Pesticides, Agent Orange and Parkinson's Disease

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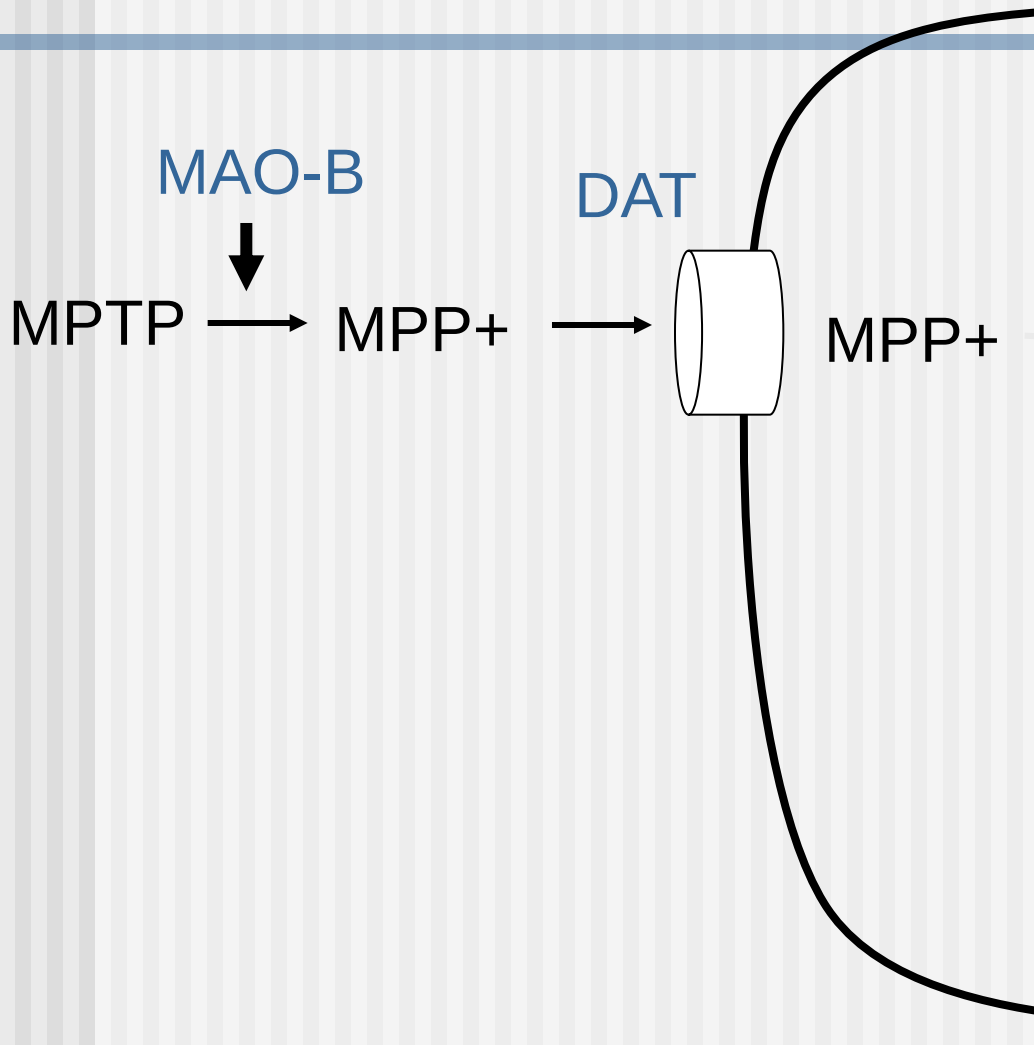
Epidemiology Studies

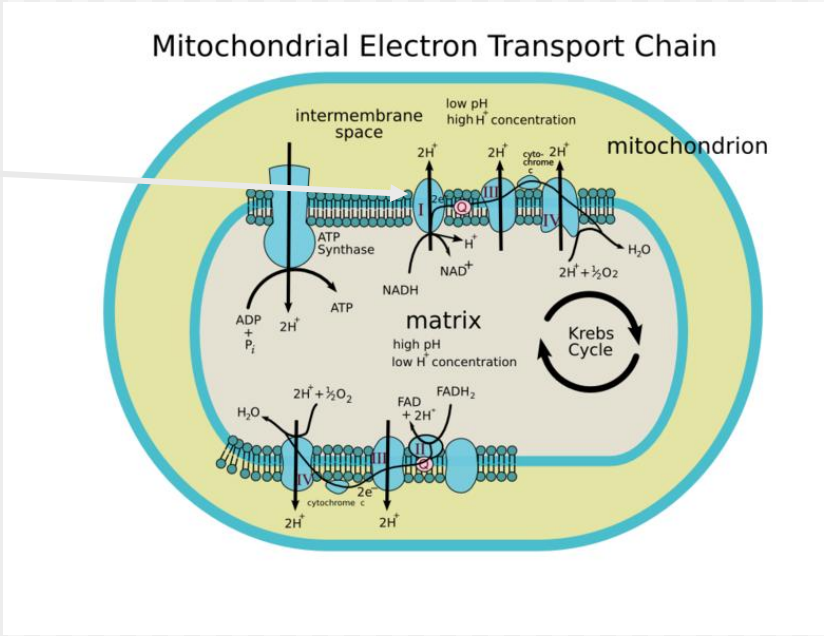
- Study disease clusters
  - Small population
- Case-control and Population-based studies
  - Larger population
- Prospective studies
  - Huge population

