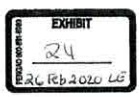
Paraquat and Parkinson's Disease Research Literature Update (External Publications)

- Maher *et. al.*, (2002) *"Epidemiological study of 203 sibling pairs with Parkinson's disease".*
  - Compared to PD patient's spouses, biological relatives (parents & siblings) of PD patients have a 2-3 times greater risk of developing PD.
  - If environmental factors are important, then spouses should display the same incidence of illness (which they don't).
  - Shifts the emphasis away from environmental factors as the cause of PD and reinforces the genetic link.
  - Does not rule out intrauterine and early life exposures to environmental factors that may predispose an individual.



Paraquat and Parkinson's Disease Research Literature Update (External Publications)

- Manning-Bog *et. al.*, (2002) *"The herbicide paraquat causes upregulation and aggregation of α-synuclein in mice".*
  - The protein α-synuclein may be involved in Lewy body (hall mark of PD) formation, and it may interact with chemical species such as paraquat initiating Lewy body formation.
  - High concentrations (up to 1 mM) paraquat accelerate the formation of α-synuclein fibrils *in vitro*.
  - *In vivo* 3 weekly doses of 10 mg/kg paraquat leads to up regulation of α-synuclein in the mouse brain levels peak 2 days after each dose, but return to control values 7 days post dose.
  - Significance of findings unclear - over expression of α-synuclein may be neuroprotective.

Paraquat and Parkinson's Disease Research Literature Update (External Publications)

- Di Monte (2001) *"Role of environmental agents in Parkinson's disease".*
  - Concise, and reasonably well balanced review article on the current research investigating the involvement of certain molecules in the possible aetiology of PD. Nothing new which we were not already aware of.
  - Paraquat gets a mention, as does the apparent similarity with MPP^+. Comments that initial *in vivo* studies linking PQ to PD did not provide convincing evidence of a link.
  - Potential synergistic effects between two chemicals discussed, including the PQ and maneb story. Mentions metal exposures such as lead-copper & lead-iron.
  - Comments on recent α-synuclein work (Di Monte lab).

Paraquat and Parkinson's Disease Research Experimental Progress

***In vivo* experimental progress**

- Development of $C_{57}Bl_{6J}$ mouse MPTP model.
- Literature reviews and expert advice suggest animals need to be left for 15-20 days post final MPTP dose before assessment of neuronal damage.
- Protocol put together taking into account this requirement start in March when new HO licence is granted.
- Established an additional endpoint for measuring damage to the nigrostriatal region of mouse brain - measurement of striatum dopamine levels and its key metabolites, DOPAC and HVA, using HPLC.

  Robust, reproducible and more sensitive than the behavioural and pathological endpoints also used.

Paraquat and Parkinson's Disease Research Experimental Progress

***In vivo* experimental progress**

- Evaluation of the role of mouse kidney toxicity in the tissue accumulation of paraquat following multiple doses.
- Kidney pathology, clinical chemistry and tissue distribution levels of paraquat determined following 1, 3 or 6 weekly doses of paraquat at 10 mg/kg ip. Final dose of paraquat was radiolabelled.
- No evidence of impaired renal renal function or toxicity following up to 6 weekly doses of paraquat.
- Amount of radiolabelled paraquat detected in tissues was the same regardless of the dosing regimen, indicating no impairment of the ability to clear paraquat.

Paraquat and Parkinson's Disease Research Experimental Progress

***In vitro* experimental progress**

- Investigation into the potential for paraquat to modulate th nigro-striatal pre-synaptic dopamine re-uptake system and the post-synaptic dopamine receptors.
- Effect of paraquat on [^{3}H]-dopamine re-uptake into rat and mouse striatal synaptosomes.
  - up to 1 mM paraquat is inactive
- Binding affinity (K_i) for paraquat at rat and mouse striatal dopamine D_1 and D_2 receptors.
  - K_i at D_1 receptors >>1 mM (inactive)
  - K_i at D_2 receptors >>1 mM (inactive)

