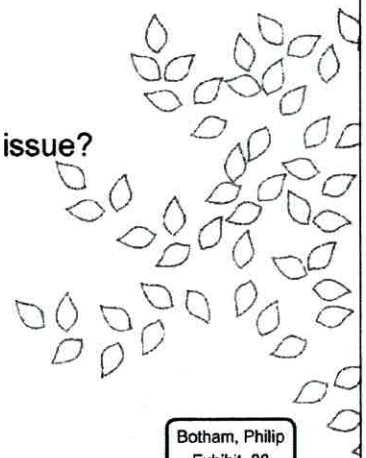
syngenta

Parkinson's Disease

What can Syngenta say about the issue?



The Issue

Paraquat exposure has been implicated in the literature as a potential contributory factor in Parkinson's disease (PD) in humans.

- There is a chemical structure association made in the literature between paraquat and MPTP
  - shown to induce symptomology of Parkinson's disease in humans
- There are literature epidemiology reports of an association between working with pesticides (and paraquat) and Parkinson's disease
- There are literature reports of an experimental model for PD in the C57Bl6 mouse where paraquat administered intraperitoneally causes a reduction in the number of neurones in the brain, together with in some studies a reduction in levels of dopamine in the brain and behavioural changes

What we cannot say

- Paraquat does not enter the brain
- Paraquat does not cause any changes in the brain
- Paraquat only causes effects in the mouse
- The mouse data on paraquat are not relevant to humans
- People are not exposed to paraquat
- There are no data reporting that paraquat may be associated with PD in humans
- The data show that paraquat does not cause PD in humans

Current position

Epidemiology

- The evidence from case reports, case series and case-control studies is mixed and without consistent exposure-response or chemical-specific pattern.
- A major problem is the absence of detailed and validated exposure information.
- Currently, the data from epidemiology studies do not provide sufficient evidence to support a causal association between exposure to agricultural chemicals, including paraquat, and PD.

Current position

Animal studies

- The mouse has been developed as a sensitive investigative tool to examine chemicals for changes considered relevant to PD.
- Paraquat administered to the mouse by the intraperitoneal (ip) route leads to a limited reduction in neuronal cell number in the *S nigra*
- There are no consistent data from the mouse model indicating effects from paraquat dosing required as part of the progression and expression of PD (dopamine changes or behavioural effects).
- There is limited evidence for paraquat causing a reduction in neuronal cell number in species other than the mouse.
  - CTL has examined the rat using a protocol taken from the literature that claimed an effect. There was no reduction in neuronal cell number in our study.
- The relevance of the observations in this investigative model to humans who may be exposed to low levels of paraquat mainly by the dermal/oral route is considered to be limited
- There is no evidence that paraquat causes biological changes related to PD by a relevant route for human exposure, or at doses approaching those to which humans may be exposed.

Current position

Structural association with MPTP

- The structural association of paraquat to MPTP is superficial and is inappropriate for drawing any conclusions on potential similar activity in regard to PD

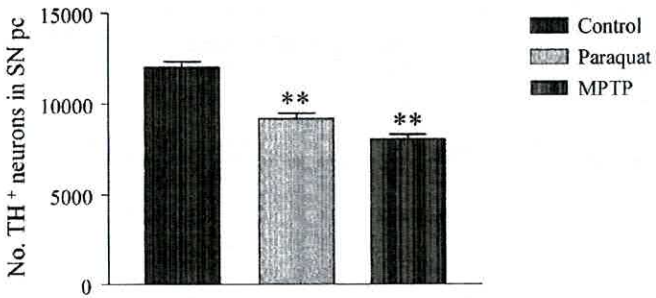
Ongoing work

- External labs will continue to generate and report data:
  - Epidemiology
    - Ag Health study USA
    - Farm workers (x2?) France
    - DEFRA (Geoparkinson study) UK
  - Animal studies
    - Di Monte
    - Corey Slechta
    - Others
- Syngenta
  - Evaluation of PQ in the rat (negative)
  - Kinetics of PQ uptake into the brain of the mouse

