

Paraquat Exposure & Human Health

Paraquat & Parkinson's Disease

2018

Parkinson's Disease - What is it?

- Parkinson's disease is a degenerative disease of the nervous system, caused by dopamine deficiency in the *striatum* and loss of neurones in the *substantia nigra*.
- Symptoms include tremors, muscle rigidity, involuntary movements and postural changes.
- Affects 1-2 % of the elderly population > 65 years, mean age for onset of symptoms is 60 years old.
- No clear genetic component to idiopathic Parkinson's disease, except in cases of early-onset.
- "Environmental factors" may play a role in the aetiology of the disease?

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Possible “Environmental” Risk Factors Associated with Parkinson’s Disease

Increased Risk

Exposure to toxins

- Pesticides
- Consumption of well water
- Pollution from industrial plants

Trauma

- Head injury (boxing)

Personality

- Introvert personality

Infection

- Bacterial (*Nocardia asteroides*) or viral (influenza)

Parkinson’s Disease

Decreased Risk

Cigarette smoking

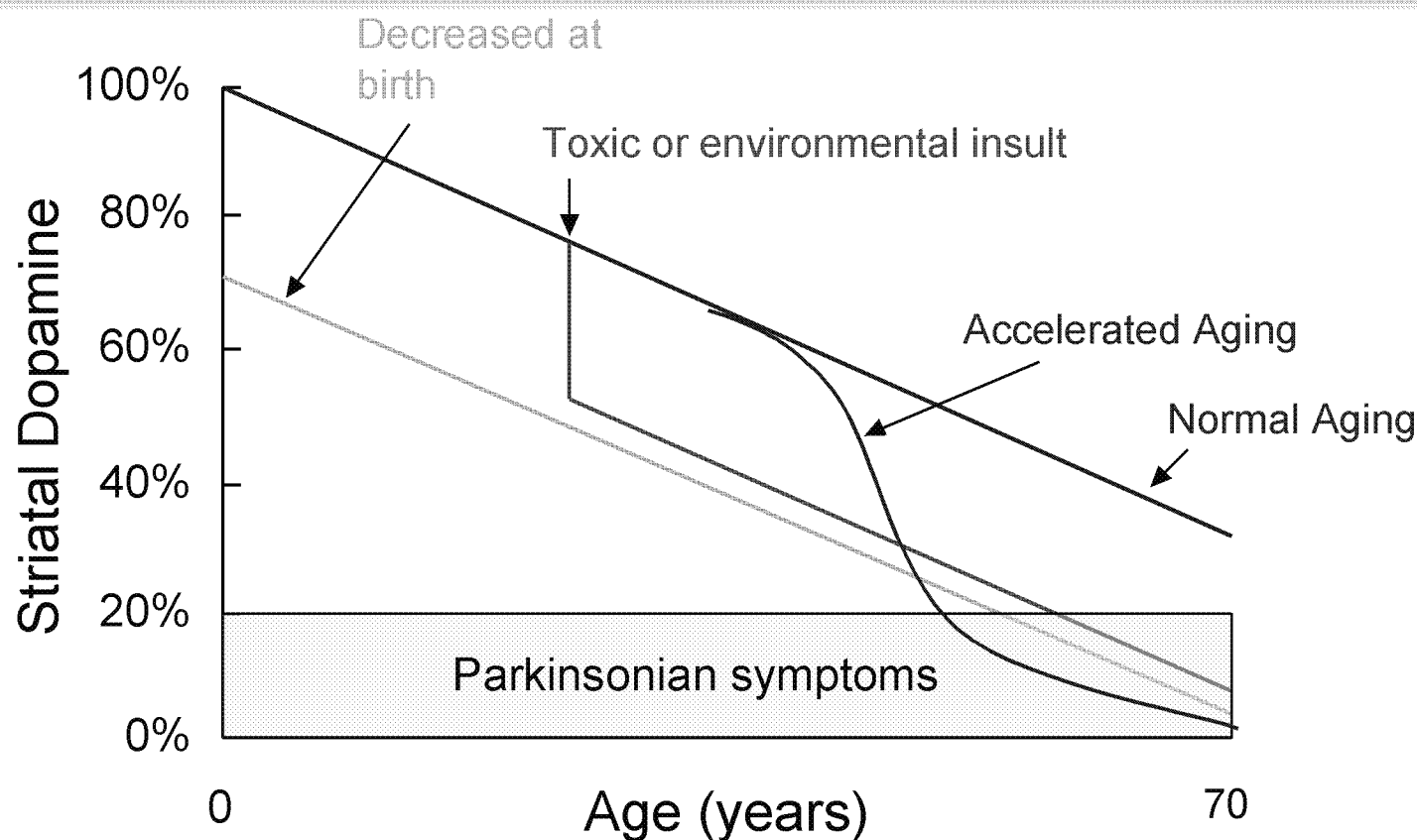
- Nicotine effects?
(nicotinic receptors)

