Parkinson's disease

- Parkinson's disease is a neurodegenerative disease caused by dopamine deficiency in the *striatum* and loss of pigmented dopaminergic neurones in the *substantia nigra* (mainly the *pars compacta*).
- These brain regions play an important role in mammalian movement control and motor co-ordination.
- Resulting symptoms include tremors, muscle rigidity, involuntary movements and postural changes.
- 70-80% loss of striatal dopamine before symptoms occur.
- Pathological hallmark - presence of Lewy bodies (protein aggregates) in surviving neurones of the *substantia nigra* detected at autopsy.

Paraquat & Parkinson's disease

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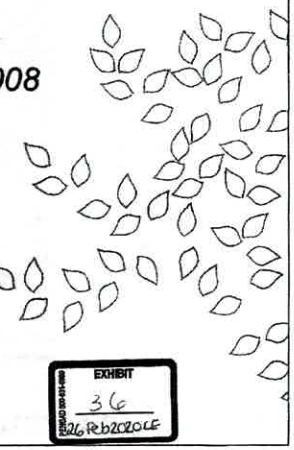
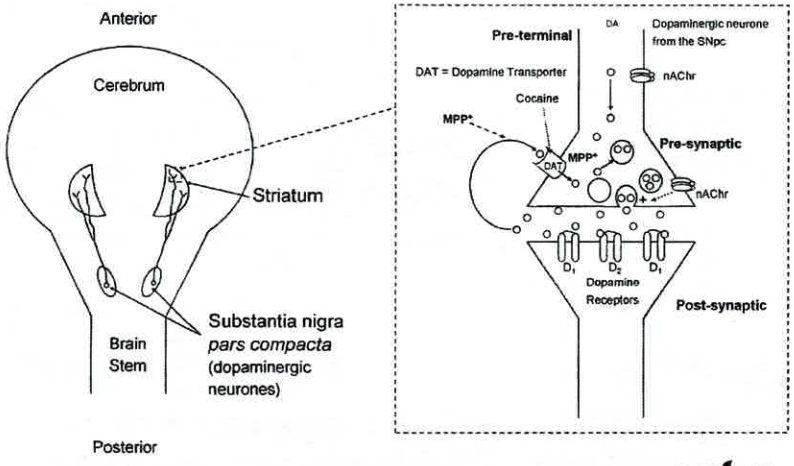
Atlanta meeting, Feb 13th-14th 2008



Nigrostriatal Dopaminergic System



Parkinson's disease

AN
ESSAY
ON THE
SHAKING PALSY.

CHAPTER I.

DEFINITION–HISTORY–ILLUSTRATIVE CASES.

SHAKING PALSY. (*Paralysis Agitans.*)

Involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forward, and to pass from a walking to a running pace: the senses and intellects being uninjured.

First described in 1817 by the London physician, **James Parkinson** in his *"Essay on the shaking palsy"*.

Possible "Environmental" Risk Factors Associated With Parkinson's Disease

Claims of Increased Risk

Exposure to toxins
- Pesticides
- Consumption of well water
- Pollution from industrial plants (metals e.g. Mn)
- Diet - consumption of >7 portions of tropical fruit daily

Trauma
- Head injury (boxing)

Infection
- Bacterial (*Nocardia asteroides*)

Decreased Risk

Cigarette smoking
- Nicotine effects? (nicotinic receptors)

Coffee drinking
- Caffeine effects? (adenosine A_2 antagonist)

Parkinson's Disease

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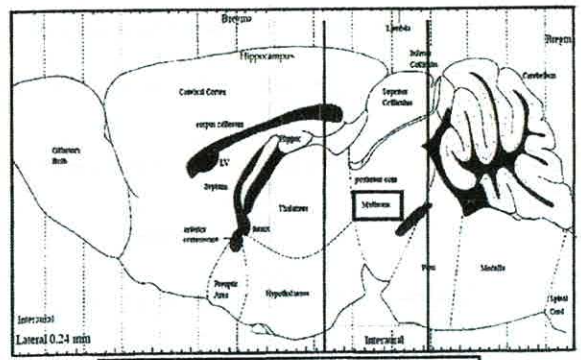

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Paraquat & Parkinson's Disease

Why have pesticides been linked to Parkinson's Disease?

Some epidemiological studies have implicated pesticides in the aetiology of Parkinson's disease.