Paraquat and Parkinson's Disease Research Experimental Work - Next Steps

Data that will be reported in the near future

- *In vivo* - MPTP model validation in $C_{57}Bl_{6J}$ mice with behavioural, pathological, neurochemical endpoints (2Q02).
- *In vivo* - pathology assessment of paraquat exposed brains and dosing of paraquat, MPTP and MPP^+ investigating changes in striatum dopamine and metabolites (3-4Q02).
- *In vitro* - determine whether [^{14}C]-paraquat is actively transported into mouse striatum and compare findings reported in rat cortex (*Smith & Wyatt, 1981*) (3Q02).
- Investigate the nature of any paraquat uptake into striatum with blockers of amine transport systems (3-4Q02).

Paraquat and Parkinson's Disease Research Experimental Work - Next Steps

Longer term studies to be reported

- *In vivo* toxicokinetic studies in the mouse examining levels of paraquat in the brain and other tissues following administration of paraquat via different routes of exposure.

  Compare with data already known for the rat (4Q02).

Paraquat and Parkinson's Disease Investigative Toxicology Research

Investigative Toxicology Input

- Investigative toxicology is involved in establishing whether there is a sound scientific basis for the claims by some research groups that exposure to paraquat causes Parkinsonian like effects in animal models.
- Are their findings repeatable?
- If so can we offer a mechanistic explanation for their results?
- If findings are not reproducible, can we refute the claims in the literature and offer alternative experimental findings?

Paraquat and Parkinson's Disease Investigative Toxicology Research

Investigative Toxicology Work (In vivo)

- Are literature claims robust & repeatable?
- Repeat *in vivo* experiments including dosing of paraquat (5-10 mg/kg ip) to $C_{57}Bl_6$ mice with neurochemical (striatum dopamine levels), neuropathological (TH^+ neurone counts in *substantia nigra*) and behavioural (locomotor activity) end point markers.
- Establish capability within CTL to make measurements in mice (previous experience only rat - principal tox species).
- Establish an appropriate positive control in the mouse to compare with paraquat data - MPTP mouse model.

Paraquat and Parkinson's Disease Investigative Toxicology Research

Investigative Toxicology Work (In vivo)

- How much paraquat gets into the mouse brain under different dosing regimens?
- Assess the kinetic profile of paraquat in the $C_{57}Bl_6$ mouse to understand the effects of high dose administration through different routes of exposure (ip, sc, oral & dietary) on brain exposure to paraquat.
- Compare with data already obtained in the rat.
- What are the effects of dosing other compounds (maneb) in combination with paraquat on brain exposure to paraquat?

Paraquat and Parkinson's Disease Investigative Toxicology Research

Investigative Toxicology Work (In vitro)

- Investigate the potential for paraquat to interact with and modulate the nigro-striatal pre-synaptic terminal dopamine re-uptake system and post-synaptic dopamine receptors in rat and mouse.
- Determine the affinity of paraquat for dopamine and monoamine transport systems in the striatum using selective radioligands for these transport proteins.

Paraquat and Parkinson's Disease Investigative Toxicology Research

Investigative Toxicology Work (In vitro)

- Paraquat may gain access to the brain, particularly in the very young owing to an incomplete BBB.
- Is there a paraquat transport system in the brain that results in paraquat being transported into dopaminergic neurones?
- Determine whether [^{14}C]-PQ is actively transported into mouse neurones (synaptosomes / brain tissue slices) and compare findings with those reported in rat cortex (*Smith & Wyatt, 1981*).
- Investigate the nature of any paraquat uptake into the striatum with blockers of amine transport systems.

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Paraquat and Parkinson's Disease Investigative Toxicology Research

Investigative Toxicology Work

- The issue around the claims that paraquat exposure and Parkinson's disease are linked needs to be addressed if the future Syngenta aspirations for the product are to be realised.
- Data generated will be used to build a scientifically robust, defensive position for paraquat in response to the issues already in the scientific literature, and to questions raised by the media, customers and regulatory authorities.

