Using Gene-Environment Interactions and Omics Approaches to Understand Neurodegenerative Disease Etiology

UCLA FIELDING SCHOOL OF PUBLIC HEALTH

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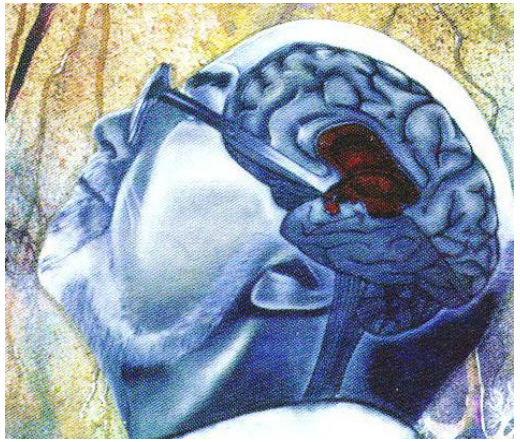
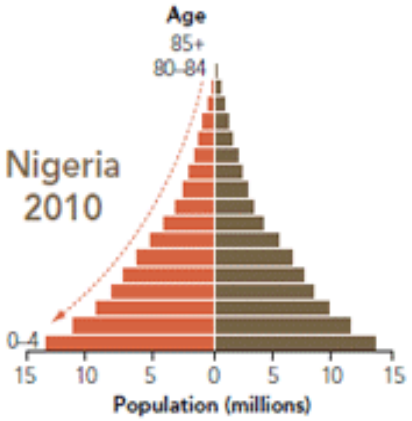
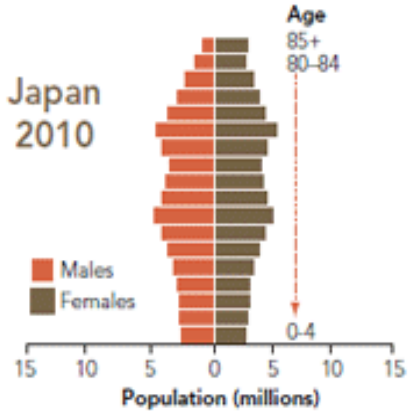
COEH Center for Occupational & Environmental Health

Why neurodegeneration?







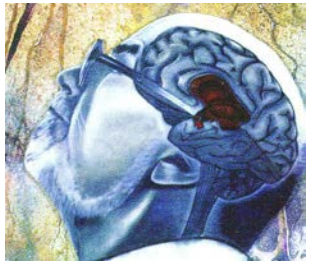
Aging may constitute a heightened state of vulnerability to neurotoxic agents

Healthy aging is important

- Expanding human lifespan & ever growing number of elderly

- Restrictions in cognitive and physical (motor) abilities, and mood have large impact on health related quality of life, disability, and high care giver burden

