Exhibit 38

Case ID: 220300429
Control No.: 26064959

From:
Dr E A Lock
Research Toxicology Section

ZENECA Central Toxicology Laboratory

Alderley Park, Macclesfield
Cheshire SK10 4TJ

Tel:
Telex:
Fax:
EDT:

Redacted - EU PII

To:
Stan Chart

Copies to
R Scott
K Atherton
F Brown
I Kimber

Your ref	Our ref	Tel ext	Date
	EAL/NWJ/	Redacted - EU PII	8 Apr 93

RE: PARAQUAT NEUROTOXICOLOGY STUDIES

Enclosed are proposals for the above studies as discussed when we visited Fernhurst last month. I am looking for approval and support to start the blood-brain barrier studies as soon as possible. The MPTP comparison with paraquat is to table that I will be seeking business support in 1994.

Regarding the cost Dr's Atherton and Scott will discuss this with you at the Liaison Meeting on 20th April. I will not be available but I hope we can do business.

Kind regards

Ted

E A Lock

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Alderley Park, Macclesfield
Cheshire SK10 4TJ

Tel:
Telex:
Fax:
EDT:

Redacted - EU PII

To:
Mr I S Chart
Agrochemicals

Copies to
Dr I Kimber
Dr E D Brown
Dr R C Scott
Dr K T Atherton

Your ref	Our ref	Tel ext	Date
	EAL/NWJ/1424	Redacted - EU PII	13 Apr 93

PARAQUAT NEUROTOXICOLOGY STUDIES

Following discussion with the Agrochemicals business on the above topic I would recommend that the following studies should be undertaken. The objectives of the work are:

1. to determine whether paraquat can enter the brain, to ascertain at what dose(s) this may occur and what effects it produces.

2. to undertake a comparative study of paraquat and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and/or MPP^+ toxicity to the nervous system with a view to publication of this information to support our defence that they have different modes of action.

By its very nature this work requires a multidisciplinary approach to explore the neurochemical, neurophysiological and neuropathological changes that may occur. It is also very important that a careful stepwise approach is taken so as to give the business confidence that our approach will benefit as opposed to undermine their position on the neurotoxic potential of paraquat. My starting position is that paraquat is toxic to brain cells (neurotoxic) if sufficient of the chemical is able to reach the brain. This is based on the known toxicology of paraquat, following its direct injection into the brain of rats (Jenner and Marsden 1987. DeGori et al 1988, Calo et al 1990, Bagetta et al 1992, Corasaniti et al 1992), administration systemically at high doses to rats (Clark et al 1966) or when deliberately ingested at very high doses by humans (Grant et al 1980, Hughes, 1988).

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1. Does paraquat cross the blood-brain barrier following systemic administration?

There have been an increasing number of recent publications primarily from an Italian group showing that paraquat given systemically enters the brain of rats and produces both behavioural and pathological changes (Corasaniti et al 1988, 1991, and 1992, Bagetta et al 1992). Much of their early work was not very rigorous and it is possible to interpret their findings to indicate that paraquat may not have actually entered the brain. Instead it could be entrapped in the blood still present in tissue at termination or bound to endothelial cells, as apposed to entering glial or neuronal cells. Their more recent work reported that paraquat at a median lethal dose (20mg paraquat cation Kg^{-1}, sc and above) produced behavioural changes which were associated with increased electrocortical activity and the development of neuronal necrosis. As with the earlier papers, this paper is poorly documented and there is the possibility that the neuronal necrosis could be due to a fixation artifact. Without our own data we cannot easily refute their findings.

Early studies on the systemic toxicity of paraquat in rats, reported behavioural signs indicative of neurotoxicity after single large doses of 30 to 75 $mgkg^{-1}$ ip (Clark et al 1966). Few studies have looked for paraquat in the brain. Whole body autoradiography in mice given a single large iv dose of 20 $mgkg^{-1}$ showed little evidence for entry of radioactivity into the brain (Litchfield et al 1973). Rose and coworkers (1976) determined paraquat concentrations in the brain following a median lethal oral dose of paraquat (126 mgkg-1) to rats and found values of 1-6 nmol/g wet weight over a 30hr time period. Dey et al (1990) determined brain levels of around 10 nmol/g wet weight during the first hour after 13.4 $mgkg^{-1}$ paraquat, sc, the values then declined to around 1 nmol/g and persisted for up to 7 days. These latter studies plus some of those from the Italian group did not take into account any entrapped paraquat in blood, in the brain, and this could account for a large component especially at the earlier times.

If paraquat were to cross the blood-brain barrier there is evidence in vitro for a transport system for paraquat into brain cells analogous to that in the lung (Rose et al 1976). Once inside the cell paraquat would readily accept an

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electron and redox cycle, which if marked and sustained would be expected to cause cell death. It is thus very important to ascertain whether this highly charged, water soluble cation, is actually entering the brain and if so, at what dose is it producing neuropathological and behavioural effects.

Programme of Work

Initial studies will focus on determining whether paraquat enters the brain. The approach will be to use radiolabelled paraquat and to examine its distribution in rat and mouse brain at various times after dosing. The initial experiments will focus on high doses starting at 20 mgkg-1 sc, to repeat the work of Corasaniti et al (1992). Care will be taken to remove any entrapped blood in the brain, by perfusion, and to confirm this by tagging the red cells with a marker such as ^{51}Cr.