The MPTP Story

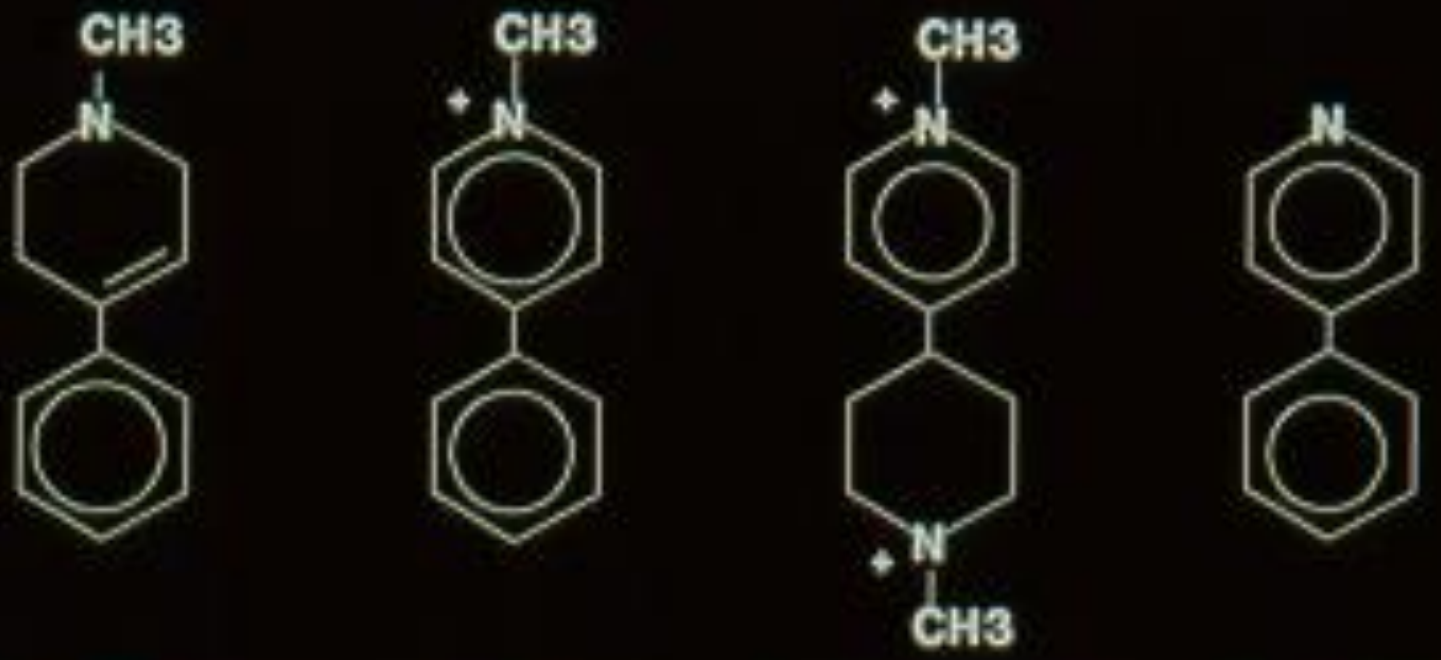
- 1976: Barry Kidston in Maryland acute Parkinsonism trying to make MPPP (an analogue of Demerol).
- 1982: 4 patients in Santa Clara with acute Parkinsonism. MPTP discovered to be the cause.

How MPTP causes Parkinsonism





MPTP AND STRUCTURALLY-RELATED COMPOUNDS



MPTP

MPP+
(CYPERQUAT)

PARAQUAT

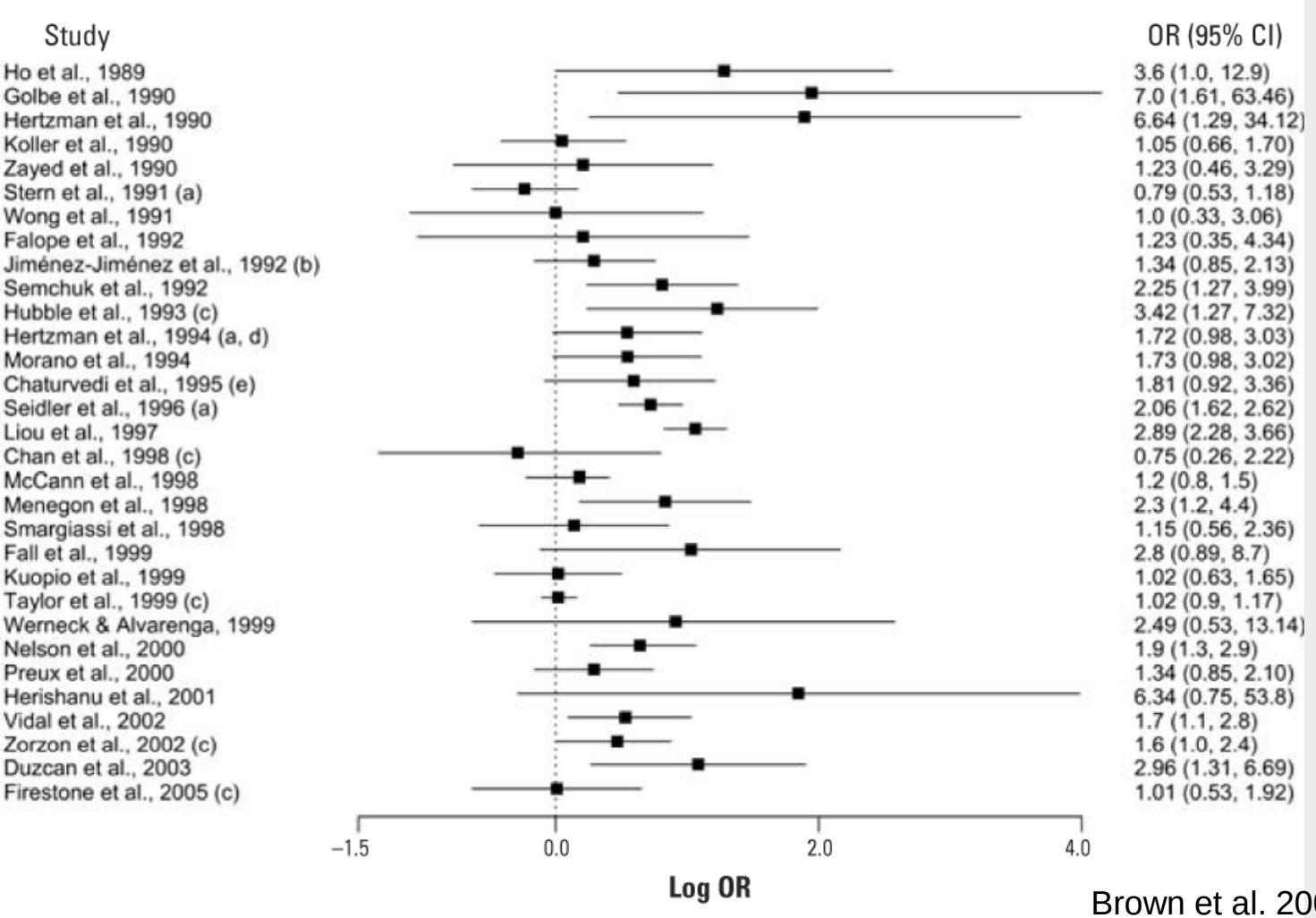
4-PHENYL
PYRIDINE

Farming, Pesticides, and PD

- Most studies have found positive associations.
  - Farming: OR 1.4 (95% CI = 1.1-1.9)
  - Well water: OR 1.3 (95% CI = 0.9-1.6)
  - Professional pesticide use: OR 1.9 (95% CI = 1.5-2.5)