Stud_ - Effects of Dosing PQ 1x / week for 3 weeks

TH⁺ cell counts in SNpc

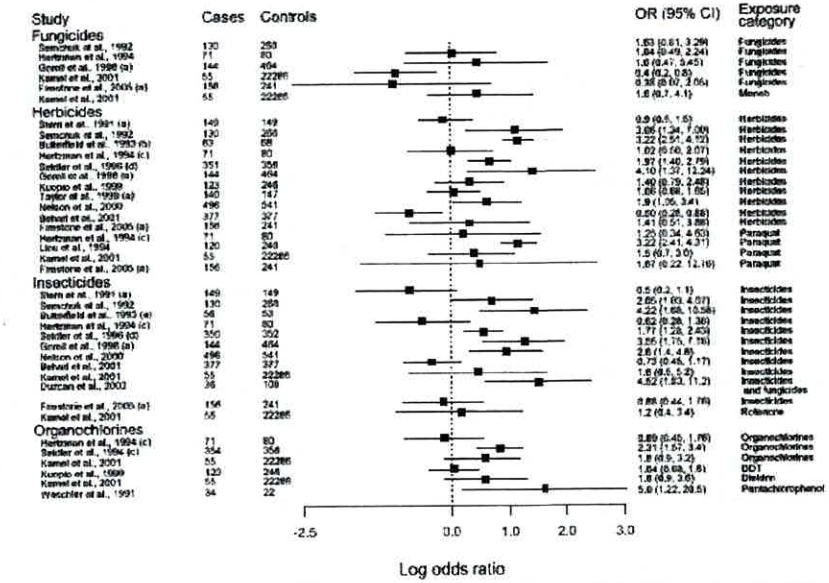
Using modified stereology parameters and new set up – 24% ↓ TH⁺ cells (PQ) ; 33% ↓ TH⁺ cells (MPTP):



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 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

syngenta

SYNG-PQ-00481045



syngenta

SYNG-PQ-00481046

Studies specifically focusing on paraquat

Study	Country	No. Exposed Cases/Total Cases	No. Exposed Controls/Total Controls	OR (95% CI)	*P* - Value
Hertzman 1990	Canada	4/57	0/122	NR	0.01
Hertzman 1994	Canada	6/127	4/124 (cardiac)	1.25 (0.34 – 4.63)	
			5/121 (voters)	1.11 (0.32 – 3.87)	
Seidler 1996	Germany	1/380	0/755	NR	NR
Liou 1997	Taiwan	31/120	22/240	3.22 (2.41 – 4.31)	< 0.01
Kuopio 1999	Finland	3/123	5/246	NR	NR
Engel 2001*	USA	20/49	NR	0.8 (0.5 – 1.3)	
Kamel 2001**	USA	?/55	?/22286	1.5 (0.7 – 3.0)	
Firestone 2005	USA	?/250	?/388	1.67 (0.22 – 12.76)	

syngenta

*Cross-sectional study **Conference presentation

SYNG-PQ-00481047

Possible outcomes

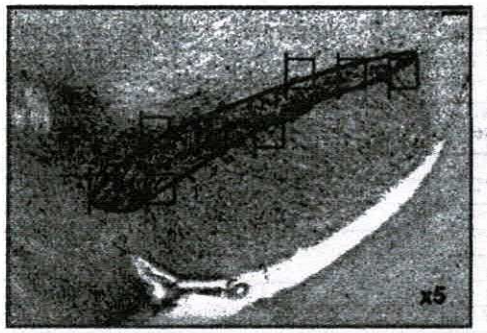
TABLE 4. Ever use of specific pesticides by self-reported Parkinson's disease (PD) cases and controls identified in the Agricultural Health Study at enrollment in 1993–1997 (prevalent PD) or at follow-up in 1999–2003 (incident PD)*