- Barbeau *et al,* (1986) investigated the role of pesticides in Parkinson's in different geographical regions in Quebec, Canada. Regional prevalence of PD was found to be non-uniform and correlated with areas of pesticide use.

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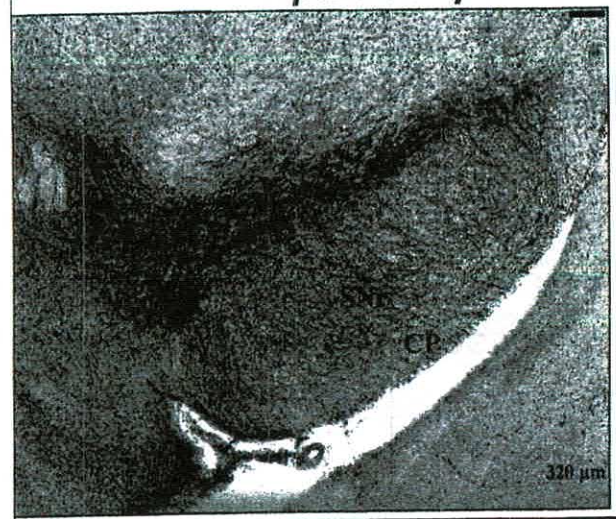
TH^+ Cells In The Substantia Nigra *pars compacta*



VTA- ventral tegmentum/ ventral tegmental area

SNr- SN reticularis

CP- Cerebral peduncle

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The MPTP Story

- In 1976 the first recorded human case of toxin-induced parkinsonism occurred in a chemistry student (Barry Kidston) from Bethesda, Maryland.
- Used a home laboratory to make analogues of the analgesic meperidine for his own recreational use.
- Changed the synthetic route conditions and unknowingly contaminated his MPPP with MPTP.
- Upon i.v. injection he developed severe parkinsonism within 3 days - unable to move or speak - responded to L-DOPA treatment.
- Summer of 1982 - six more cases observed in Californian heroin addicts. Contamination of heroin with MPTP.

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Paraquat & Parkinson's Disease

Why has paraquat been linked to Parkinson's Disease?

Apparent structural similarity to compounds MPTP & MPP^+

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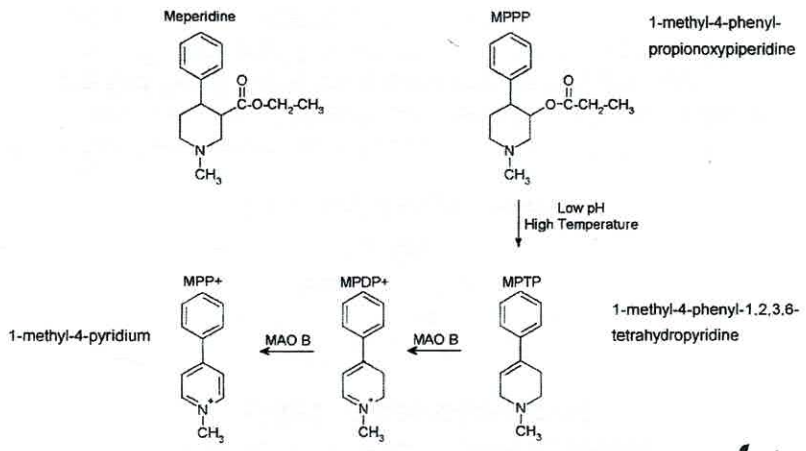
Conversion of MPPP to MPTP & MPP^+



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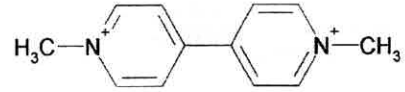
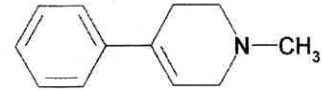
Apparent Structural Similarity Of Paraquat With MPTP & MPP^+

Paraquat



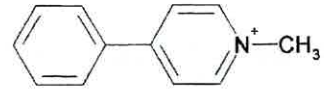
Hydrophilic, charged di-cation

MPTP



Monoamine, uncharged lipophilic molecule

MPP^+



Charged, neurotoxic metabolite of MPTP

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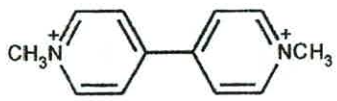
Paraquat and neurotoxicity (historical position)