Elbaz and Tranchant. 2007

Pesticide Use and PD



Brown et al. 2006

Pesticides and PD

- Associations found in 4 continents (North America, Europe, Asia, Australia).
- Appears to be independent of farming

BUT

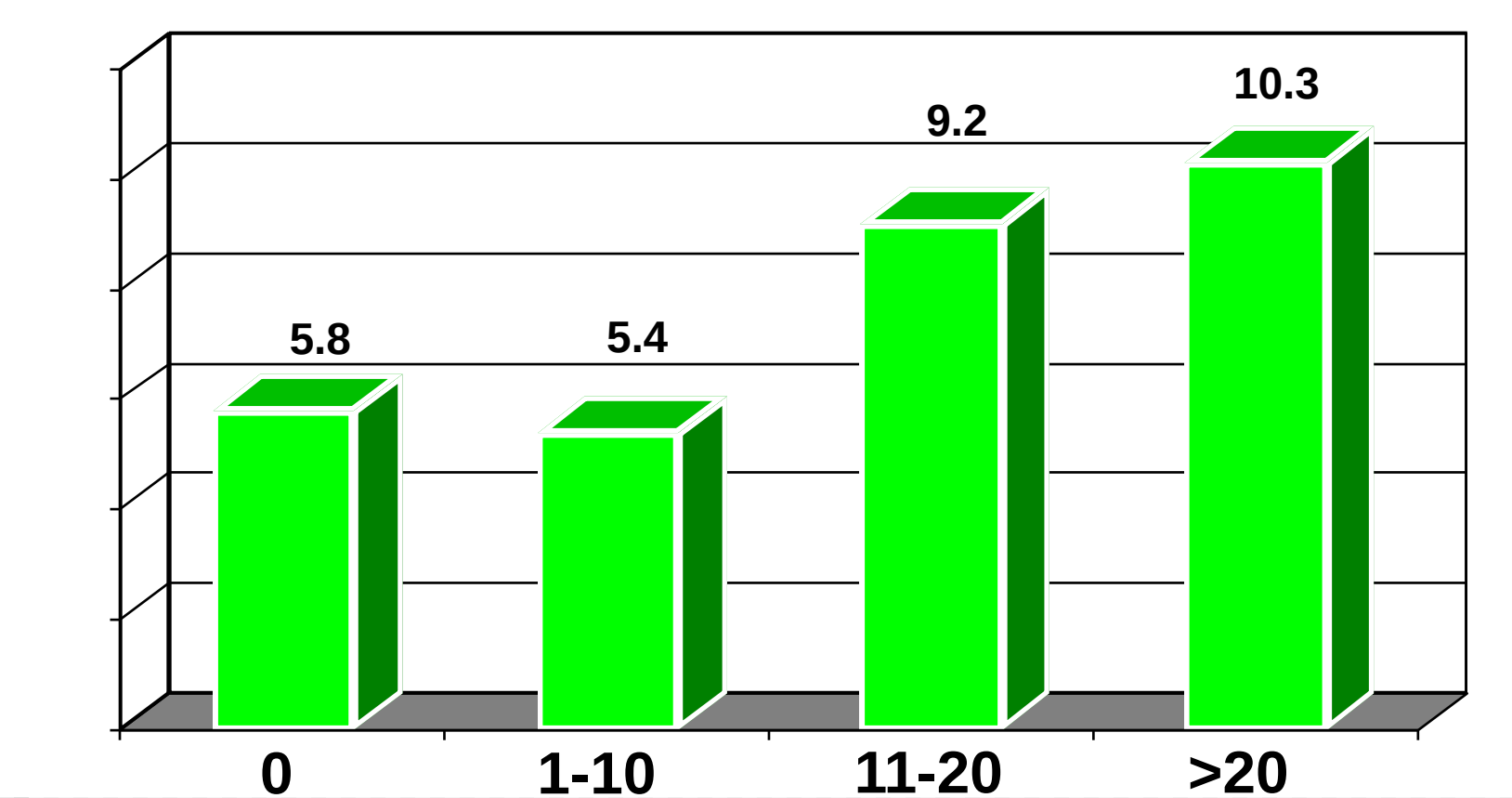
- Methodological concerns
  - Recall bias
  - Control population
- Problems identifying individual agents

Addressing Recall Bias:
Prospective Cohort Study

- Honolulu-Asia Aging Study
- Prospective cohort of 8006 Japanese-American men born 1900-1919
- Years on plantation assessed in 1965
- Self report on pesticide exposure (occupation and home exposure) assessed in 1971
- 116 incident cases

