

## Paraquat & Parkinson's Disease

26-841000 LG

## Paraquat & Parkinson's Disease Running Order of Presentations

Introduction to PQ & PD:	Nick Sturgess
<i>In vivo</i> studies with PQ:	Louise Marks
<i>In vitro</i> studies comparing PQ with MPTP / MPP <sup>+</sup> :	Alison Foster
Summary comments:	Nick Sturgess

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### What is Paraquat?



Paraquat

1,1-dimethyl-4,4'-bipyridinium ion

Paraquat is a non-selective herbicide.

Molecule first described in 1882, but herbicidal properties not discovered until 1955 by ICI.

Became commercially available in 1962 following experimental use in Malaysian rubber plantations (1959) as a replacement for sodium arsenite. Dichloride salt formulation - many trade names, *Gramoxone*.

Found in some OTC herbicide treatments (*Pathclear* & *Weedol*).

Very safe if used correctly - rapidly deactivates on contact with the soil, and does not leach into ground water.

Syngenta's No.2 selling product (\$360 million in 2003).

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### Parkinson's Disease - What is it?

- Parkinson's disease is a neurodegenerative disease caused by dopamine deficiency in the *striatum*, and loss of dopaminergic neurones in the *substantia nigra*.
- Symptoms include tremors, muscle rigidity, involuntary movements and postural changes.
- Affects approximately 1.8% of the elderly population >65 years equates to 1.5 million people in the US.
- Mean age for onset of symptoms is typically between 60-65 years old, and only 5% of Parkinson's patients develop symptoms before age 40.
- No clear genetic component to idiopathic Parkinson's disease, except in some cases of early-onset.
- "Environmental factors" may play a role in the aetiology of the disease?

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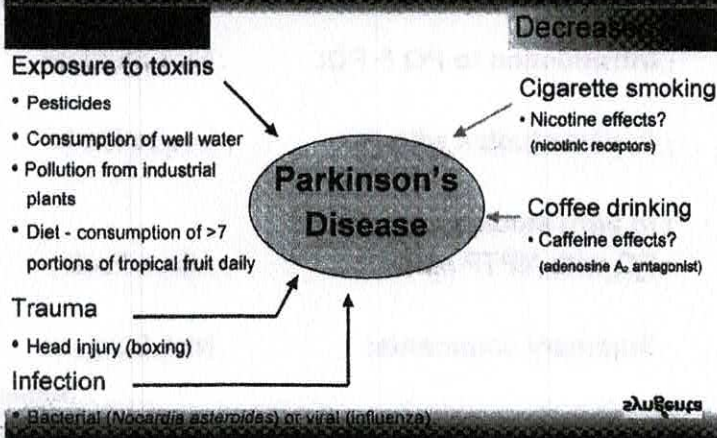
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## Possible "Environmental" Risk Factors Associated with Parkinson's Disease



## Why have pesticides been associated with Parkinson's disease?

- Barbeau *et al.*, (1986) carried out an epidemiological study investigating the role of pesticides in PD. Regional prevalence of PD in Canada non-uniform.
  - correlated with areas of pesticide use.
- Other workers followed this lead - further epidemiology studies - found prevalence of PD correlated with industrial areas and logging regions.
- Tanner *et al.*, (1999) epidemiological study revealed no overall difference in concordance for PD between non-identical and identical twins.
- Data were interpreted as evidence that genetic factors do not play a major role in PD aetiology, thus focusing attention back onto environmental factors.

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## GENETICS and PD: RESEARCH POINTS to ENVIRONMENTAL CAUSES

by Caroline M. Tanner, M.D., Ph. D., and J. William Langston, M.D., The Parkinson's Institute, Sunnyvale, California, a National Parkinson Foundation Center of Excellence

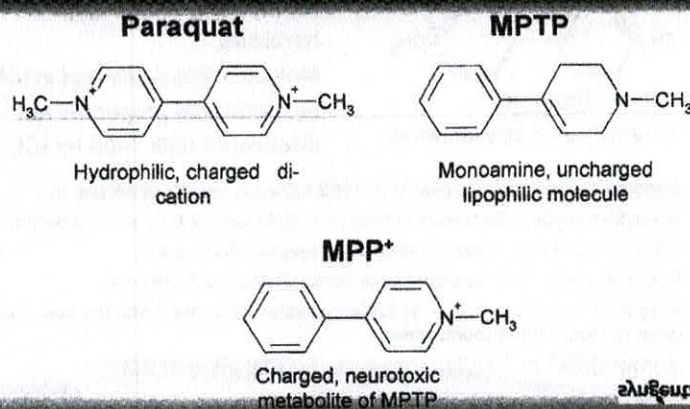
For 150 years, there has been an ongoing debate in the scientific community on the cause of Parkinson's disease. Both environmental factors and inheritance were early suspects as causes. Our discovery in the early 1980s that the chemical MPTP (1-methyl, 4-phenyl, 1,2,3,6-tetrahydropyridine) could create parkinsonism supported the view that environmental factors were paramount. Recently, the identification of several mutations that are known to be cause of disease in specific families has supported the genetic viewpoint.

Thanks to the completion of the World War II Veteran Twins Study on the etiology of Parkinson's disease (published in the January 27, 1999 issue of the *Journal of the American Medical Association*), we can say for the first time with confidence that environmental factors are the most common cause of disease in "typical onset" patients (those diagnosed after 50 years of age). At the same time, our results confirm that genetic factors are the cause of disease in most "young onset" patients (those diagnosed prior to age 50). The road we followed to obtain these results has been a long one, involving contacting 19,842 of the veteran twins, but the journey has been worthwhile since the study clearly distinguishes the conditions under which genetic or environmental factors would apply to onset of the disease.



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## Why has paraquat been associated with Parkinson's disease?