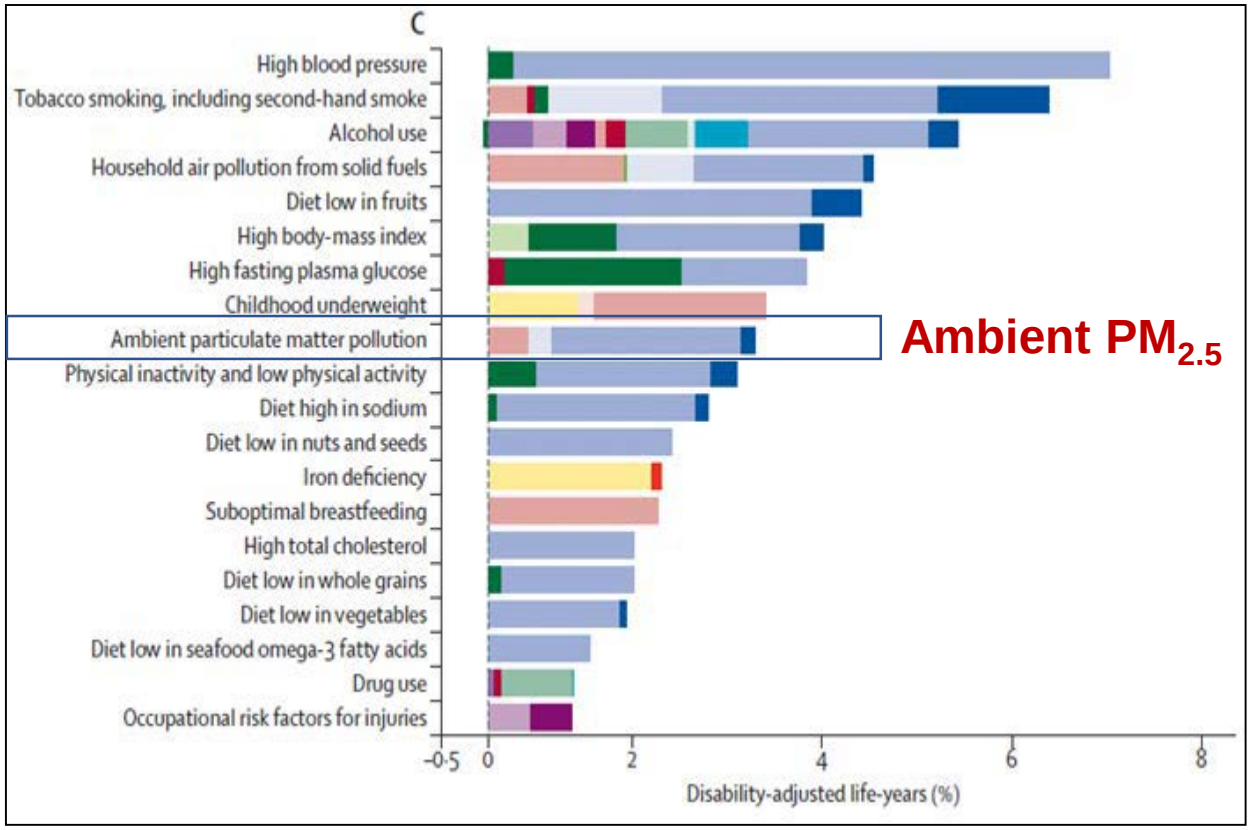
Environmental factors and neurodegeneration

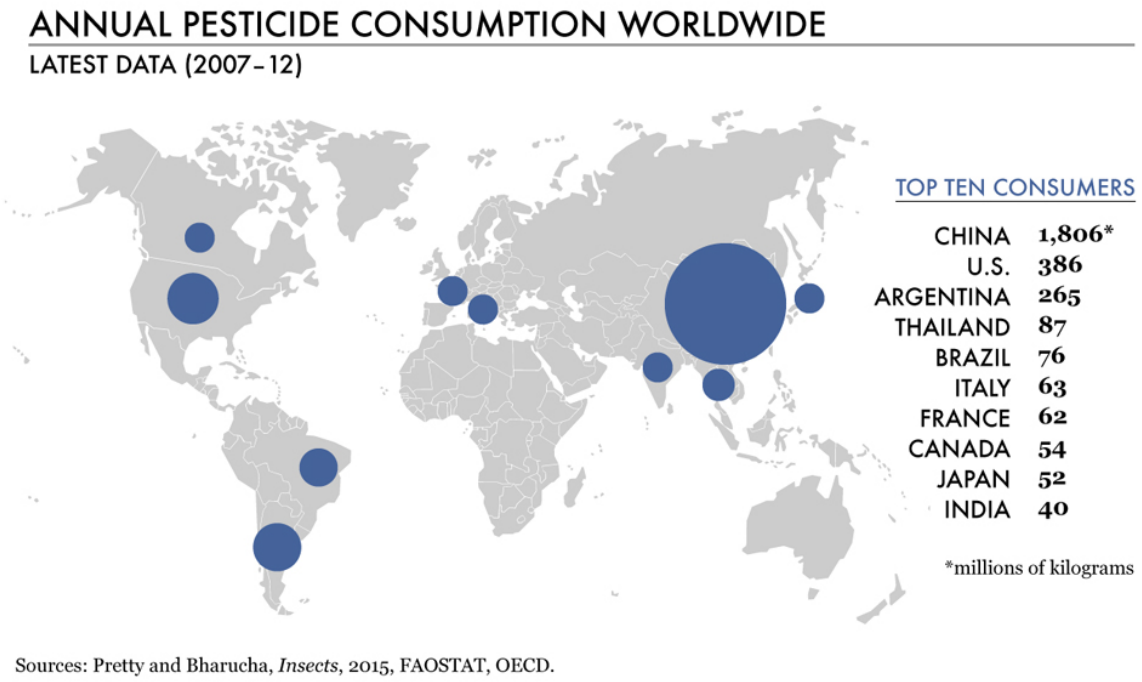


World Health Organization

Global Burden of Disease 2010 identifies air pollution

Fine particles contribute annually to over 3.2 million premature deaths worldwide and over 74 million years of healthy life lost





Sources: Pretty and Bharucha, *Insects*, 2015, FAOSTAT, OECD.