Arch Neurol. 2002;59:1787-1792

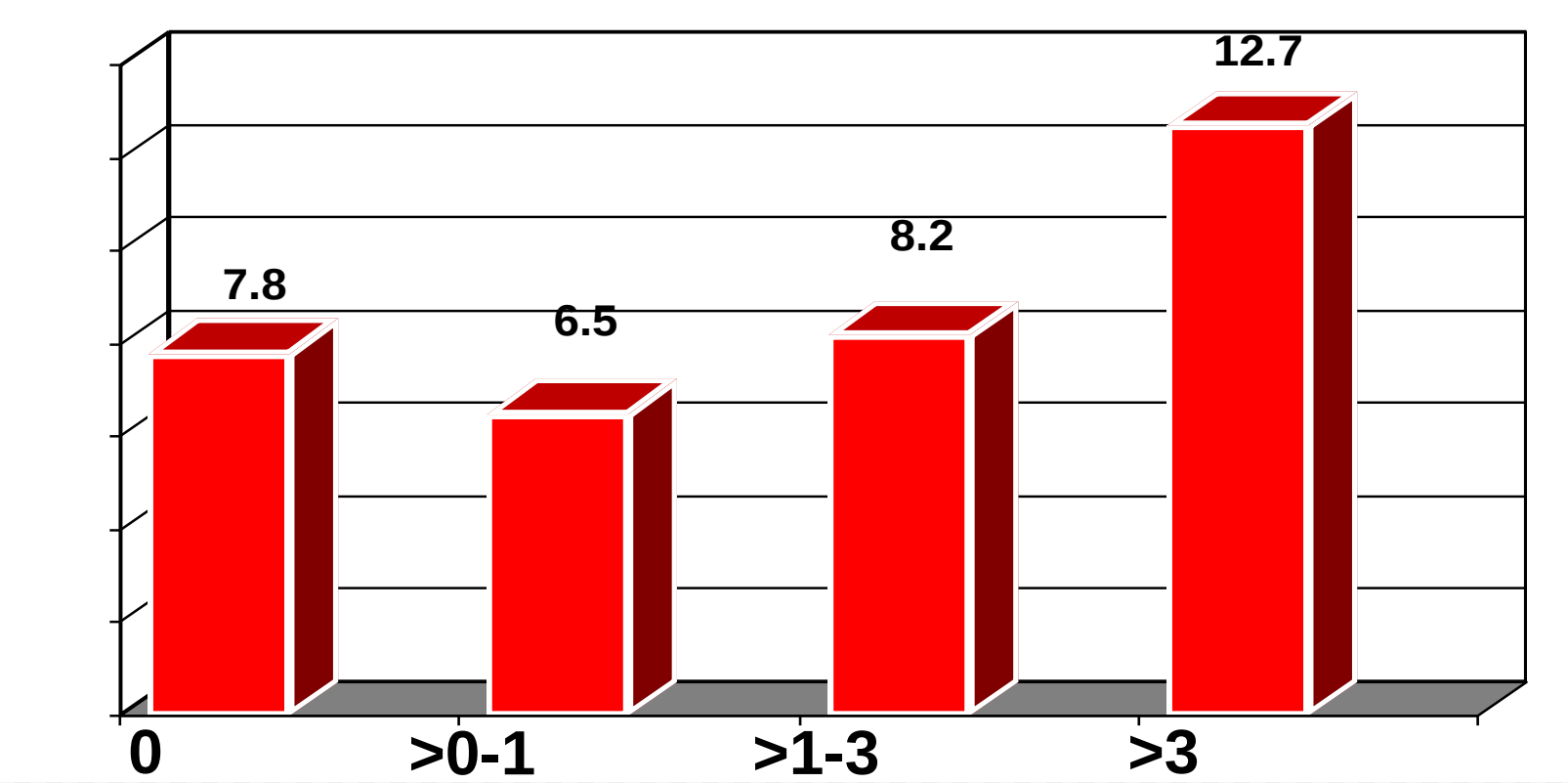
Age-adjusted Incidence of PD* by Years of Plantation Work (follow-up = 30 yrs)



Years of Plantation Work

***Rate per 10,000 person-years**
Test for Trend - p=0.011

Age-adjusted Incidence of PD* by Years of Pesticide Exposure (follow-up = 24 yrs)



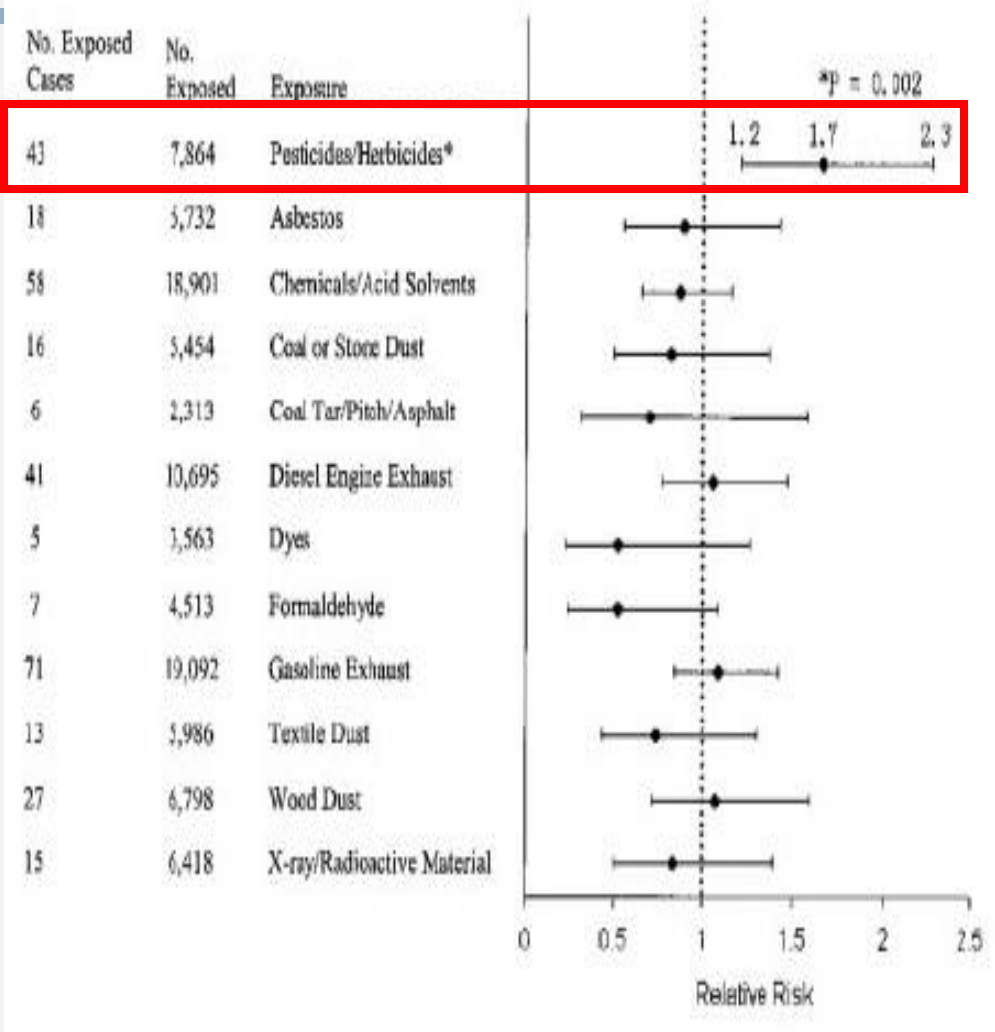
***Rate per 10,000 person-years**
Test for Trend - p=0.100

Cancer Prevention Study II Nutrition Cohort

- 143,325 individuals
- Enrolled in 1992
- Follow up 1997, 1999, 2001
- Exposure was assessed by a questionnaire (yes or no, length of exposure?)
- Case identification by questionnaire, medical records then reviewed.