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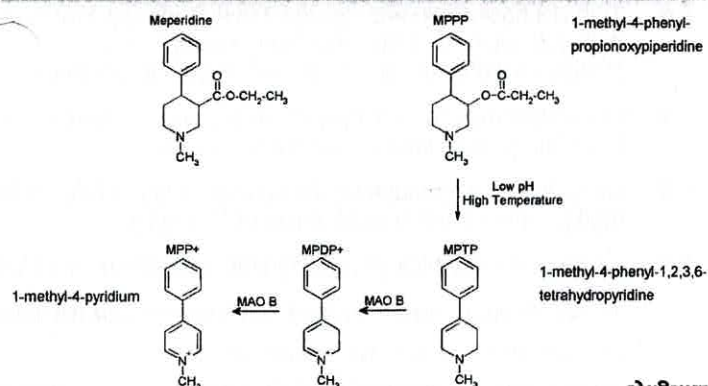
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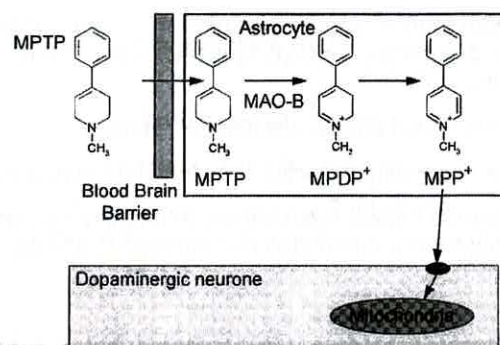


## Conversion of MPPP to MPTP and MPP<sup>+</sup>



## MPTP Mechanism of Toxicity

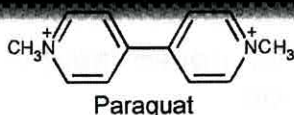
### Metabolic activation and mechanism of toxicity of MPTP



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## Is Paraquat likely to be neurotoxic?



1,1-dimethyl-4,4'-bipyridinium ion

Paraquat is unlikely to be neurotoxic owing to the fact that it has a chemical structure and physical properties (charged, polar molecule) which mean it will not readily cross the blood brain barrier (BBB).

In addition it has a non-metabolised, diamine structure, which mean it will not be a substrate for neuronal monoamine transport systems in the CNS.

## Does Paraquat cross the BBB and cause neurotoxicity?

- Long term (1-2 year) Syngenta regulatory studies with paraquat (PQ), involving oral or dietary administration to rats and dogs, have shown no signs of neurotoxicity or neuropathological changes indicative of neurotoxicity.
- Oral dosing of paraquat to rats at 5 mg/kg/day for 14 days (Widdowson *et al.*, 1996) produced no evidence of neuronal damage (including the nigrostriatal region), or behavioural changes indicative of neurotoxicity.
- [<sup>14</sup>C]-PQ dosing studies with rats and mice reveal very little PQ (<0.05% of total dose) gets into the brain as we predict.
- Concluded that in the rat & dog (primary toxicology species), there was no evidence to indicate that PQ is neurotoxic via relevant routes of exposure.

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## Literature Developments of Concern

- Not a recent problem - issue arose 10 years ago!
- Fredriksson *et al.*, (1993) Eriksson's group (Uppsala). Two oral doses of PQ (0.36 mg/kg at days 10 & 11) to young C<sub>57</sub>Bl<sub>6</sub> mice.
  - behavioural effects observed at 60 days
  - corresponding reductions in striatal dopamine content
- Syngenta funded follow up work conducted to see if findings were repeatable - no conclusive effects.

2003

## Recent Literature Developments Of Concern

- Two US based research groups have produced a series of publications since 1999 implicating paraquat in a Parkinson's disease animal model - work still on going.
- Cory-Slechta group - Rutgers, NJ, (University of Rochester, NY). Di Monte group - Parkinson's Institute, Sunnyvale, CA.
- Using the C<sub>57</sub>Bl<sub>6</sub> mouse model and i.p. dosing of PQ (1-30 mg/kg) - typically 3 weekly doses of 10 mg/kg.
- Looking at three biological endpoints as markers of toxicity:
  - neuropathological - loss of neurones from substantia nigra (stereology)
  - neurochemical - loss of dopamine from the striatum
  - neurobehavioural - reduction in locomotor activity

2003

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## Recent external pressures on paraquat quoting links with Parkinson's disease

### Pesticides Action Network Europe PAN Europe

To promote sustainable alternatives,  
PAN Europe co-ordinates and strengthens  
activities of European NGOs  
addressing pesticide problems.

PAN Europe is affiliated to PAN Germany and PAN UK.

### Open letter for an EU-wide paraquat ban 08/04/03

To: The Member States' representatives of the Commission's Standing Committee on the Food Chain and Animal Health involved in examining active substances in plant protection products (Directive 91/414/EEC).  
From: Swedish Society for Nature Conservation (SNV), European Environmental Bureau (EEB), Pesticide Action Network Europe (PAN-E)

Date: 8 April 2003

Dear Mrs/Mr,

We are writing this letter because we are highly concerned about the on-going discussions to include the herbicide paraquat in Annex 1 to Council Directive 91/414/EEC at the next meeting of the Standing Committee on the Food Chain and Animal Health 14 - 15 April 2003.

2003

## Recent external pressures on paraquat quoting links with Parkinson's disease

### The Swedish government is suing the EU Commission

The Swedish government decided today to sue the EU Commission for their decision to approve paraquat in pesticides with the EU. The decision was made by the commission last December.

The suit means that the EU Court of Justice is trying the government's petition to nullify the commission's decision.

6th February 2004

2004

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## Recent external pressures on paraquat quoting links with Parkinson's disease

### Stockholm to seek ban on paraquat herbicides.

By NICHOLAS GEORGE

6 February 2004  
Financial Times

2004/02/06

## Research Activity at Syngenta CTL Strategy Being Followed

- Establish whether there is a sound scientific basis for the claims being made. Are the findings repeatable?
- If findings are not reproducible, aim to publicly refute the claims in the literature by offering our own alternative experimental findings.
- If findings are repeatable, Syngenta CTL generated data will be used to build a defensive position for PQ based on establishing a no effect dose (under various dosing regimens) in the C<sub>57</sub>Bl<sub>6</sub> mouse model, based on a biological endpoint - neuronal cell loss in the *substantia nigra*.
- Avoided measuring PQ levels in the brain, since the detection of any PQ in the brain (no matter how small) will not be perceived externally in a positive light.

2004/02/06

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## Research Activity at Syngenta CTL *in vivo* Studies (Louise Marks)

- Repeat of published *in vivo* experiments with PQ alone being dosed to C<sub>57</sub>Bl<sub>6</sub> mice.
- Established in CTL the MPTP mouse model as a positive control - 10 daily doses of 20 mg/kg MPTP i.p.
  - 70% reduction in striatal dopamine
  - 30% reduction in TH<sup>+</sup> neurones in the *substantia nigra*
- 3 weekly doses of PQ (10 mg/kg i.p.) to C<sub>57</sub>Bl<sub>6</sub> male mice with neurochemical (striatal dopamine levels) and neuropathological (TH<sup>+</sup> neurone counts in the *substantia nigra*) end point markers.
- Intend to seek peer review of our findings.