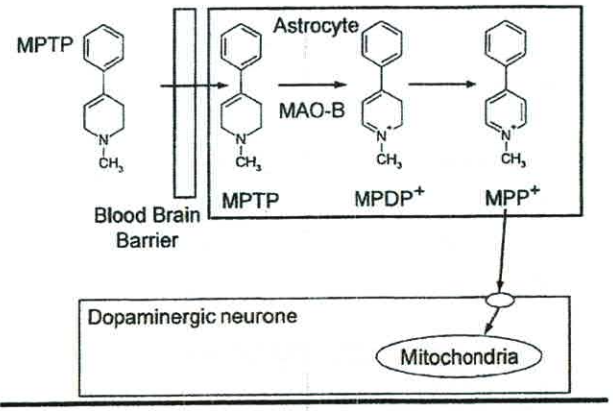
Paraquat

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

- It has been considered that paraquat is unlikely to be neurotoxic (unless directly injected into the brain) 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 would not be an obvious substrate for neuronal monoamine transport systems in the CNS.

Mechanism of MPTP Toxicity

Metabolic activation and mechanism of toxicity of MPTP



Does Paraquat cross the BBB and cause neurotoxicity?

- Long term Syngenta regulatory studies with paraquat, involving oral or dietary administration to rats and dogs, show no clear signs of neurotoxicity or neuropathology.
- Oral dosing of paraquat to rats at 5 mg/kg/day for 14 days (Widdowson *et. al.*, 1996) produced no evidence of neuronal damage or behavioural changes indicative of neurotoxicity.
- [^{14}C]-PQ dosing studies with rats and mice (Naylor *et al*, 1995; Widdowson *et al*, 1996) reveal that PQ can get into the brain, but only to a very limited extent (<0.05% of total dose).
- Concluded that in the rat & dog (primary toxicology species), there was no evidence to indicate that PQ is neurotoxic via relevant routes of exposure.

MPTP & Use In Animal Models of Parkinson's disease

- **Toxicity:** Human >NHP >Mouse >Rat
- Pigmented mouse ($C_{57}Bl_6$) used as an animal model for PD.
- Use of non-human primates (marmosets & macaques) can do good behavioural studies and considered closest to humans.

Recent Literature Developments

- 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 $C_{57}Bl_6$ 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

Paraquat & Parkinson's Disease

Developments in the scientific literature

Summary of findings from PQ mouse model

Primarily (although not exclusively) derived from the findings reported by the Di Monte group (Parkinson's Institute)

- $C_{57}Bl_{6J}$ mouse; 8-12 weeks old; male
- i.p. dosing of 10 mg/kg PQ (dichloride salt) once a week for 3 weeks (3 x 10 mg/kg)
- Primary endpoint TH^+ neuronal cell counts in the SNpc
- Striatal dopamine (& metabolites); behavioural (locomotor activity)
- 25 - 30% loss of TH^+ neurones
- TH^- neurones not affected

Early Literature Developments

- Fredriksson *et. al.*, (1993) Eriksson's group (Uppsala). Two oral doses of PQ (0.36 mg/kg at days 10 & 11) to young $C_{57}Bl_6$ mice.
  - behavioural effects observed at 60 days
  - corresponding reductions in striatal dopamine content
- Syngenta (Zeneca) funded follow up work conducted to see if findings were repeatable - no conclusive effects - not reported because negative data.