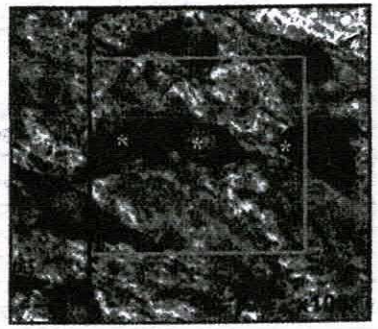
Pesticide class/factor†	Chemical‡	Prevalent PD						Incident PD					
		Cases		Controls		OR‡§	95% CI‖	Cases		Controls		OR¶	95% CI
		No.	%	No.	%			No.	%	No.	%		
Herbicides													
Chloroacetanilide	Alachlor	30	39	25,590	34	0.8	0.5, 1.5	34	49	17,399	34	1.1	0.6, 1.9
	Metolachlor	22	29	22,202	29	0.9	0.5, 1.6	30	45	14,856	29	1.3	0.7, 2.3
Benzoic acid	Dicamba	26	35	23,847	32	0.9	0.5, 1.6	32	47	16,454	32	1.5	0.8, 2.8
Dinitroaniline	Pendimethalin	23	32	21,386	28	1.4	0.8, 2.6	17	25	13,603	27	0.7	0.4, 1.2
	Trifluralin	31	40	25,787	34	0.9	0.5, 1.6	32	48	17,406	34	1.7	1.0, 3.2
Imidazolinone	Imazethapyr	19	25	20,461	27	0.9	0.5, 1.7	22	32	13,747	27	1.2	0.6, 2.1
Mixture	Petroleum oil	22	30	22,295	30	0.5	0.3, 0.9	28	41	15,224	30	1.1	0.6, 1.9
Organophosphonate	Glyphosate	45	55	46,687	60	1.0	0.6, 1.7	49	67	32,686	66	1.1	0.6, 2.0
Phenoxyacetate	2,4-D‡	47	58	40,405	52	0.9	0.5, 1.6	49	68	28,118	52	1.0	0.5, 2.1
	2,4,5-T‡	16	22	9,824	13	0.9	0.5, 1.7	24	35	6,961	14	1.8	1.0, 3.3
	2,4,5-TP‡	4	5	4,229	6	0.8	0.3, 1.9	7	10	2,909	6	0.9	0.4, 1.8
Quaternary ammonium	Paraquat	14	20	11,266	15	1.8	1.0, 3.4	11	16	7,382	14	1.0	0.5, 1.9
Thiocarbamate	EPTC‡	6	8	9,160	12	0.6	0.3, 1.3	14	21	5,409	13	1.1	0.6, 2.1
	Butylate	17	23	14,726	20	0.7	0.3, 1.3	24	35	10,087	20	1.4	0.8, 2.5
Sulfonylurea	Chlorimuron-ethyl	16	22	17,552	23	0.8	0.4, 1.5	16	24	11,535	23	1.0	0.6, 1.8
Triazine	Atrazine	40	49	35,377	45	1.0	0.5, 1.9	43	59	24,232	45	1.1	0.5, 2.2
	Cyanazine	30	39	19,702	26	2.6	1.4, 4.9	26	38	13,504	26	1.0	0.5, 1.8
Triazinone	Metribuzin	28	36	20,879	28	1.5	0.8, 3.0	19	28	14,251	28	0.5	0.3, 1.0
Insecticides													
Carbamate	Aldicarb			Not calculated				5	7	3,436	7	0.5	0.2, 1.3
	Carbaryl	35	46	35,262	46	1.0	0.6, 1.7	37	51	24,775	47	0.7	0.4, 1.2
	Carbofuran	21	27	12,908	17	1.3	0.7, 2.5	21	31	8,993	18	1.1	0.6, 2.1
Organochlorine	Aldrin	23	31	8,804	12	1.2	0.7, 2.3	22	31	6,136	12	1.1	0.6, 2.0
	Chlordane	15	21	12,731	17	0.1	0.4, 1.4	23	32	9,131	18	0.9	0.4, 1.5
	Dieldrin	9	13	3,128	4	0.9	0.4, 2.0	8	11	2,303	5	0.8	0.4, 1.8
	DDT‡	25	33	12,620	17	1.0	0.6, 1.8	29	40	8,870	17	1.0	0.6, 1.9
	Heptachlor	15	21	7,144	10	1.1	0.6, 2.2	16	23	5,159	10	0.7	0.4, 1.4
	Lindane	14	19	8,883	12	1.0	0.5, 1.9	19	28	6,460	13	1.4	0.8, 2.5
	Toxaphene	5	7	6,719	9	0.3	0.2, 1.0	9	13	4,632	9	0.6	0.3, 1.3

ngenta

SYNG-PQ-00481048

The Optical Fractionator Method of Stereology





$$N = \text{Sum } Q^{-} \times 1/\text{ssf} \times 1/\text{asf} \times 1/\text{tsf}$$

syngenta

SYNG-PQ-00481049

Proposed Exponent Protocol

1) Toxicokinetic Study (single dose of 7.4 & 74 μmol/kg - propose to dose the Gramoxone formulation)

PND 5 mice
Saline
0.3 mg/kg PQ
PQ + 1 mg/kg maneb
i.p. & oral

Adult mice
Saline
10 mg/kg PQ
PQ + 30 mg/kg maneb
i.p., oral, dermal & inhalation
(direct application to the nasal epithelium)

Concentration of PQ in plasma and different brain regions using [^{14}C]-PQ and autoradiography (WBA for young mice)

2) Use PBPK modelling to determine the equivalent human exposure doses via the relevant routes (dermal / oral - seem to think inhalation route is relevant).

syngenta

SYNG-PQ-00481050

Proposed Exponent Protocol

3) Toxicology Study (i.p. dosing to mice using the Cory-Slechta dosing regimen)

