


LS DB.ppt

CMS briefly presented proposals for the structure and maintenance of the PQ/PD database. The procedure and search method utilised for scanning the literature were described and suggestions put forward for the ongoing scientific review and positioning of published findings.

Location and disposition of LAN storage of key documents and consideration for wide(r) sharing of data and documents were also discussed. Any future provisions for PQ/PD may be able to take advantage of wider initiatives on document handling and sharing in Syngenta. The team will be updated on developments in this area as appropriate.

In the meantime, a CTL-only project area will be established in the G:\ drive XSHARE folder.

11. Proposal for Influencing Strategy **MJLC**

A science-based approach to an influencing strategy was proposed. This should be supported by position statementsPosition statements should support this. Any development of the strategy must consider how best to influence academia, and regulatory and NGO 'environments'.

It was agreed that a techno-regulatory team is required that can identify the threats to paraquat from the PD hazard models. The team should promote a science-based understanding of the issues surrounding the implication of paraquat in PD-like effects in man in order to maintain and safeguard paraquat registrations.

Action: MJLC would set up an initial meeting with appropriate techno-regulatory input from individuals before the year-end, to discuss the key issues and start to formulate PQ influencing strategy. Review the HAFS statement pertaining to this project. **MJLC**

Action: The position statement on PQ & PD would also be reviewed and updated. **ALL**

Formatted

Formatted

Unless other issues arise in the mean time, the next meeting will be organised for early in 2002 when we will hopefully have some more experimental data to discuss.

Formatted

Formatted

All