Summary of findings from PQ + maneb mouse model

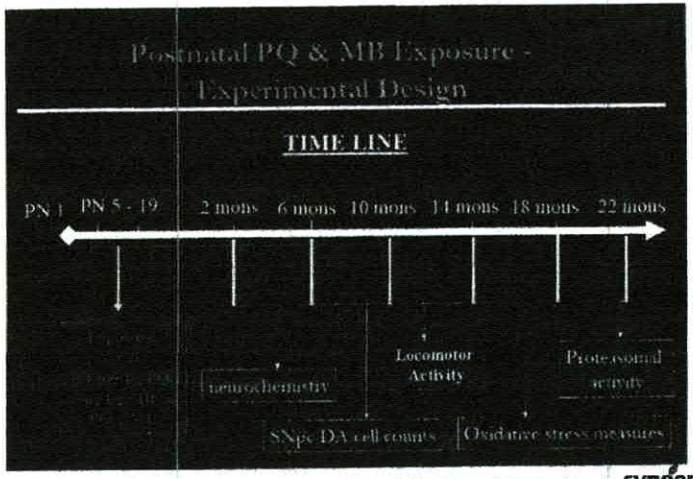
Cory-Slechta group

- Combination experiments with i.p. PQ (10 mg/kg) & the fungicide maneb (30 mg/kg).
- Neuronal cell loss greater in mice exposed to PQ + maneb compared to just PQ or maneb alone.
- Older mice (18 month old) exposed to PQ, or PQ and maneb are more susceptible than those of 6 wks or 5 months of age.
- Examined the effects of developmental exposure to paraquat and maneb by exposing as neonates (PN5-19; 0.3 mg/kg PQ & 1 mg/kg maneb i.p. route) or as fetus (GD10-17; s.c. via the dam) and subsequent re-exposure as adults (i.p. route).
- Lower doses (0.1 mg/kg PQ) reported effects at 22 months.

Summary of findings from PQ mouse model

- No significant effect on striatal dopamine concentration
- No significant behavioural deficits, although some groups report reduced locomotor activity
- Reduction in neuronal cell counts observed only in SNpc, not in other dopaminergic brain areas e.g. VTA & hippocampus
- Dose response - 1, 5 & 10 mg/kg reduced by 8, 18 & 27% respectively
- Pre-dosing with L-dopa or L-valine is neuroprotective
- Increasing frequency of dosing does not increase the magnitude of cell loss (remains at 25-30%)

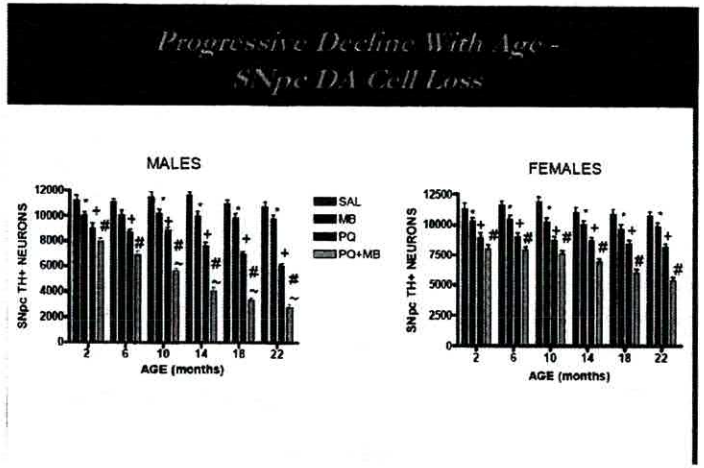
Paraquat mouse model (Cory-Slechta group)



Summary of findings from PQ mouse model

- Most of neuronal cell loss occurs following the 2nd dose. Very little cell loss after 1st dose. No additional loss following 3rd dose.
- Some groups claim the neuronal cell loss is progressive over a period of 3 or more months.
- Very old (>18 months) and very young (<6 weeks) are more susceptible.
- Effect is reported to be gender specific (according to Cory-Slechta group) - males only.
- NHP work - paraquat has been dosed to NHP's but no effect on neuronal cell numbers yet reported.

Paraquat mouse model (Cory-Slechta group)



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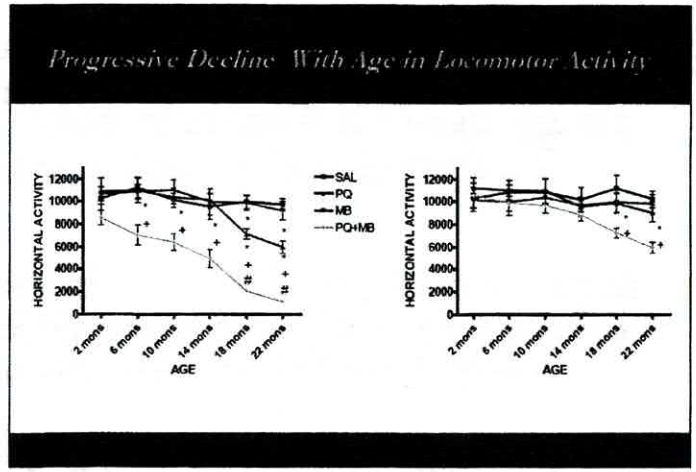
Paraquat mouse model (Cory-Slechta group)



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Summary of literature findings from the PQ rat model

Rat Studies

- Work relating to paraquat & PD has used the mouse model.
- Recent studies reported in the rat (Wistar) - *Ossowska et al, (2005) Krakow, Poland.*
- PQ dosed i.p. 10 mg/kg / week for 4-24 weeks.
- After 4 weeks no significant reduction in TH^+ neurones.
- After 24 weeks 37% reduction in TH^+ neurones, dopamine decreased by 30% and decreased TH activity.
- Rat appears to be less sensitive than the mouse with respect to nigrostriatal toxicity, although findings need confirmation.