2004/02/06

## Research Activity at Syngenta CTL *in vitro* Studies (Alison Foster)

- Does PQ behave in a similar way to MPTP and its metabolite, MPP<sup>+</sup>, as implied in some of the recent literature concerning paraquat and Parkinson's disease?
- Investigated the potential for paraquat to interact with, and modulate, the nigrostriatal dopamine re-uptake system, and dopamine receptors in rat and mouse synaptosomes.
- Demonstrates clear differences between PQ and the mechanism of action of MPTP.

2004/02/06

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## *In vivo* Studies

- The optimisation of our positive control model for dopamin neurotoxicity- MPTP model
- Data from two dosing studies investigating the effect of paraquat administration on the nigrostriatal system of the C57 black mouse

21/05/2019

21/05/2019

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## Optimisation of the MPTP Model of Parkinson's Disease

- MPTP is a neurotoxic agent which produces effects similar to Parkinson's disease.
- MPTP produces pathology similar to that in PD and is a commonly used model of the disorder.
  - ↓ striatal dopamine
  - ↓ tyrosine hydroxylase containing neurons in the substantia nigra
  - ↓ locomotor activity
- Paraquat has been reported to be neurotoxic following systemic administration in rats and mice.
- Effects reported with paraquat are similar to those seen with MPTP.

Developing an MPTP model in CTL would allow investigation of paraquat neurotoxicity when compared to a known neurotoxic agent

21/05/2019

## Optimisation of MPTP model- Experimental Design

- 10 week old male C57BL/6 mice
- 5 daily injections of 20/22.5/25 mg/kg MPTP-HCl (n=3)
- controls received equal volumes of sterile saline
- Animals continuously monitored for 2 hours post dosing for signs of MPTP toxicity:
  - piloerection, increased salivation, increased lacrimation
  - increased or decreased activity, tremor, involuntary movement, abnormal gait respiratory changes
- 7 days after last MPTP dose, striatal and cortical samples removed for HPLC analysis of:
  - Dopamine (DA)
  - 3,4-dihydroxyphenyl acetic acid (DOPAC)
  - Homovanillic acid (HVA)
  - 5-Hydroxytryptamine (5-HT)

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## Summary of MPTP Dose Optimisation Studies

Study	Dosing Regimen	Route	Total dose (dose received mg/kg)	Well tolerated	Reduction in striatal DA (target >50%)
<b>XM7115</b>	5 x 20 mg/kg	i.p.	100	Yes	47%
	5 x 25 mg/kg	i.p.	125 (75)	No	50.5%
	5 x 22.5 mg/kg	i.p.	112.5 (45)	No	57.5%
<b>XM7143</b>	10 x 20 mg/kg	i.p.	200	Yes	52%
<b>XM7190</b>	10 x 20 mg/kg	i.p.	200	OK	66%

2008

## Optimisation of the MPTP Model of Parkinson's Disease

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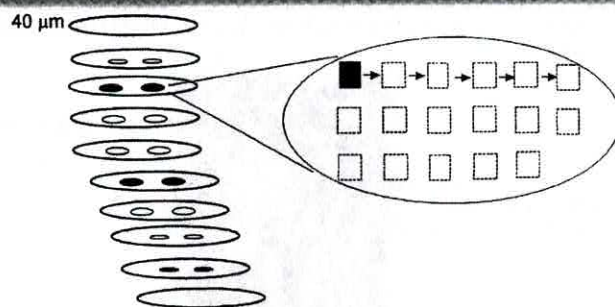
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## TH Immunohistochemistry - Methods

- Mid/hindbrain blocks immersion fixed in 4% PAM, cyoprotected and processed for immunohistochemistry
- 40µm coronal sections cut on freezing microtome
- Free floating immunohistochemistry was carried out using a tyrosine hydroxylase antibody (1:4000; Biogenesis) on sections containing s.nigra region
- Staining was visualised using 3,3'-diaminobenzidine- TH<sup>+</sup> cells brown colour
- Sections were counterstained using cresyl violet- nucleus purple colour. This allowed easier identification of TH<sup>+</sup> dopaminergic neurons in SNpc.
- Sections adjacent to those used for TH staining were stained with cresyl violet for total cell counts

2008

## Counting neurons- Optical Fractionator method of stereology



Counts neurons from a representative number of sections then uses mathematical reconstruction to acquire an estimate of the total number of cells in each animal

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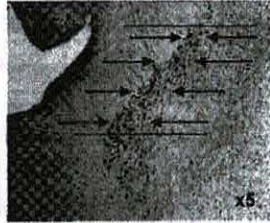
## TH Cell Counts - Methods

- Most groups have used optical fractionator method of stereology to count TH<sup>+</sup> cells:

*'... unbiased technique independent of neuronal shape or size or any conformational changes in the tissue ...'*

- Method involves counting TH<sup>+</sup> neurons from a representative number of sections (e.g. every 3rd section) throughout the SNpc region in each animal

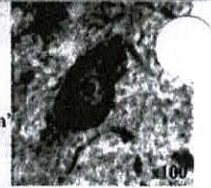
- Step 1- The pars compacta of S. nigra is identified at low magnification (X5) (Fig 1)



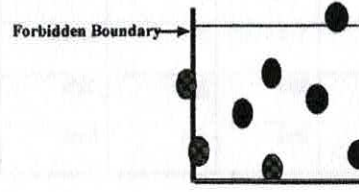
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## TH Cell Counts - Methods 2

- Step 2- A counting frame is superimposed onto s. nigra at X100 magnification and TH<sup>+</sup> neurons with a cresyl violet stained nucleus are counted (Fig 2)



- Cells lying beyond the frame or cells touching the 'forbidden' boundary line are not included in the counts



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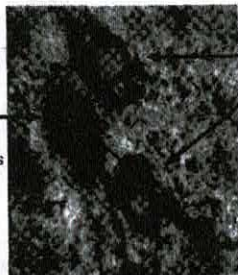
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## TH Cell Counts - Methods 3

- Step 3- Cells are counted from a 10µm thickness of the section (not including the top 2 µm )

Cell becomes visible as  
move through 10µm thickness



Cells visible initially

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## TH Cell Counts - Methods 4