PND 5-19 mice	PND 5-19 & as adults	Adult mice	PND 5-19 & as adults
Saline	(8 months old)	(8 months old)	Saline
PQ	Saline	Saline	PQ
maneb	PQ	PQ	maneb
PQ + maneb	maneb	maneb	PQ + maneb
(leave till 8 months old)	PQ + maneb	PQ + maneb	(leave till 16 months old)

Neuronal Cell Loss

(Stereology - Jim O'Callaghan (CDC); silver staining - Bob Switzer (Neuroscience Associates) & Daryl Thake (Monsanto)

3 different doses to both male & female mice:

(i) Human equivalent dose (from PBPK modelling)

(ii) Mid dose

(iii) "High" Cory-Slechta dose (0.3 or 10 mg/kg PQ, 1 or 30 mg/kg maneb)

syngenta

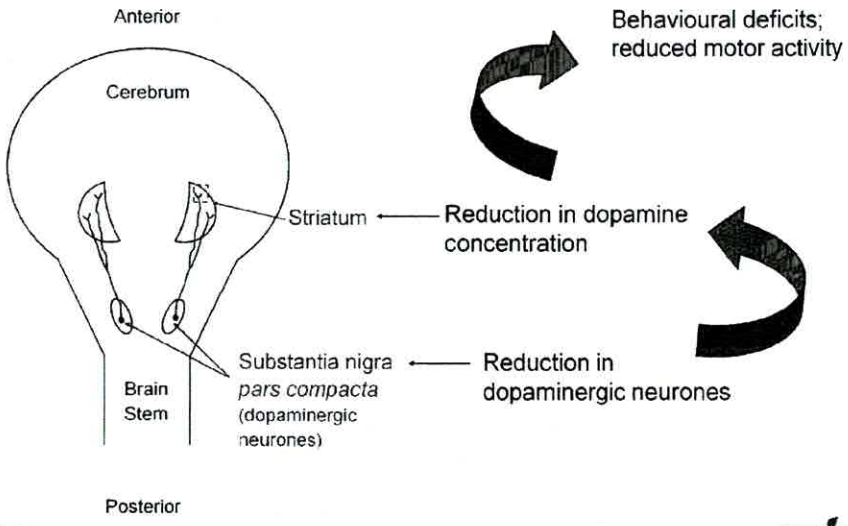
SYNG-PQ-00481051

mg/kg	10%	NO	----------	McCormack *et al* (2002)
5 mg/kg	36%	----------	YES +	Brooks *et al* (1999)
5 mg/kg	18%	NO	----------	McCormack *et al* (2002)
5 mg/kg (for 4 wks)	----------	----------	NO	Thiruchelvam *et al* (2000 b)
10 mg/kg	23-24%	NO	NO	SYNGENTA CTL
10 mg/kg	61%	----------	YES ++	Brooks *et al* (1999)
10 mg/kg	28%	NO	----------	McCormack *et al* (2002)
10 mg/kg	26%	----------	----------	McCormack *et al* (2003)
10 mg/kg	25-35%	----------	----------	Manning-Bog *et al* (2003)
10 mg/kg (for 4 wks)	----------	8%	NO	Thiruchelvam *et al* (2000 b)
2 x 10 mg/kg	14%	NO	NO	SYNGENTA CTL
2 x 10 mg/kg	21%	10% (DOPAC & HVA 15-20%)	NO	Thiruchelvam *et al* (2002)
2 x 10 mg/kg	31%	NO	YES at 24 hrs NO at 3 months	Thiruchelvam *et al* (2003)
2 x 10 mg/kg (18 mth old)	38%	NO	YES at 3 month	Thiruchelvam *et al* (2003)
2 x 10 mg/kg (for 6 wks)	9%	7%	NO	Thiruchelvam *et al* (2000 a)
2 x 10 mg/kg (for 12 wks)	----------	NO	YES but recovery by 24 hrs	Reeves *et al* (2003)

syngenta

SYNG-PQ-00481052

Nigrostriatal dopaminergic system

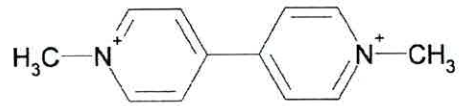


syngenta

SYNG-PQ-00481053

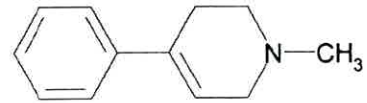
Apparent Structural Similarity Of Paraquat With MPTP & MPP+

Paraquat



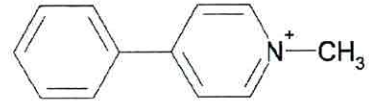
Hydrophilic, charged di-cation

MPTP



Monoamine, uncharged lipophilic molecule

MPP+



Charged, neurotoxic metabolite of MPTP

syngenta

SYNG-PQ-00481054