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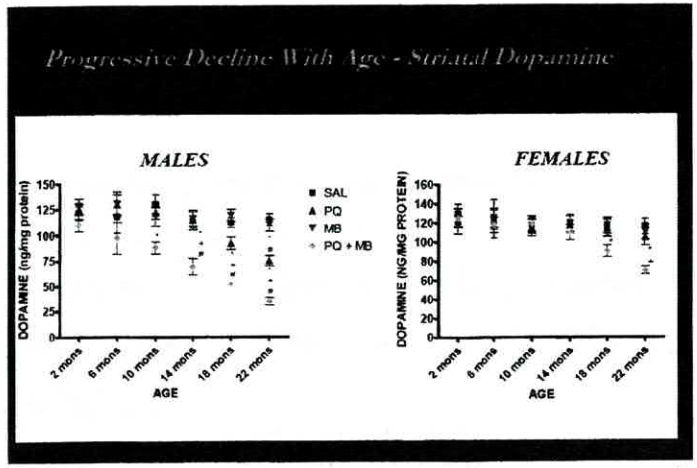
Paraquat mouse model (Cory-Slechta group)



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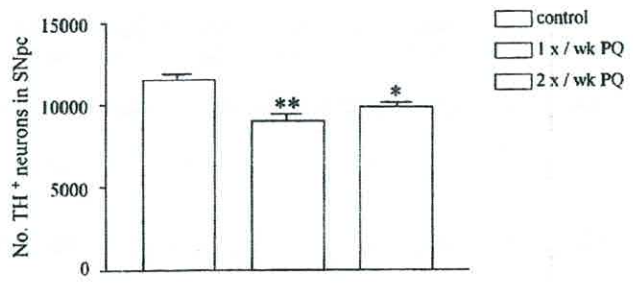
SYNG-PQ-00105738

Syngenta Study - Effect of Dosing PQ once & twice per week for 3 weeks

TH^+ cell counts in SNpc: 21% ↓ TH^+ cells (1x /wk) ; 14% ↓ TH^+ cells (2x /wk)



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 10 mg/kg PQ dichloride, n=9; PQ 2 x week group received injections of 10 mg/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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SYNG-PQ-00105743

Summary of literature findings from the PQ rat model

Rat Studies

- Sprague Dawley rat (male) *Canadian (Quebec)* group in 2006
  - PQ dosed i.p. 10 mg/kg twice a week for 4 weeks
  - 40% reduction in TH^+ neurones
  - effects on striatal dopamine not reported

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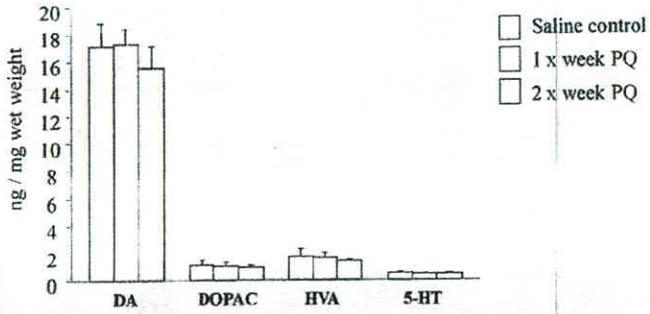
SYNG-PQ-00105741

Syngenta Study - Effect of Dosing PQ once & twice per week for 3 weeks

HPLC analysis of striatal dopamine:



Data represents mean ± SD. 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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Syngenta CTL Investigative Studies

***In vivo* studies** - replicating studies conducted in the $C_{57}Bl_6$ mouse model with paraquat to validate the literature claims

***In vitro* studies** - investigating the potential of paraquat to interact with the dopamine transporter & dopamine receptors