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Paraquat and Parkinson's Disease Investigative Toxicology Research

Investigative Toxicology Work

People involved in the investigative research work:

Nick Sturgess

Andy Gyte

Alison Foster

Louise Marks (post-doctoral neuroscientist)

Ted Lock

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Paraquat and Parkinson's Disease Investigative Toxicology Research

Investigative Toxicology Work (In vivo)

- Are literature claims robust & repeatable?
- Repeat *in vivo* experiments including dosing of paraquat (5-10 mg/kg ip) to $C_{57}Bl_6$ mice with neurochemical (striatum dopamine levels), neuropathological (TH^+ neurone counts in *substantia nigra*) and behavioural (locomotor activity) end point markers.
- Establish capability within CTL to make measurements in mice (previous experience only rat - principal tox species).
- Establish an appropriate positive control in the mouse to compare with paraquat data - MPTP mouse model.

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Paraquat and Parkinson's Disease Research Reshaped Programme Of Work For 2002

Recruitment of post-doc problematic.

Appropriately skilled post-doc now in place.

- *In vivo* - MPTP model validation in $C_{57}Bl_6$ mice with behavioural, pathological, neurochemical endpoints (*3Q02*).
- *In vivo* - pathology assessment of paraquat exposed brains and dosing of paraquat, MPTP and MPP^+ investigating changes in striatum dopamine and metabolites (*4Q02*).
- External publication of findings at scientific meetings to assist our influencing strategy (*spring 2003*).
- *Toxicokinetic studies in the mouse examining levels of paraquat in the brain and other tissues (moved back to 2Q03).*

Paraquat and Parkinson's Disease Research Reshaped Programme Of Work For 2002

- *In vitro* - investigate the potential for paraquat to interact with and modulate the nigro-striatal pre-synaptic terminal dopamine re-uptake system and post-synaptic dopamine receptors in rat and mouse (*2Q02 - update to follow*).
- Determined the affinity of paraquat for dopamine and monoamine transport systems in the striatum using selective radioligands for these transport proteins.

Paraquat and Parkinson's Disease Research Reshaped Programme Of Work For 2002

- Paraquat may gain access to the brain, particularly in the very young, owing to an incomplete BBB. Is there a paraquat transport system in the brain that results in paraquat being transported into dopaminergic neurones?
- *In vitro* - determine whether [^{14}C]-paraquat is actively transported into mouse striatum and compare findings reported previously in rat cortex (*3Q02*).
- *In vitro* - investigate the nature of any paraquat uptake into striatum with blockers of amine transport systems (*3-4Q02*).

Paraquat and Parkinson's Disease Research Plans For 2003

Toxicokinetic studies in the mouse examining levels of paraquat in the brain and other tissues (2Q03).

- How much paraquat gets into the mouse brain under different dosing regimens?
- Assess the kinetic profile of paraquat in the $C_{57}Bl_6$ mouse to understand the effects of high dose administration through different routes of exposure (ip, sc, oral & dietary) on brain exposure to paraquat.
- Compare with data already obtained in the rat.
- What are the effects of dosing other compounds (maneb) in combination with paraquat on brain exposure to paraquat?

Paraquat and Parkinson's Disease Research Plans For 2003

- Developmental neurotoxicity - literature data on synergistic effects of paraquat and maneb on very young neonates difficult to understand and is an area of comparatively little scientific understanding.
- Possible differences in BBB permeability at different ages and following exposure to maneb.
- Started to think through the issues and consequences of carrying out work in this area - need to further develop our plans for this area of research (*3-4Q03*).
- Need to make provision for resource to address this issue in 2003.

Paraquat and Parkinson's Disease Research Resource Budget For 2002 & 2003

Budget for 2002

Phase 1	220 staff days (1 man year)	*170 used 30/04/02*
Phase 2	220 staff days	
Phase 3	220 staff days (on hold)	

With post-doc now in place, will need to spend at least 110 (+ 440) staff days by end 2002 to complete work proposals.

Budget for 2003

Toxicokinetic work	1 man year
Developmental neurotoxicity	1.5 - 2.0 man years

Advice is to invest 3 man years effort for 2003.