Coffee drinking

- Caffeine effects?
(adenosine A₂ antagonist)

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The Effect of Environmental Insult On Parkinson's Disease Onset



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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 incidence of PD in Canada non-uniform.
 - correlated with areas of pesticide use.
- Other workers followed this lead - further epidemiology studies - found incidence 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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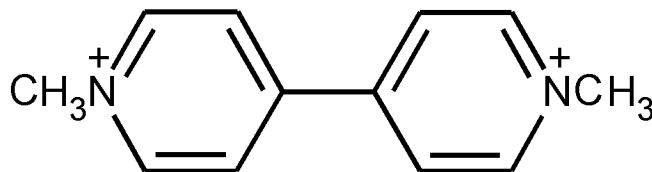
Why has Paraquat been associated with Parkinson's disease?

- Structural similarity with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine).
- 1976 chemistry student (Bethesda, Maryland) involved in synthesising analogues of Pethidine for recreational use.
- MPPP (1-methyl-4-phenyl-propionoxypiperidine) heroin substitute.
- Developed PD-like symptoms - later discovered the compound was contaminated with MPTP.
- 6 further cases of MPTP poisoning reported in 1982 in northern California.

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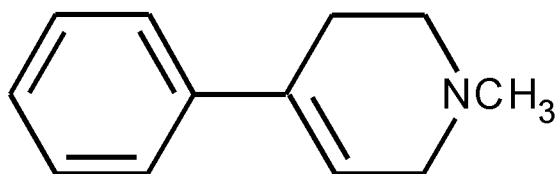
Paraquat and MPTP

Paraquat



Di-quaternary
cation

MPTP

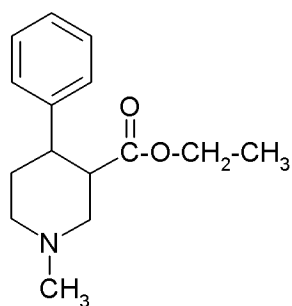


Uncharged
and lipophilic
molecule

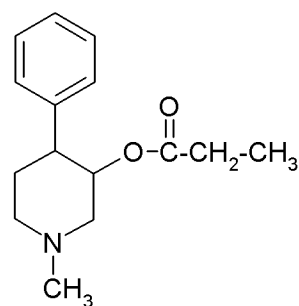
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Conversion of MPPP to MPTP and MPP⁺