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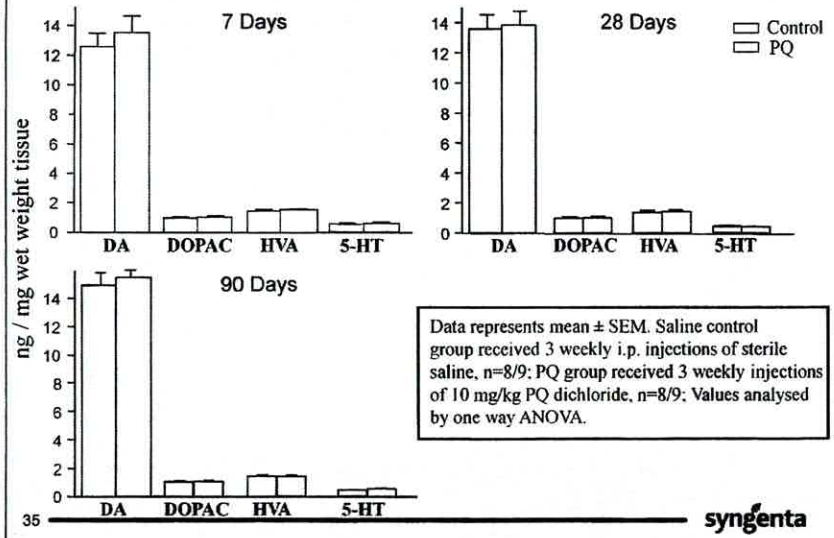
-PQ-00105742

Syngenta Study - Investigating the Time Course of PQ Effects

HPLC Analysis of Striatal Dopamine & Metabolites



Data represents mean ± SEM. Saline control group received 3 weekly i.p. injections of sterile saline, n=8/9; PQ group received 3 weekly injections of 10 mg/kg PQ dichloride, n=8/9; Values analysed by one way ANOVA.

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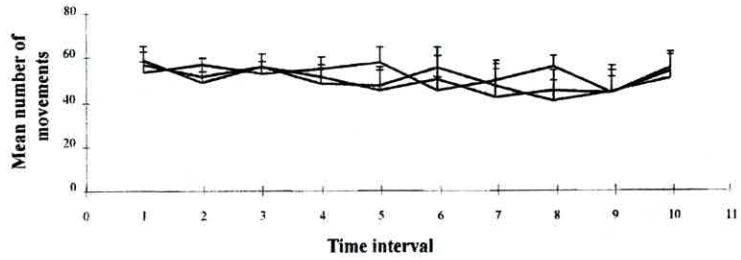
SYNG-PQ-00105747

Syngenta Study - Effect of Dosing PQ once & twice per week for 3 weeks

Behavioural - locomotor activity measured at 2, 24, 48 hours and 7 days after final dose

Mean number of total movements (large and small) 7 days after final dose



Saline

10 mg/kg PQ once per week

10 mg/kg PQ twice per week

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SYNG-PQ-00105745

Syngenta Study - Does the cell loss observed following PQ dosing occur as a result of a general toxicity?

- The 10 mg/kg PQ dose is high (i.p. LD_{50} = 30 mg/kg)
- Could nigrostriatal deficits be observed when any compound is administered at its maximum tolerated dose?
- Administered 4 different compounds at MTD
  - Caffeine & paracetamol - general population exposed
  - Antimycin-A & N-ethylmaleimide (NEM) - oxidative stressors
- Compounds dosed at MTD, once per week for 3 weeks
  - neuronal cell counts and striatal dopamine analysis

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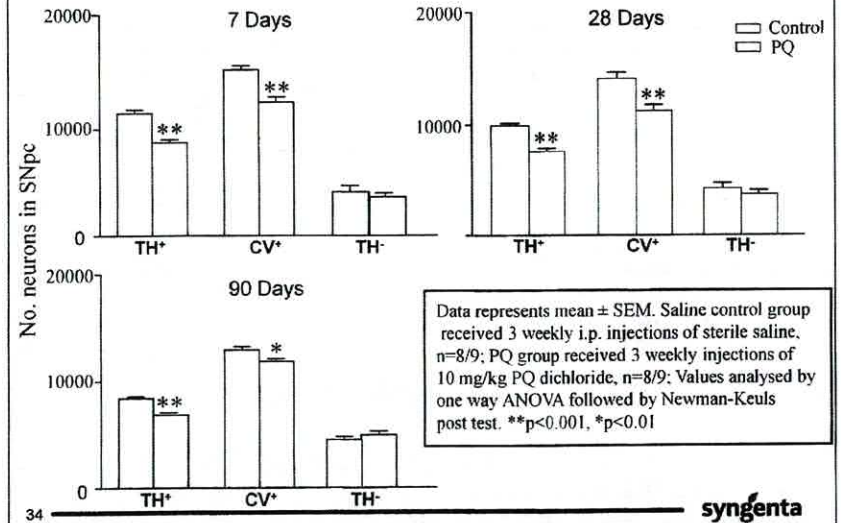
Syngenta Study - Investigating the Time Course of PQ Effects