- Step 4

From these counts, the optical fractionator method can be used to estimate the total number of TH<sup>+</sup> neurons in the pars compacta of each animal:

$$N = \sum Q^- \times 1/tsf \times 1/asf \times 1/ssf$$

Where:

tsf- thickness sampling fraction

asf- area sampling fraction

ssf- section sampling fraction

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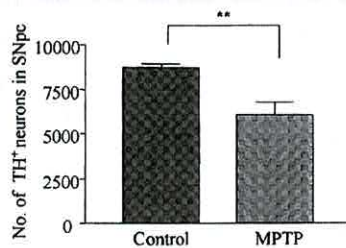
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## Study XM7190 - Effect of MPTP on TH<sup>+</sup> cell number in the Substantia Nigra pars compacta



Data represents mean  $\pm$  SEM. Saline control group received 3 weekly i.p. injections of Sterile saline, n=6; MPTP group received 10 daily injections of 20mg/kg MPTP, n=7. Values analysed by one way ANOVA followed by Student's t-test. \*\*p<0.01

2000

## Reductions in TH reported with Paraquat

- Thiruchelvam *et al.*, 2000- reported a 22% reduction in TH cell number with combined PQ + Mb (6 twice weekly injections of 10mg/kg PQ + 30mg/kg Mb)
- McCormack *et al.*, 2002- reported a 32% reduction in TH cell number following 3 weekly injections of 10mg/kg PQ
- Thiruchelvam *et al.*, 2002- around a 25% reduction in TH cell number following adult exposure to PQ
- McCormack & DiMonte, 2003- reported a 25% reduction in TH cell number following 3 weekly injections of 10mg/kg PQ
- XM7190 produced a 30% reduction in TH cell number following MPTP administration. This has demonstrated that the sensitivity of the endpoint should be sufficient to allow detection of any PQ induced changes in TH cell number of the magnitude previously reported.**

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## Investigation of Paraquat Neurotoxicity – Experimental Design

- 10 week old male C57Bl<sub>6</sub> mice received 3 weekly injections of 10 mg/kg PQ dichloride
- Mice were killed 7 days after the final injection and brains removed on ice
- Forebrain- striata removed for HPLC analysis of striatal DA and metabolites
- Mid/hindbrain- immersion fixed in 4% PAM and 40 $\mu$ m sections cut for TH IHC and for cresyl violet staining
- The optical fractionator method of stereology was used to estimate the numbers of TH<sup>+</sup> and CV<sup>+</sup> neurons in the SNpc to determine whether PQ has the ability to reduce the number of dopaminergic neurons in the pars compacta

2000

## XM7229-Effect of Paraquat on the concentration of Striatal Dopamine and its metabolites

200 mg/kg total dose MPTP

Slight-moderate toxicity.

67% reduction in striatal DA

50% reduction in DOPAC

30% reduction in HVA

DA turnover 153% control

no change in 5-HT

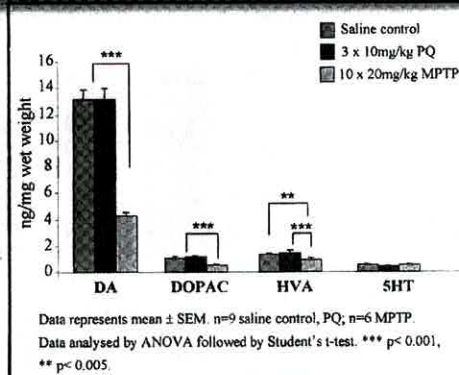
30mg/kg total dose PQ dichloride.

No signs of toxicity

No sig. reduction in striatal DA,

DOPAC or HVA.

No sig. change in DA turnover



2000

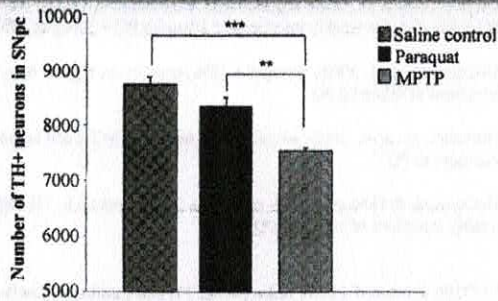
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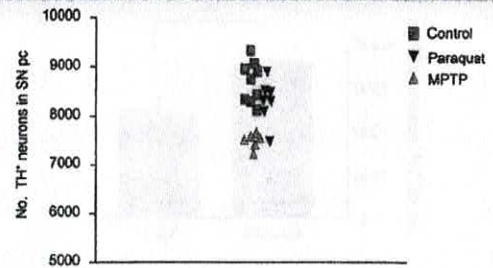
# Study XM7229 - Effect of paraquat on TH<sup>+</sup> cell number in the Substantia Nigra pars compacta



Data represents mean ± SEM. Saline control group received 3 weekly i.p. injections of Sterile saline, n=9; PQ group received 3 weekly injections of 10mg/kg PQ dichloride, n=7; MPTP group received 10 daily injections of 20mg/kg MPTP, n=6. Values analysed by one way ANOVA followed by Newman-Keuls post test. \*\*\*p<0.001 \*\*p<0.01

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# Study XM7229 - Effect of paraquat on TH<sup>+</sup> cell number in the Substantia Nigra pars compacta



Saline control group received 3 weekly i.p. injections of sterile saline, n=9; PQ group received 3 weekly injections of 10mg/kg PQ dichloride, n=7; MPTP group received 10 daily injections of 20 mg/kg MPTP, n=6.

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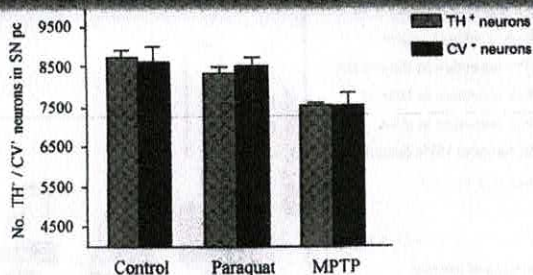
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# Study XM7229 - Effect of paraquat on TH<sup>+</sup> and CV<sup>+</sup> cell number in the substantia nigra pars compacta



Data represents mean ± SEM. Saline control group received 3 weekly i.p. injections of Sterile saline, n=9,8; PQ group received 3 weekly injections of 10mg/kg PQ dichloride, n=7,7; MPTP group received 10 daily injections of 20mg/kg MPTP, n=6,6.