Meperidine



MPPP

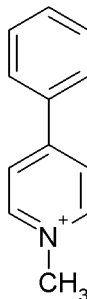


1-methyl-4-phenyl-propionoxypiperidine

Low pH
High Temperature

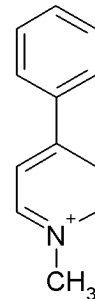


MPP⁺



1-methyl-4-pyridium

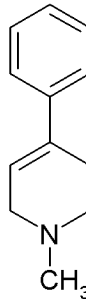
MPDP⁺



← MAO B

← MAO B

MPTP

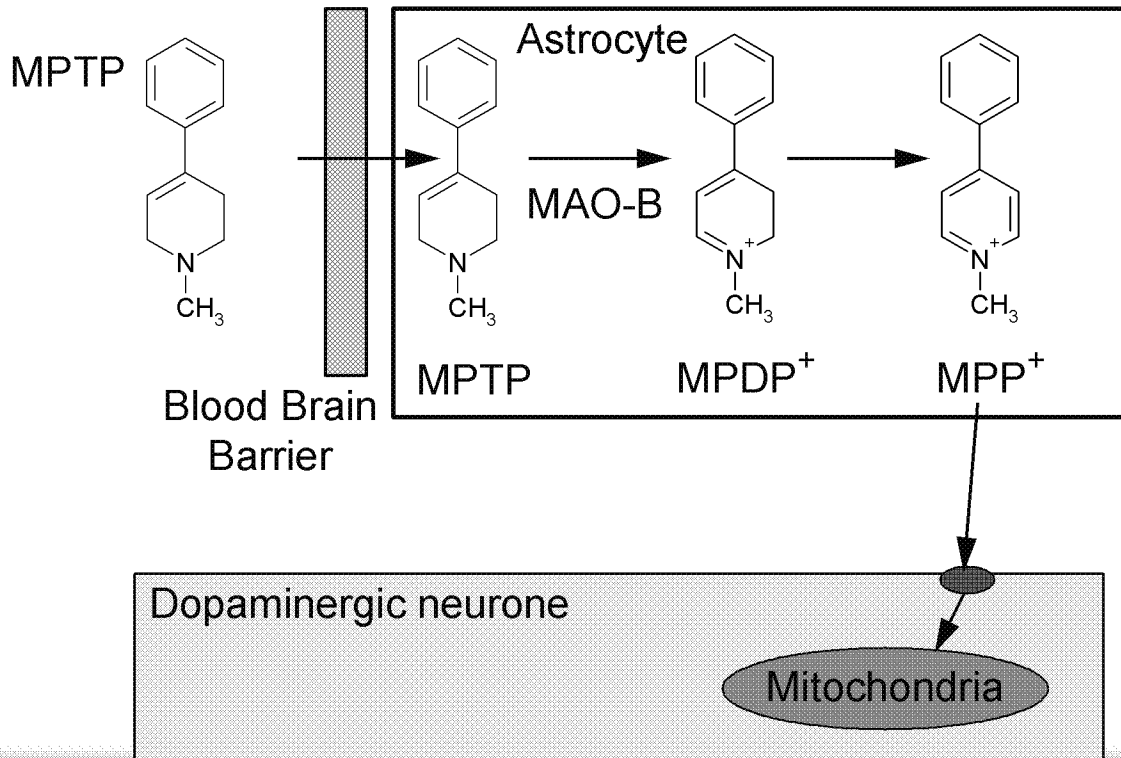


1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

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MPTP Mechanism of Toxicity

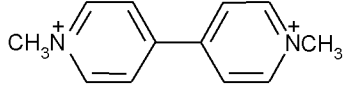
Metabolic activation and mechanism of toxicity of MPTP



2008

Paraquat and MPTP

Paraquat



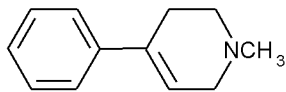
Charged molecule

Polar (water soluble)

Diamine

Not Metabolised

MPTP



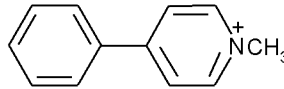
Uncharged molecule

Lipophillic

Crosses the BBB

Metabolised

MPP⁺



Does not cross BBB

Neurotoxic

Monoamine

glaxo

Does Paraquat cross the BBB and cause neurotoxicity?

- Long term (1-2 year) Syngenta regulatory studies with PQ, involving oral or dietary administration to rats and dogs, have shown no clear signs of neurotoxicity or neuropathological changes indicative of neurotoxicity.
- Oral dosing of PQ 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.
- [¹⁴C]-PQ dosing studies with rats and mice reveal very little PQ (<0.05% of total dose) gets into the brain.
- Concluded that in the rat (primary toxicology species), there was no clear evidence to indicate that PQ is neurotoxic via relevant routes of exposure.

2018

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 C₅₇Bl₆ 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.
- Work subsequently repeated by Ding *et. al.*, (2001).

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Recent Literature Developments of Concern

- Two US based research groups have produced a series of publications since 1999 - work still on going.
- Cory-Slechta group - *University of Rochester, NY (Rutgers, NJ)*.
Di Monte group - *Parkinson's Institute, Sunnyvale, CA*.
- Using the C₅₇Bl₆ 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

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Recent Literature Developments of Concern

- Cory-Slechta group (Rochester, NY; Rutgers, NJ)
Brooks *et. al.*, (1999), Thiruchelvam *et. al.*, (2000; 2001; 2002),
Barlow *et. al.*, (2003)
- PQ alone given to adult C₅₇Bl₆ mice caused neuropathology and behavioural changes consistent with PD.
- Results probably artefact due to disruption of BBB with injection into the brain of fluorogold (neuronal marker) prior to PQ dosing.
- Subsequent publications from this group involving the dosing of PQ (10 mg/kg i.p.), and the dithiocarbamate fungicide, maneb (30 mg/kg i.p.), in combination.
- Combination effect greater than with either compound alone, and effects are greater than additive.
PQ alone relatively inactive.

2008

Recent Literature Developments of Concern (Developmental Effects)

- Cory-Slechta group (Rochester, NY; Rutgers, NJ)
- Exposure of neonates to PQ (0.3 mg/kg ip) + maneb (1 mg/kg ip) on post-natal days 5-19. Effects are potentiated when mice are exposed to higher doses of PQ (10 mg/kg) + maneb (30 mg/kg) in later life as an adult.
- Development of mouse BBB not complete on post-natal day 5, so there is likely to be greater brain exposure to PQ and maneb in the young neonate, than as an adult.
- Prenatal exposure to maneb increases adult vulnerability to neurotoxic effects of PQ.
- But prenatal exposure to PQ did not enhance vulnerability to PQ as an adult.

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Recent Literature Developments of Concern (Prenatal Exposure)

- Cory-Slechta group (Rochester, NY; Rutgers, NJ)
- Pregnant mice exposed to maneb (1 mg/kg/day) on prenatal days 10-17. Subsequent offspring exposed to PQ (5 mg/kg/day for 8 days) produces 95% reduction in locomotor activity, 60% reduction in dopamine and 40% reduction in neurone counts.
- Deficit is greater than with PQ exposure as an adult only.
- Males only affected - neuroprotective effects of estrogen?
- Implies prenatal maneb exposure impairs development of BBB.