Ascherio et al. 2006

Cancer Prevention Study II Nutrition Cohort



Ascherio et al. 2006

Prospective Cohort Studies

- Strengths
  - Large study population
  - Controlled for many possible confounds (e.g. smoking, NSAIDs)
  - Prospective (no recall bias)
- Weaknesses
  - Little info on duration, intensity and type of exposures
  - No info on specific pesticides
  - Potential misdiagnosis of cases

Ascherio et al. 2006

Agricultural Health Study

- Prospective cohort study examining relationship between pesticide use and human disease
- 52,000 pesticide applicators and 32,000 spouses from Iowa and N. Carolina enrolled 1993-97
- Detailed pesticide use information
- PD participants almost twice as likely to be exposed to 2,4,5-T and to trifualin than non-PD (control) participants

Kamel et al. 2007

Agricultural Health Study

- Strengths
  - Incident cases were prospective
  - Identified specific pesticides
  - Controlled for other confounds
- Weaknesses
  - Small number of cases (self reported), limited power
  - Exposures self reported (agents, durations etc)
  - Diagnosis self reported

Kamel et al. 2007

Parkinson's (PEG) Study (Beate Ritz MD,Ph.D.)

Goal:

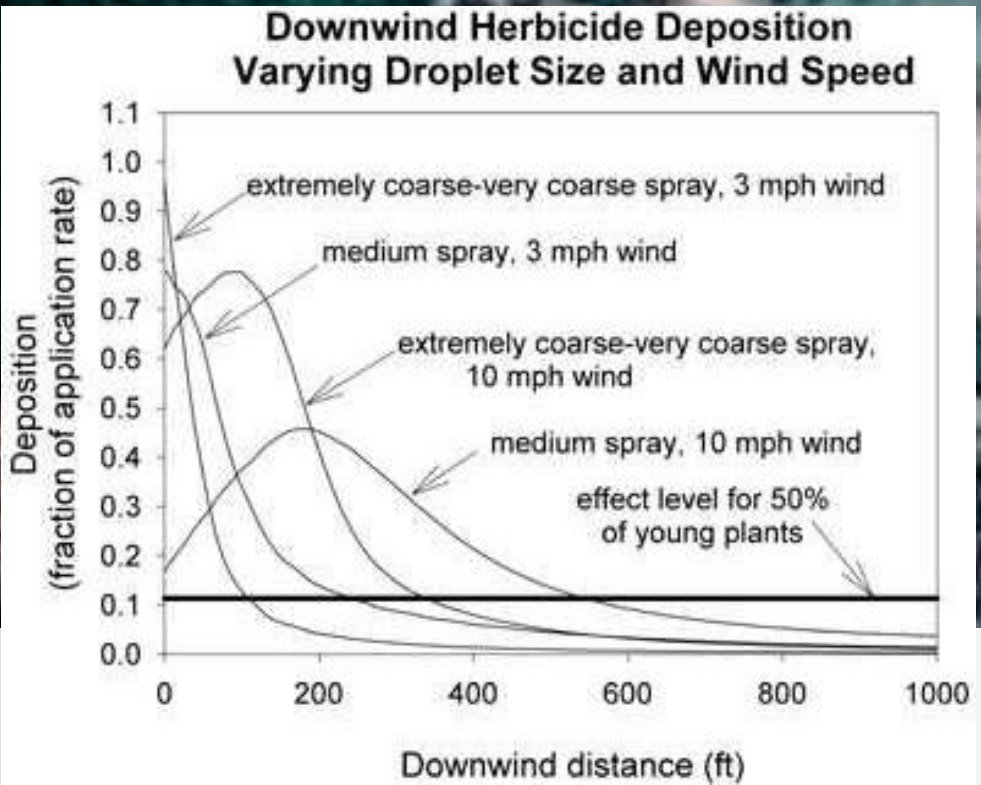
Identify all newly diagnosed cases of PD over a 4-year period (2001-2005)
- 400 cases
- 400 population controls and 200-300 (unaffected) sibling controls

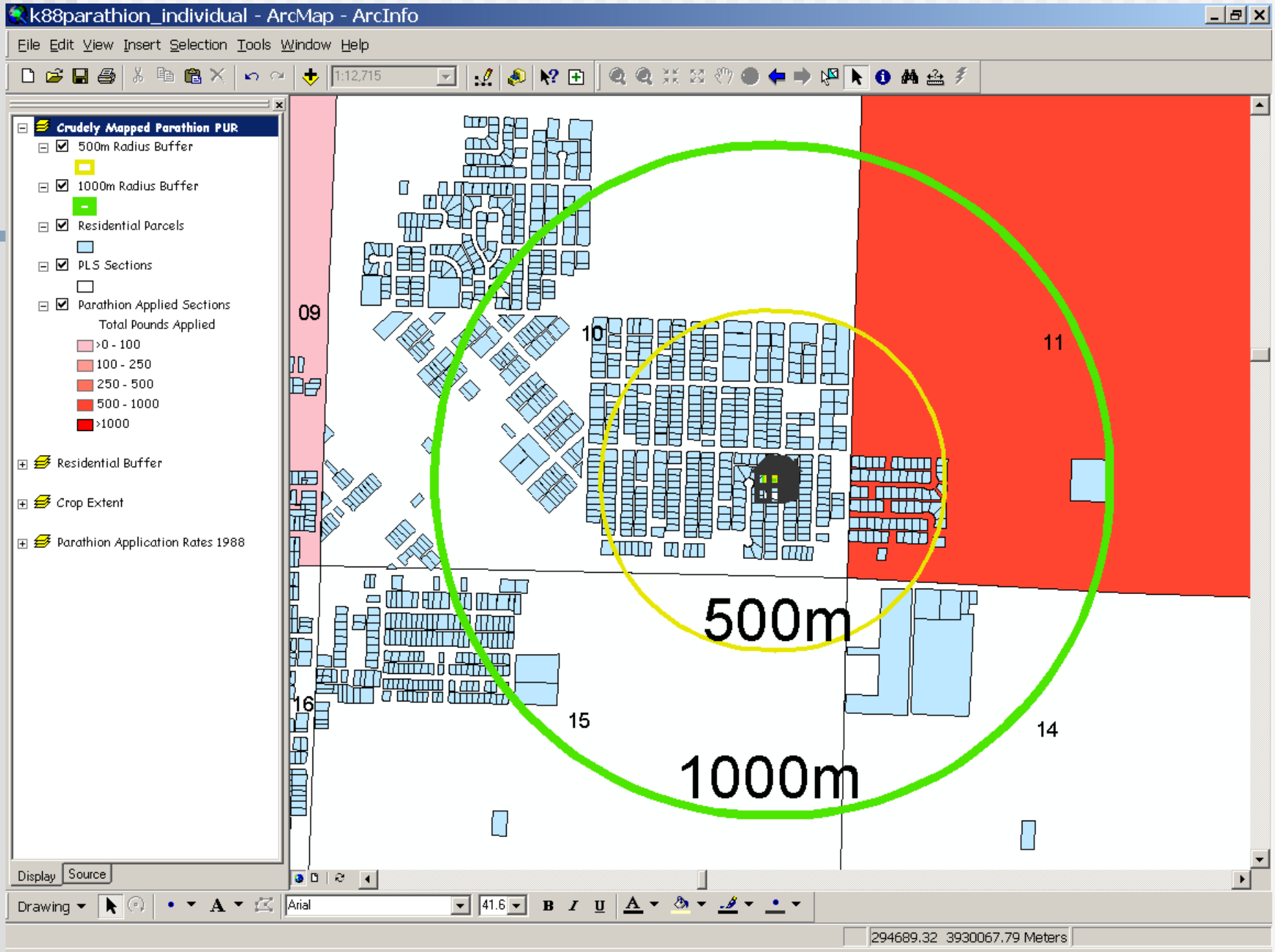
Establish a lifetime history of exposure to pesticides
- recollections of residential history
- historical exposure records for pesticide use (PUR)