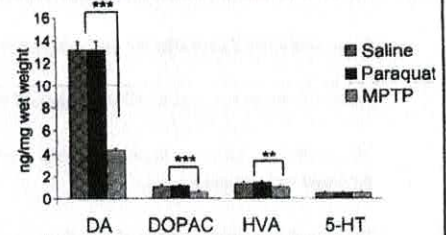
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# XM7258-Effect of Paraquat on concentration of Striatal Dopamine and its metabolites

20.0 mg/kg paraquat (PQ) dichloride (MPTP)  
Slight-moderate toxicity (TAPk)  
80% reduction in striatal DA  
66% reduction in DOPAC  
46% reduction in HVA  
DA turnover 194% of control  
no change in 5-HT

30 mg/kg total dose PQ dichloride

No signs of toxicity  
No sig. reduction in striatal DA, DOPAC or HVA.  
No sig. change in DA turnover



Data represent mean ± SEM. Saline control, n=9; PQ dichloride, n=9; MPTP, n=8. Data analysed using one way ANOVA followed by Student's t-test, \*\*\* p<0.0001, \*\* P<0.05

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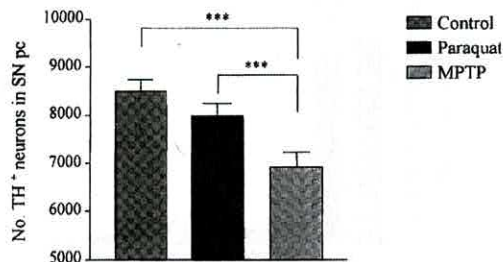
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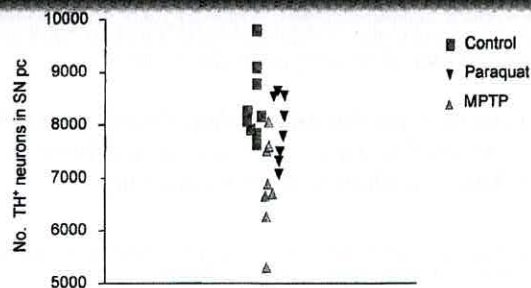
# Study XM7258 - Effect of paraquat on TH<sup>+</sup> cell number in the Substantia Nigra pars compacta



Data represents mean ± SEM. Saline control group received 3 weekly i.p. injections of Sterile saline, n=9; PQ group received 3 weekly injections of 10mg/kg PQ dichloride, n=8; MPTP group received 10 daily injections of 20mg/kg MPTP, n=9. Values analysed by one way ANOVA followed by Newman-Keuls post test \*\*\*p<0.001

2000000

# Study XM7258 - Effect of paraquat on TH<sup>+</sup> cell number in the Substantia Nigra pars compacta



Data represents individual animal values. Saline control group received 3 weekly i.p. injections of sterile saline, n=9; PQ group received 3 weekly injections of 10 mg/kg PQ dichloride, n=8; MPTP group received 10 daily injections of 20mg/kg MPTP, n=8.

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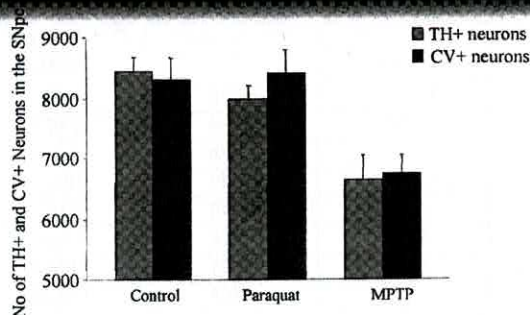
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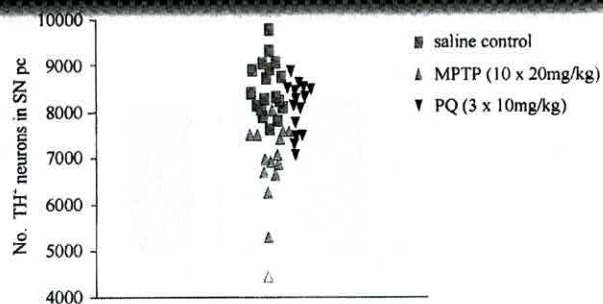
# Study XM7258 - Effect of paraquat on CV<sup>+</sup> cell number in the Substantia Nigra pars compacta



Data represents mean ± SEM. Saline control group received 3 weekly i.p. injections of Sterile saline, n=9,7; PQ group received 3 weekly injections of 10 mg/kg PQ dichloride, n=8,8; MPTP group received 10 daily injections of 20mg/kg MPTP, n=9,8.

2000000

# Study XM7229 + XM7258 combined- Effect of paraquat on TH<sup>+</sup> cell number in the Substantia Nigra pars compacta



Data represents individual animal values from studies XM7229 and XM7258. Saline control group received 3 weekly i.p. injections of sterile saline, n=18; PQ group received 3 weekly injections of 10 mg/kg PQ dichloride, n=15; MPTP group received 10 daily injections of 20 mg/kg MPTP, n=14 and one MPTP animal received 3 daily injections of 20 mg/kg MPTP (Δ).

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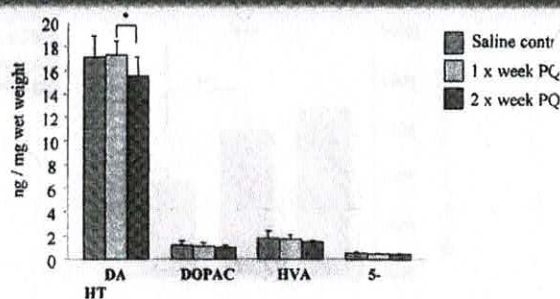
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## Summary and Conclusions

- In two separate studies, XM7229 and XM7258 we have failed to reproduce the PQ induced neurotoxicity reported in the literature.
  - The two studies have demonstrated a lack of PQ neurotoxicity in mice from two suppliers – suggests that subpopulation differences did not contribute to our initial lack of PQ induced toxicity.
- ↓
- Attempt to understand areas where differences may exist between our studies and the groups reporting PQ toxicity.
  - Continue to investigate this reported toxicity- increase the frequency of dosing to determine whether this might induce changes to the nigrostriatal system.
  - Addition of a behavioural endpoint- measurement of locomotor activity.

21080000

## Study XM7371 - Effect of Paraquat on the Concentration of Striatal Dopamine and its Metabolites



Data represents mean  $\pm$  SD. Data analysed using one way ANOVA followed by Student's t-test, \*  $p < 0.05$ . Control,  $n=8$ ; 1 x week PQ animals received 3 weekly injections of 10 mg/kg paraquat dichloride,  $n=9$ ; 2 x week animals received 10 mg/kg paraquat dichloride twice a week for 3 weeks,  $n=8$ .