In addition, studies will be performed with [^{14}C] inulin to measure the extracellular space and in concert with paraquat to determine whether or not entry into brain cells has occurred. Inulin has been used by others to determine the integrity of the blood-brain barrier (eg Berardi et al 1987). Other large molecular weight markers such as horseradish peroxidase or vital dyes may also be used to ascertain whether the barrier has been damaged. Parallel studies, at selected times and doses, will be conducted for histopathological assessment with particular emphasis on certain brain regions eg pyriform cortex where the Italian group claim neuronal necrosis occurs. Key studies may also be undertaken to look at electrocortical activity in the brain following paraquat administration to support and complement the tissue distribution and pathology studies.

Once we have some indication as to whether paraquat enters the brain and what the consequences are we will conduct studies at other doses and via other routes of administration to put the findings into perspective. This latter aspect of the work will only be undertaken once we know the results at large toxic doses (eg 20 mgkg-1 sc and above) and following discussion with the Product Manager and Business.

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Proposed time scale of studies

May 93 July 93 Oct 93 Dec 93 Mar 94

develop methods to determine whether paraquat enters the rat brain after 20 mgkg-1 sc or above. Examine early time point and after 24 hr.

assess the brains for neuropathological changes

determine temporal relationship and explore dose route in the rat.

assess the brains for neuropathological changes.

conduct experiments to measure electrocortical responses to paraquat in the rat.

extend key findings in the rat to the mouse. Explore any unexplained findings.

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2. Comparison of the neurotoxicity of MPTP and/or MPP+ with paraquat

MPTP produces neurotoxicity in man and certain laboratory animals following parenteral administration. The clinical signs of neurotoxicity are characteristic of Parkinson's disease. The structural similarity between MPP^+, the active metabolite of MPTP, which has been shown to cause selective damage to dopaminergic in man and experimental animals (eg Jenner & Marsden 1987) and paraquat has been widely noted. Certain claims have been made regarding the likely effect of paraquat on the nervous system following chronic occupational exposure (Barbeau et al 1987). While others have disputed the role of herbicides and pesticides as the likely cause, of an increased incidence of Parkinson's disease in certain rural regions of Canada (Rajput et al 1987). During the last 5-10 years considerable progress has been made in understanding the mechanism whereby MPTP causes selective necrosis to dopaminergic neurons. The key factors are as follows:

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1. MPTP is sufficiently lipid soluble that it can enter the brain and glial cells in particular.

2. MPTP then undergoes metabolism via the enzyme monoamine oxidase B to form the monocation MPP^+, this enzyme is located at extraneuronal sites which are in close proximity to nigral dopamine neurons.

3. MPP^+ once formed, is actively accumulated into dopaminergic neurons by a transport system, where it accumulates to high concentrations and causes cell death.

4. The precise cellular toxicity is not understood but both mitochondrial dysfunction and redox cycling have been implicated.

The active metabolite MPP^+ is very polar and its penetration into the brain is poor. For this reason much of the experimental work with MPP^+ has been done using direct intracerebral injection, where not surprisingly it is neurotoxic when infused into selected brain regions (Bradbury et al 1986, Cavalla et al 1985). Comparative studies with the two charged molecules (MPP^+ and paraquat) following subcutaneous or oral administration do not appear to have been conducted. Hence I recommend we design a study to compare and contrast the effect of MPTP and MPP^+ and paraquat on the nervous system. Namely assessment of neurobehavioural function, detailed measurements of dopamine and its metabolites in selected brain regions and neuropathology.

The likely outcome is that we should be able to show that paraquat does not share the prediliction of MPP^+ for the dopaminergic nigro-striatal system. However we would expect at high doses, should paraquat enter neurones in sufficient concentration, that it could redox cycle and may cause cellular injury. In my judgement this is only likely to occur at lethal doses, but our studies as described in part one should give us a clearer position on that.

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Programme of work

Prior to starting this work, we will need to select the best animal model of MPTP-induced neurotoxicity as there are certain species differences in response with regard to the spectrum of changes in behaviour, neurochemistry and neurodegeneration. This will necessitate a more detailed review of the literature and discussion with key experts such as Professor Peter Jenner (Kings College London) and with Professor Alan Crossman in Manchester. It is important that we have the most appropriate model for MPTP which is recognised as such by the neuroscience community.

The programme would then involve (1) establishing the model in CTL and determining the relevant neurobehavioural sequelae, neurochemical changes and neuropathology and then (2) comparing these findings with that following paraquat administration. Dose levels and time courses will be established based on our current knowledge of paraquat toxicity and the schedule developed for MPTP.

Prior to initiating studies with paraquat, consultation with the product manager and business will take place.

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Proposed time scale of studies

Jan 1994 June 94 Dec 94 ----> 95

Define appropriate model for MPTP and set up methodology

conduct animal studies MPTP/MPP+, measure behavioural, neurochemical and neuropathological consequences

Validate for dose-response and temporal effects

conduct appropriate studies with paraquat

depending on the outcome. Conduct further studies Publish findings.

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