Collect biological samples
- DNA for genotyping
- Organochlorine serum analyses
- Lymphocytes for GEP and oxidative stress markers

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Determining Pesticides Exposure

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DAT Polymorphisms, Maneb and Paraquat Exposure

Table 4. Associations with PD by number of risk alleles* in the 5Õand 3Õ rgion of DAT and residential paraquat and maneb exposure in the California Central Valley study

	Zero/Low			High		
Risk Alleles	Cases (n= 286)	Controls (n= 319)	OR[a] 95% CI	Cases (n= 38)	Controls (n= 15)	OR[a] 95% CI
0	65	80	ref	4	5	0.88 (0.22, 3.48)
1	99	117	0.98 (0.63, 1.52)	10	4	2.99 (0.88, 10.21)
2+	122	122	1.30 (0.85, 2.00)	24	6	4.53 (1.70, 12.09)

[a] *Odds Ratio (OR) adjusted for age (continuous), race, years of schooling, smoking (ever/never)*

Ritz et al, 2009

How can we prove that a toxin contributes to the pathogenesis of PD?

- A plausible mechanism of action.
- Association between a toxin and PD in epidemiological studies.
- Recapitulation of behavioral and pathological features in cellular and animal models.

Mitochondrial Dysfunction and PD

- MPTP is a complex I inhibitor
- Decreased complex I and II in brains and peripheral platelets in PD
- Mitochondrial-associated genes and PD (PINK1, DJ1, POLG, cybrids)

Rotenone Exposure and PD

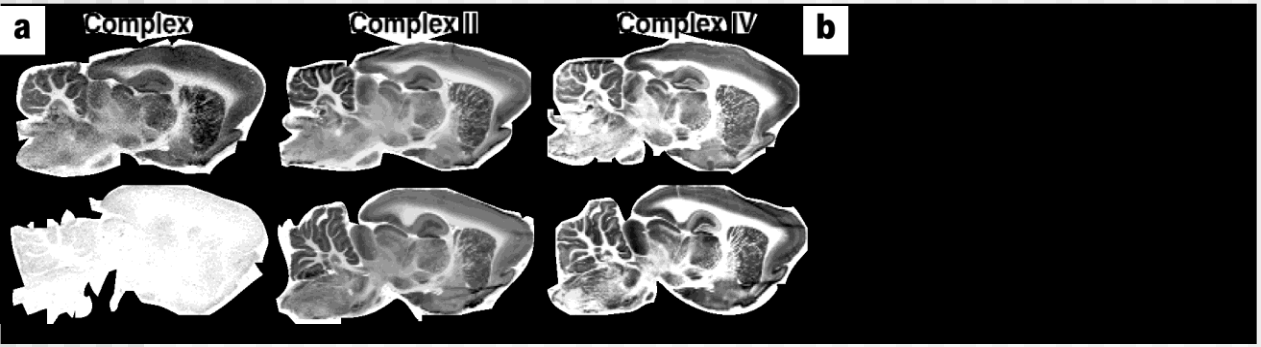
- Residential use more common than commercial use.
- A few case-control studies support increase incidence but not conclusive.
  - Dhillon et al 2008: OR 10.9 (2.5-48)
  - Agriculture Health Study: OR 1.7 (few cases)
  - Anecdotal reports