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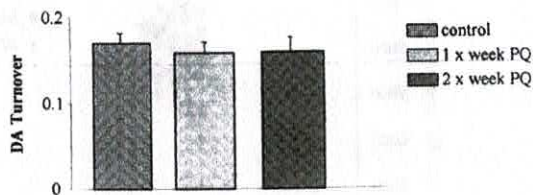
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## Study XM7371 - Effect of Paraquat on Dopamine Turnover in the Striatum



Data represents mean  $\pm$  SEM. Data analysed using one way ANOVA followed by Student's t-test. Control,  $n=8$ ; 1 x week PQ animals received 3 weekly injections of 10 mg/kg paraquat dichloride,  $n=9$ ; 2 x week animals received 10 mg/kg paraquat dichloride twice a week for 3 weeks,  $n=8$ .

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## XM7371- Effect of paraquat on TH<sup>+</sup> cell number in the SNpc

Saline Control	PQ 1 x week	PQ 2 x week
11480	7239	8183
9425	8336	9223
11518	8498	10627
12314	11406	9412
11662	9969	10415
11242	9240	9247
12621	7366	11257
12363	8791	10553
	9568	10426
Mean:		
11578 $\pm$ 354 SEM / 1001 SD	8935 $\pm$ 434 SEM / 1303 SD (23% ↓)	9927 $\pm$ 321 SEM / 962 SD (14% ↓)

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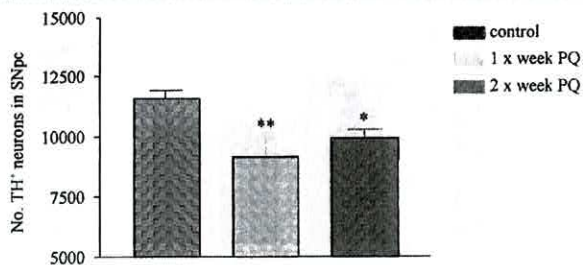
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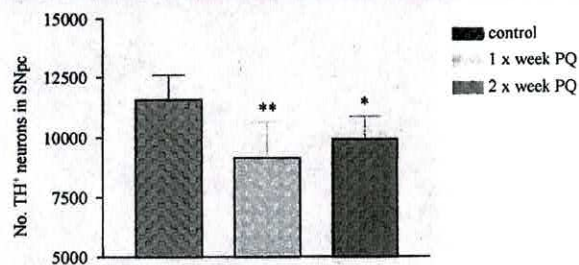
# Study XM7371 - Effect of Paraquat on TH<sup>+</sup> Cell Number in the Substantia Nigra Pars Compacta



Data represents mean ± SEM. Saline control group received 3 weekly i.p. injections of Sterile saline, n=8; PQ 1 x week group received 3 weekly injections of 10mg/kg PQ dichloride, n=9; PQ 2 x week group received injections of 10mg/kg PQ dichloride twice weekly over 3 weeks, n=9. Values analysed by one way ANOVA followed by Newman-Keuls post test. \*\*p<0.001 \*p<0.01

2000000000

# Study XM7371 - Effect of Paraquat on TH<sup>+</sup> Cell Number in the Substantia Nigra Pars Compacta



Data represents mean ± SD. Saline control group received 3 weekly i.p. injections of Sterile saline, n=8; PQ 1 x week group received 3 weekly injections of 10mg/kg PQ dichloride, n=9; PQ 2 x week group received injections of 10mg/kg PQ dichloride twice weekly over 3 weeks, n=9. Values analysed by one way ANOVA followed by Newman-Keuls post test. \*\*p<0.001 \*p<0.01

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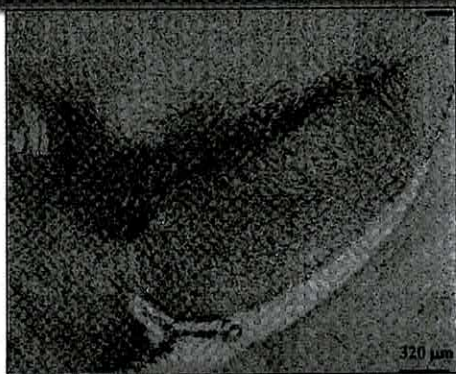
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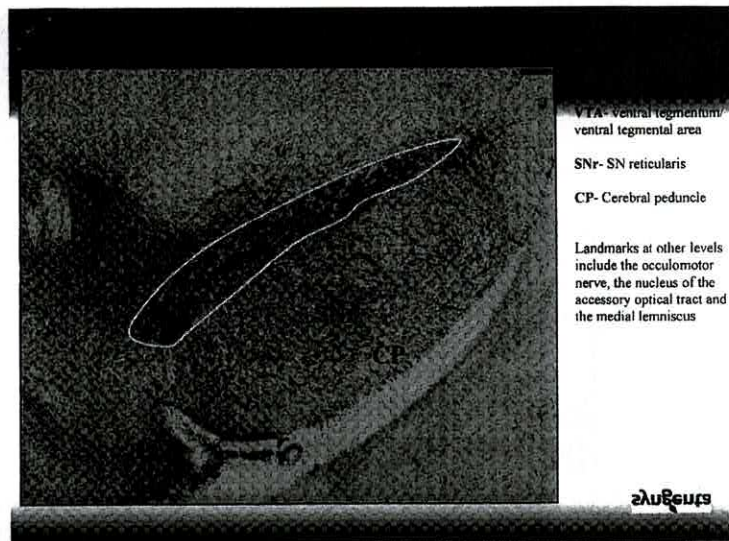
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## TH<sup>+</sup> Cells in the Substantia Nigra



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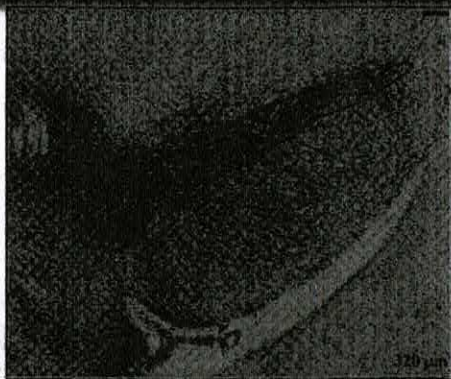
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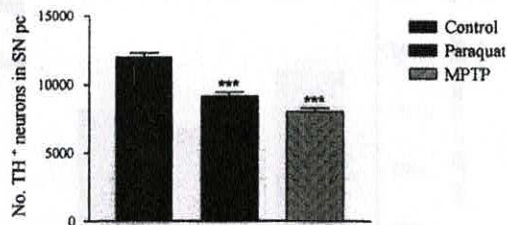
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# Delineation of TH<sup>+</sup> Cells in the Substantia Nigra Pars Compacta