Cell Count Data



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

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Findings From Paraquat Rat Model

Rat Studies

- Sprague Dawley rat (male) *Canadian (Quebec)* group in 2006
  - PQ dosed i.p. 10 mg/kg twice a week for 4 weeks
  - 40% reduction in TH⁺ neurones
- Syngenta repeated the above study.....

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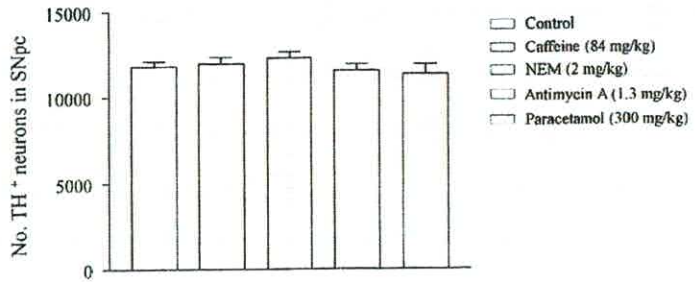
SYNG-PQ-00105751

Syngenta Study - Does the Cell Loss Observed Following PQ Dosing Occur as a Result of a General Toxicity?

TH⁺ Cell Counts in the SNpc



Data represents mean ± SEM. n=10 per group. Control control group received 3 weekly i.p. injections of sterile saline. Data analysed by one way ANOVA followed by Newman-Keuls post test.

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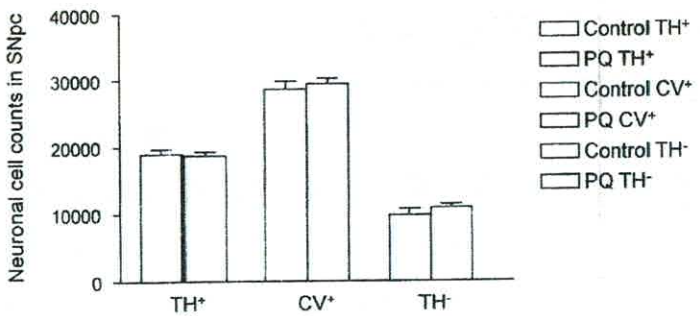
Syngenta Study - Effect of 10 mg/kg PQ dosed i.p. twice a week for 4 weeks to the rat



Data represents mean ± SEM. Data analysed using one way ANOVA followed by Newman–Keuls post test. PQ treated animals received 10 mg/kg PQ dichloride i.p. twice a week for 3 weeks (n=8/9). Controls received injections of sterile physiological saline (n=9/10). Animals were killed 24 hours after the final injection.

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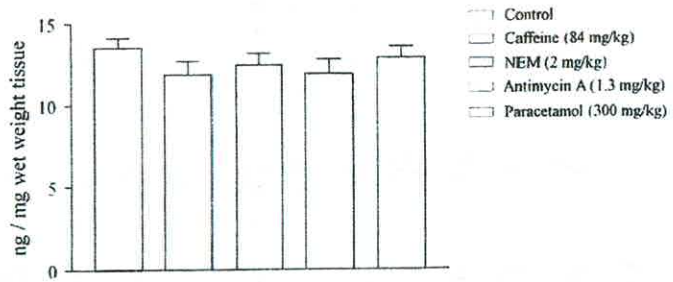
Syngenta Study - Does the Cell Loss Observed Following PQ Dosing Occur as a Result of a General Toxicity?

HPLC analysis of striatal dopamine



Data represents mean ± SEM. Data analysed using Student's t-test. Compounds were administered i.p. once a week for 3 weeks at MTD doses and animals terminated 7 days after the last dose. n = 10 per group, control, caffeine, NEM and paracetamol; n = 9 antimycin A.

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