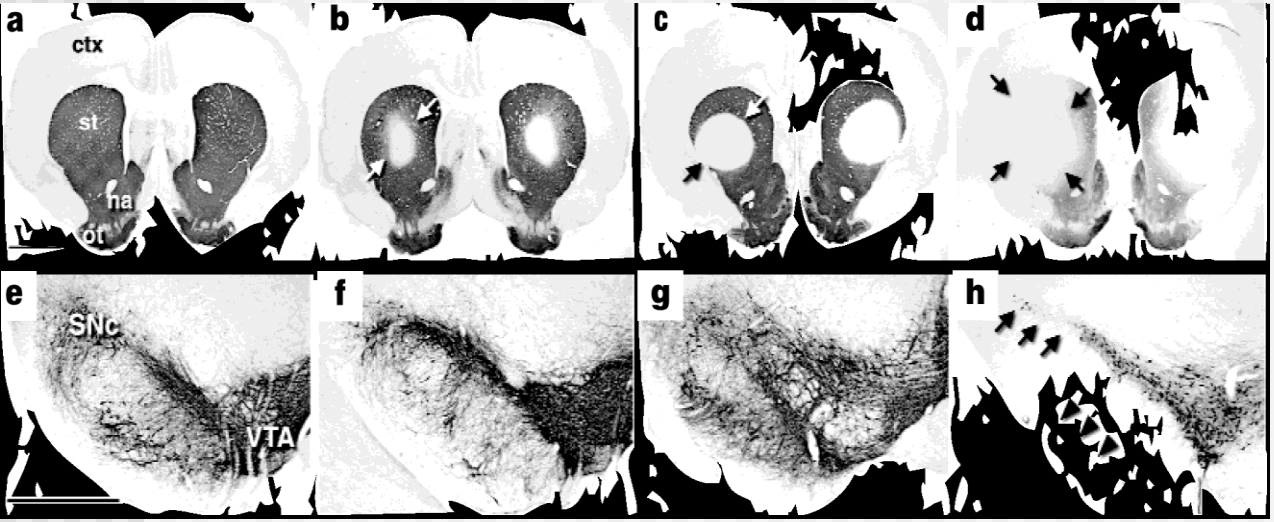
Mechanisms of Rotenone Toxicity

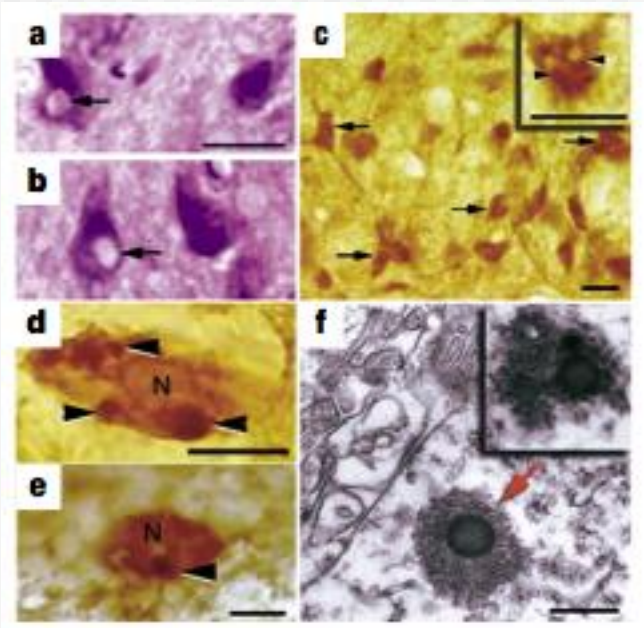
- Complex I inhibition leads to oxidative stress and energy failure at low concentrations
- Proteasome inhibition
- Microtubule inhibition

Chronic systemic pesticide exposure reproduces features of Parkinson's disease

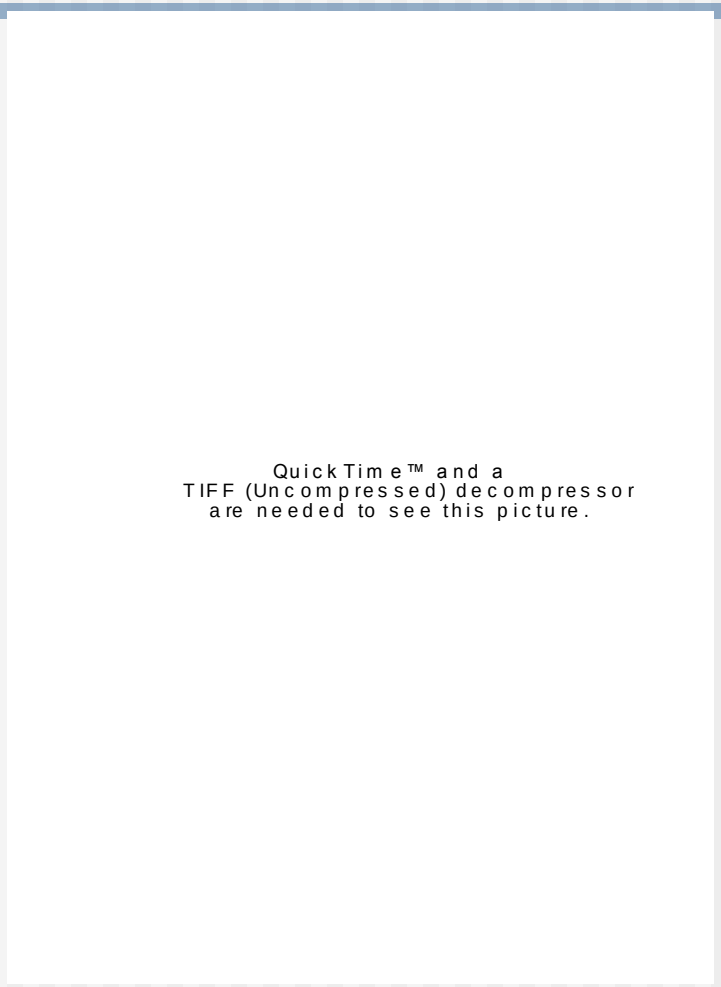
Ranjita Betarbet, Todd B. Sherer, Gillian MacKenzie, Monica Garcia-Osuna, Alexander V. Panov and J. Timothy Greenamyre







Progression of Parkinson's Disease Pathology Is Reproduced by Intragastric Administration of Rotenone in Mice



Francisco Pan-Montojo et al. PLOS One 2010

Proteasome Dysfunction and PD

- Proteasome-associated genes and PD (Parkin, UCH L1)
- Decreased activity in brains and blood in PD
- Alpha-synuclein is at least partially degraded by the proteasome.

Pesticides that Lead to UPS Inhibition

Rotenone
- Complex I inhibitor

Ziram and other dithiocarbamates
- Fungicide
- Multivalent interactions

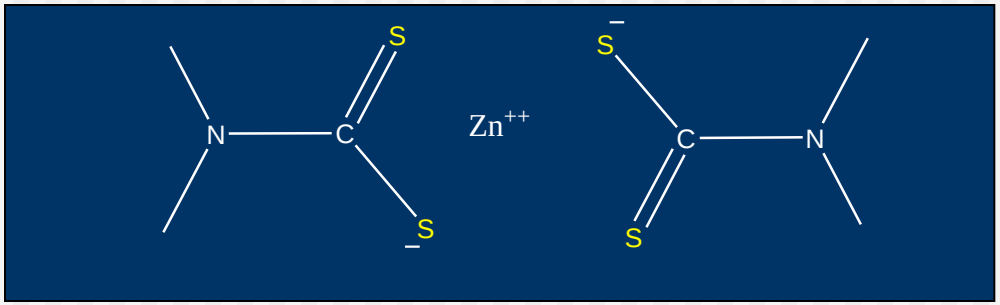
Benomyl
- Fungicide (Benzimidazole)
- Binds to tubulin

Dieldrin and Endosulfan
- Organochlorines (epoxicide)
- Inhibits GABA-gated chloride channels

Wang et al, 2006

Ziram and Related Compounds

- Dimethyl- and diethyldithiocarbamates
- Widely used fungicides on fruits and nuts.
- Approximately 15 million lbs were used in the US in 2002.