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# XM7258 - Effect of paraquat and MPTP on TH<sup>+</sup> cell number in the substantia nigra pars compacta



Data represents mean  $\pm$  SEM. Saline control group received 3 weekly i.p. injections of sterile saline, n=9, PQ group received 3 weekly injections of 10 mg/kg PQ dichloride, n=9, MPTP group received 10 daily injections of 20 mg/kg MPTP, n=9. Values analysed by one way ANOVA followed by Newman-Keuls post test \*\*\*p<0.001

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# XM7258 - Effect of paraquat and MPTP on TH<sup>+</sup> cell number in the substantia nigra pars compacta

Saline Control	Paraquat	MPTP
12480	8882	6986
11789	7966	7144
11887	9437	9179
11595	10360	8364
12766	8468	8840
11499	9746	8647
13470	8916	7594
10126	10231	7756
12374	8392	7591
Mean: 11998 $\pm$ 314 SEM / 942 SD	9155 $\pm$ 280 SEM / 840 SD (24% ↓)	8011 $\pm$ 258 SEM / 774 SD (33% ↓)

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# XM7258 - Effect of paraquat and MPTP on CV<sup>+</sup> cell number in the substantia nigra pars compacta

Saline Control	Paraquat	MPTP
17460	11638	9871
16957	12551	8818
14504	11240	8760
14511	10452	9435
17398	13506	12882
16106	10841	14493
17642	12118	11509
12840	13569	10248
	13187	13301
Mean: 15927 $\pm$ 628 SEM / 1777 SD	12122 $\pm$ 387 SEM / 1160 SD (24% ↓)	11035 $\pm$ 701 SEM / 2102 SD (31% ↓)

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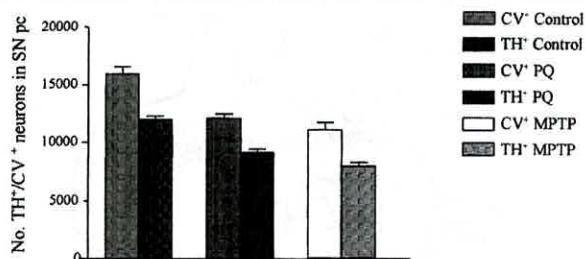
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SYNG-PQ-00493373



## XM7258 - Effect of paraquat and MPTP on TH<sup>+</sup> & CV<sup>+</sup> cell number in the substantia nigra pars compacta



Data represents mean ± SEM. Saline control group received 3 weekly i.p. injections of sterile saline, n= 8 (CV), 9 (TH); PQ group received 3 weekly injections of 10 mg/kg PQ dichloride, n=9; MPTP group received 10 daily injections of 20 mg/kg MPTP, n=9.

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## XM7480 - Effect of paraquat on cell number in the SNpc

	7 Day				28 day				90 day			
	Control		PQ		Control		PQ		Control		PQ	
			Diff.	% ↓			Diff.	% ↓			Diff.	% ↓
TH <sup>+</sup>	11430 ± 314	8723 ± 268**	-2707	23.7	8927 ± 222	7087 ± 280**	-2330	23.5	6364 ± 188	6076 ± 104**	-1518	18.1
CV <sup>+</sup>	15484 ± 364	12474 ± 435**	-3020	19.5	14163 ± 574	11255 ± 551**	-2698	20.5	12927 ± 332	11846 ± 169*	-1081	8.4
TH <sup>+</sup>	4084 ± 580	3743 ± 486	-313		4227 ± 505	3657 ± 404	-570		4533 ± 286	4970 ± 219	+437	

Animals terminated 7, 28 or 90 days after the final PQ injection.

Data represents mean ± SEM. Control group received 3 weekly injections of sterile saline, n= 8 (7d), n=9 (28d, 90d). PQ group received 3 weekly injections of 10 mg/kg PQ dichloride, n=9 (7d, 28d), n=8 (90d). Data analysed using one way ANOVA followed by Newman-Keuls post test, \*\* p < 0.001, \* p < 0.01.

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## The Effect of Paraquat on the Nigrostriatal Dopaminergic System in the Rat and Mouse

### An *In Vitro* Study

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## Aim of the *in vitro* experiments

- Investigate the potential for paraquat to interact with, and modulate, the nigrostriatal pre-synaptic dopamine re-uptake system and post-synaptic dopamine receptors in rat and mouse synaptosomes.
- Does paraquat behave in a similar way to MPTP (MPP<sup>+</sup>), as implied in some of the recent literature concerning paraquat and Parkinson's disease?
- Compare the effects of paraquat with those of the "structurally related" compound MPTP, and its metabolite, MPP<sup>+</sup> *in vitro*.

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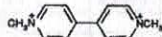
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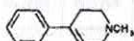
## Paraquat and MPTP

### Paraquat



Charged molecule  
Polar (water soluble) – poor BBB permeability  
Diamine – unlikely to be a substrate for monoamine transporter  
Not Metabolised  
Does not inhibit complex 1 ETC in mitochondria

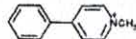
### MPTP



Uncharged molecule  
Lipophilic  
Crosses the BBB  
Metabolised

MAO-B

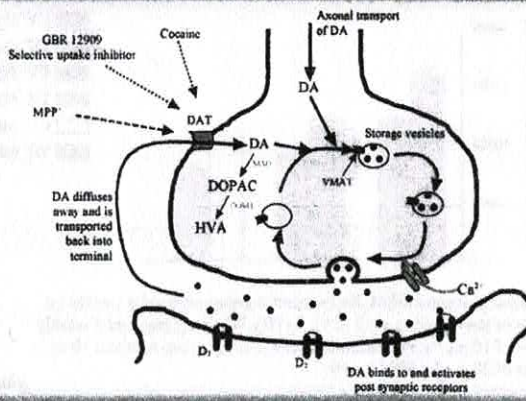
### MPP<sup>+</sup>



Neurotoxic metabolite  
Monoamine  
Does not cross BBB  
Inhibits complex 1 ETC  
Accumulated by DAT

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## Dopaminergic neurone terminal



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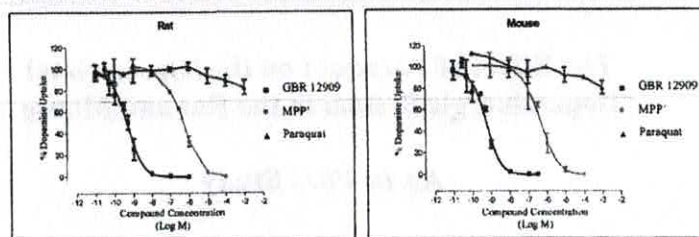
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## [<sup>3</sup>H]-dopamine re-uptake assay

- Striata from rats and mice were excised, homogenised and synaptosomes (fractions of broken nerve endings which represent pre-synaptic nerve terminals) isolated by differential centrifugation.
- The synaptosomes were pre-incubated with test compound for 30 min at 30 °C prior to the addition of [<sup>3</sup>H]-dopamine. After 8 minutes the reaction was terminated by rapid filtration using the Brandel Harvester.
- The uptake of [<sup>3</sup>H]-dopamine into the synaptosomes retained on the filter was then determined by scintillation counting.