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Recent Literature Developments of Concern

- Di Monte group (Parkinson's Institute, Sunnyvale, CA) McCormack *et. al.*, (2002), McCormack & Di Monte, (2003)
- PQ alone dosed ip to C₅₇Bl₆ male mice (3 weekly doses of 1,5 or 10 mg/kg i.p.) produces a dose related loss of neurones in the substantia nigra *pars compacta*.
- 1,5 & 10 mg/kg PQ resulted in 10, 18 & 28% loss of neurones respectively.
- L-valine (high affinity substrate for the neutral amino acid transporter in the BBB) protects against PQ-induced dopaminergic injury. D-valine and L-lysine ineffective.
- Implies that PQ is transported across the BBB to the brain by a neutral amino acid transporter.

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Ongoing Research Activity (External)

- NIEHS funding (\$20 million over 5 years) to investigate environmental and genetic determinants of Parkinson's disease.
- *The Parkinson's Institute, Sunnyvale, CA (Di Monte)* will examine risks associated with pesticides & metals, and the possible protective effects of tobacco and caffeine.
- *Emory University, Atlanta (Miller & Greenamyre)* will develop new animal and cellular models to study the gene-environment interactions involved in the development of PD, and will focus on how pesticides interact with vesicular monoamine transport (VMAT) mechanisms.
- *UCLA (Chesselet)* will study how genetic polymorphisms play a role in the increased risk of PD associated with exposure to pesticides.
- Verbal contact has been made with these groups, and publications originating from them are likely to continue over at least the next five years.

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Ongoing Research Activity (External)

- David Ray - University of Nottingham, UK.
- Investigating the effects of PQ (3-20 mg/kg s.c.) on rats & mice on neurochemical and neurobehavioural endpoints.
- Plans to make measurements of brain levels of PQ under different dosing regimens, with and without maneb and L-valine.
- Has access to a very sensitive 2-D radioactivity detector, with very fine spatial resolution, for examining levels of PQ within different regions of the brain. Requires access to radiolabelled PQ - already approached Syngenta CTL.
- Currently examining ways in which we may be able to collaborate, or work with him at a scientific level in the future.

20100609

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 C₅₇Bl₆ 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.

20180619

Research Activity at Syngenta CTL *in vivo* Studies

- Repeat of published *in vivo* experiments with PQ alone being dosed to C₅₇Bl₆ 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⁺ neurones in the *substantia nigra*
- 3 weekly doses of PQ (10 mg/kg i.p.) to C₅₇Bl₆ male mice with neurochemical (striatal dopamine levels) and neuropathological (TH⁺ neurone counts in the *substantia nigra*) end point markers.
- In life phase complete - report findings in July 2003.
- May seek expert peer review of neuropathology.

200300429

Research Activity at Syngenta CTL *in vitro* Studies

- Does PQ behave in a similar way to MPTP and its metabolite, MPP^+ , 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.
- Unlike the MPTP metabolite, MPP^+ , PQ does not act as a substrate for the dopamine transporter, and does not bind to dopamine D_1 and D_2 receptors in rat and mouse striatum.
- Demonstrates clear differences between PQ and the mechanism of action of MPTP.
- Data to be published at *Neurotox 2003*.

2003

Scientific Influencing Strategy (Ongoing)

- Regularly updating position statements for Regulatory defence and the basis of PR statements.
- Conducting scientific evaluation of existing and new scientific publications.
- Established contact with the key external researchers and have been mapping their future research activity.
- Influence future work by external researchers where possible (e.g. David Ray, UK).
- Establish credibility with external researchers by publishing scientific findings from *in house* research.

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Research Publication Plan

- *In vitro* work on the differences between PQ and MPTP
 - *Neurotox 2003, UK.*
 - poster presentation and abstract in *Toxicology*
- *In vivo* work on PQ effects on SN neurone counts in C₅₇Bl₆
 - publish in peer reviewed journal, particularly if literature claims not reproducible *in house* (2003/2004)
 - establish no effect dose for loss of TH⁺ neurones in SN and publish in peer reviewed journal (2003/2004)
- Make presentations at SOT and SFN meetings in 2004.
- Consider publishing the factual exposure position, and an independent scientific review of the epidemiology literature.

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Influencing Strategy - Regulators

- Latest position statement document used as a brief for EU.
- Section on Parkinson's disease included in the JMPR submission June 2003.
- Be proactive with the Regulators by seeking opportunities to make them aware of developments in the science and our positions.
- Consideration of appropriate timing for conducting regulatory neurotoxicity studies with PQ.

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