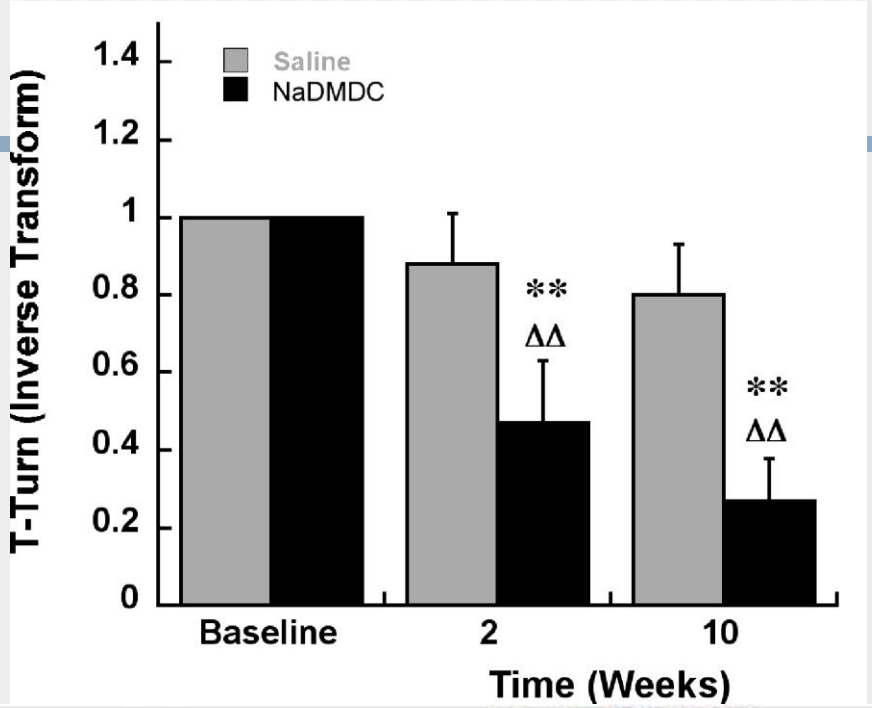
Ziram Exposure and PD (PEG Study)

Table 3. Ambient Occupational and Residential Maneb, Ziram, and Paraquat Exposure by Time Window of Exposure and Age of Central Valley of California Study Population

	Occupational**				Residential ***			
	Case	Control	OR*	95% CI	Case	Control	OR*	95% CI
Ziram and paraquat exposure								
1974-1999 Time Window								
60 years old or younger								
No exposure to ziram or paraquat	28	53	1.00	ref	21	38	1.00	ref
Ziram or paraquat exp only	30	29	1.90	(0.92,3.94)	35	37	1.66	(0.80,3.47)
Ziram and paraquat exp	19	5	5.97	(1.94,18.33)	21	12	2.76	(1.09,7.00)
Over 60 years old								
No exposure to ziram or paraquat	137	141	1.00	ref	103	99	1.00	ref
Ziram or paraquat exp only	84	76	1.17	(0.78,1.76)	113	112	0.88	(0.59,1.31)
Ziram and paraquat exp	64	37	1.93	(1.18,3.15)	69	43	1.41	(0.86,2.29)

Wang A, Costello S, Cockburn M, Zhang X,Bronstein ,Ritz B (submitted)

Systemic Administration of Ziram



region	mean ± SEM (n=5)			
	2W		2 + 9W	
	control	treated	control	treated
TH immunoreactivity fiber density				
dorsolateral	1515 ± 207	1456 ± 221	1934 ± 299	1951 ± 269
dorsomedial	1048 ± 149	843 ± 117	1435 ± 199	1358 ± 130
ventrolateral	1439 ± 142	1290 ± 140	1833 ± 254	1409 ± 166*
ventromedial	815 ± 103	696 ± 62	1073 ± 174	946 ± 152
TH-IR positive neurons				
SNc	7980 ± 491	7832 ± 574	8251 ± 214	7897 ± 247

* $p \leq 0.05$

Chou et al, 2009

Other Pesticides and PD

- Paraquat
  - Associated with increased risk of PD (in combination with maneb and ziram)
  - Mechanism: Redox-cycling?
  - Animal model; DA cell loss and behavior
- Benomyl
  - Associated with increased risk of PD (PEG and Ag Health)
  - Mechanisms: UPS-I, ALDH-I, and MT-I
  - Primary culture DA loss but no animal models

What about Agent Orange ?

The New York Times
October 12, 2009

- "…the Department of Veterans Affairs plans to add Parkinson's disease, ischemic heart disease and hairy-cell leukemia to the growing list of illnesses presumed to have been caused by Agent Orange, the toxic defoliant used widely in Vietnam."

Agent Orange

- Code name for a defoliant herbicide used during the Vietnam War.
- Name derived from orange drums it is shipped in
- Mixture of 2 herbicides:
  - 2,4-dichlorophenoxyacetic acid (2,4-D)
  - 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).
- Contaminant: 2,3,7,8-TCDD (dioxin)

Herbicide use during Vietnam War

- **19 million gallons of herbicides (11.2 of Agent orange) sprayed over 3.6 million acres in Vietnam 1962 – 71.**
- **86% from large scale spraying operations (operation Ranch Hand) – good documentation**
- **Rest from trucks and back packs – poor documentation**

Source: Science 2007; 315:176-79

Agricultural Health Study

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 classification†	Chemical†	Prevalent PD						Incident PD					
		Cases		Controls		OR‡,§	95% CI‡	Cases		Controls		OR§	95% CI
		No.	%	No.	%			No.	%	No.	%		
Herbicides													
Chloroacetanilide	Alachlor	30	39	25,599	34	0.8	0.5, 1.5	34	49	17,359	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
Dinotroaniline	Pendimethalin	23	32	21,386	28	1.4	0.8, 2.6	17	25	13,893	27	0.7	0.4, 1.2
	Trifuralin	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
Organophosphorus	Glyphosate	45	55	46,687	60	1.0	0.6, 1.7	49	67	32,686	60	1.1	0.6, 2.0
Phenoxyacetate	2,4-D‡	47	58	40,405	52	0.9	0.5, 1.8	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	6,409	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
Sulfonyl urea	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	38	20,879	28	1.5	0.8, 3.0	19	28	14,251	28	0.5	0.3, 1.0

Agent Orange Neurotoxicity

- 2,4-D: mechanism of injury is unknown and evidence for effects on dopaminergic system is inconsistent
  - Inhibits microtubule assembly in cerebellar granule cell culture
  - Mild, transient locomotor effect in acutely exposed rats
  - Striatal dopamine depletion in rats administered intracerebral injection, but no toxicity noted in mice administered sub q injection.
- TCDD: mechanism of injury is unknown but linked to oxidative stress

Conclusions: Do pesticides contribute to the pathogenesis of PD?

- Plausible mechanisms of action. YES
- Association between a toxin and PD in epidemiological studies. YES
- Recapitulation of behavioral and pathological features in cellular and animal models. YES

Question & Answers

Thank You