21065075

## The effect of Paraquat, MPTP and MPP<sup>+</sup> on dopamine uptake in rat and mouse striatal synaptosomes *in vitro*



### Rat IC<sub>50</sub> values

GBR 12909 0.46 ± 0.15 nM  
MPP<sup>+</sup> 0.51 ± 0.04 μM  
PQ > 1,000 μM

### Mouse IC<sub>50</sub> values

GBR 12909 0.48 ± 0.16 nM  
MPP<sup>+</sup> 0.56 ± 0.16 μM  
PQ > 1,000 μM

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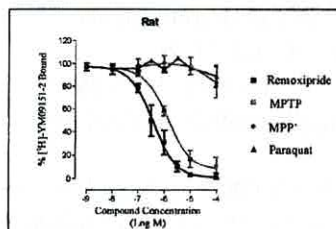


## Competitive binding assays

- Measure the ability of an unlabeled compound (e.g. paraquat) to compete with a radiolabelled ligand which selectively binds to a given receptor.
- Radioligands, selective for the dopamine transport system (DAT) and the dopamine D<sub>1</sub> and D<sub>2</sub> receptors, were used to determine the affinity of paraquat, MPTP and MPP<sup>+</sup> for these respective receptors.
- Striata from rats and mice were excised, homogenised and then the membrane preparations were isolated by centrifugation prior to use in competitive binding assays.
- The membranes were incubated with a fixed concentration of radiolabelled ligand, and a range of concentrations of test compound, for 1 hour to reach equilibrium. The reaction was then terminated by rapid filtration using the Brandel Harvester, followed by several washes to remove any unbound radiolabel and test compound.
- The binding of the radioligand to the membrane receptors retained on the filters was then quantified by scintillation counting, and the binding affinity (K<sub>i</sub>) calculated.

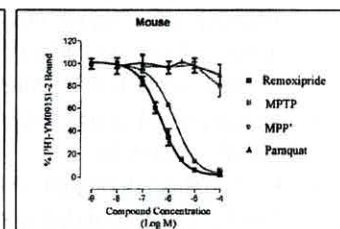
21085012

## The effect of Paraquat, MPTP and MPP<sup>+</sup> on [<sup>3</sup>H]-YM-09151-2 binding to the dopamine D<sub>2</sub> receptor in rat and mouse striatal membranes *in vitro*



### Rat K<sub>i</sub> values

Remoxipride 130 ± 70 nM  
MPP<sup>+</sup> > 100 μM  
MPTP 0.47 ± 0.09 μM  
PQ > 100 μM



### Mouse K<sub>i</sub> values

Remoxipride 130 ± 30 nM  
MPP<sup>+</sup> > 100 μM  
MPTP 0.41 ± 0.09 μM  
PQ > 100 μM

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## Summary of the binding data for the D<sub>1</sub> and D<sub>2</sub> receptors and the DA transport site in the rat and mouse

	D <sub>1</sub> receptor K <sub>i</sub>	D <sub>2</sub> receptor K <sub>i</sub>	DAT K <sub>i</sub>
Paraquat Rat	>100 μM	>100 μM	>100 μM
Paraquat Mouse	>100 μM	>100 μM	>100 μM
MPTP Rat	>50 μM	0.47 ± 0.09 μM	>50 μM
MPTP Mouse	>50 μM	0.41 ± 0.09 μM	>100 μM
MPP <sup>+</sup> Rat	>100 μM	>100 μM	>100 μM
MPP <sup>+</sup> Mouse	>100 μM	>100 μM	>100 μM
Positive Controls	Butaclamol	Remoxipride	GBR 12909
Rat	7.2 ± 2.9 nM	130 ± 70 nM	4.3 ± 1.7 nM
Mouse	9.5 ± 3.0 nM	130 ± 30 nM	5.0 ± 0.6 nM

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## Conclusion

PQ may have apparent structural similarity to MPP<sup>+</sup> however:

- PQ does not act as a substrate for the dopamine transporter (DAT)
- PQ shows no binding affinity for DAT
- PQ shows no binding affinity for the dopamine D<sub>1</sub> and D<sub>2</sub> receptors

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## Is The Incidence of Parkinson's Disease Increasing?

- If Parkinson's disease is caused by general exposure to pesticides one might expect to see an increase in the incidence of the disease over the last 50 years.
- Is the incidence of Parkinson's disease on the increase?
- Incidence in EU and US is approximately 16 to 19 per 100,000 per year.
- Few studies investigating any change in incidence with time.
- The Mayo Clinic database from Olmsted County, Minnesota has the world's oldest database on Parkinson's disease - monitoring area residents since 1935.
- It shows no long term trends in the incidence of Parkinson's disease. Therefore any influence pesticides may have on the development of PD must be small.

## Paraquat & Parkinson's Disease

"But before throwing away your pesticides remember Parkinson's disease existed before pesticides were manufactured"

Abraham Lieberman MD

(National Parkinson Foundation USA website)

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## Human Exposure to Paraquat & Parkinson's Disease

- Human exposure to Paraquat by the general population is minimal - people don't eat weeds!
- No significant residues of paraquat are found in food or water. In cases where it has been used as a desiccant (grain crops), residue levels are very low and do not represent a significant source of exposure.
- Greatest exposure is to spray workers (dermal contact), or by oral ingestion (accidental or intentional).

## Human Exposure to Paraquat & Parkinson's Disease

- Human data from paraquat poisoning cases (although very limited numbers of individuals) provides further evidence that paraquat is not neurotoxic.
- Neuropathological examination of 15 human brains from acute paraquat poisoning deaths in Malaysia, revealed no significant quantitative loss of neurones in the *substantia nigra* (Wong *et al*, 1997).
- Neurological follow-up, 5-10 years post exposure, in 4 survivors of paraquat poisoning and 3 with paraquat skin contact, excluded Parkinsonism in all patients.

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