Pesticides, why we should care…

Organophosphates:

Insecticides previously and currently used in agriculture have known acute neurotoxicity

1999-2000 NHANES (US population survey, aged 6-59) urine pesticide metabolites:

- Chlorpyrifos (TCPY) > **96%** of samples
- Diazinon (IMPY) **29**% samples

Neonicotinoids:

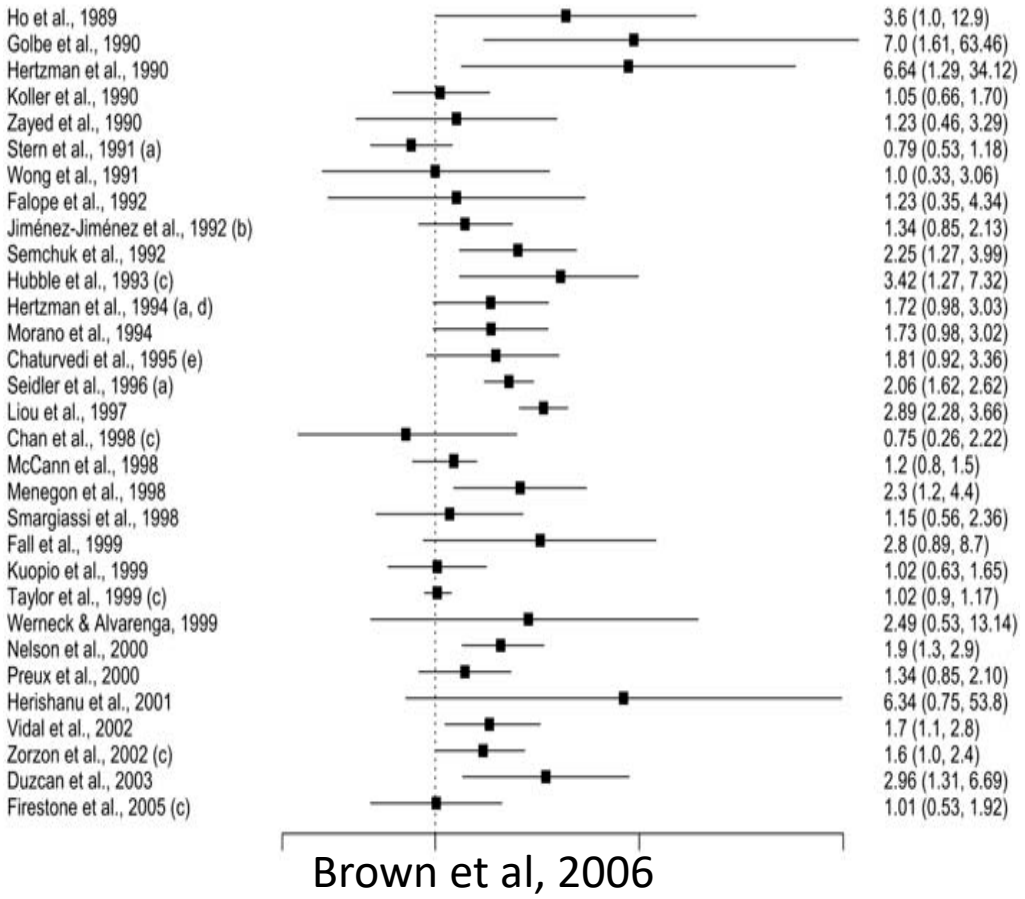
Insecticides currently widely used in agriculture

2015-2016 NHANES (US population survey, participants aged 6-59) urine pesticide metabolites :

- **Half** of the U.S. population 3+ years of age are exposed to neonicotinoids

Barr et al.. Environ Res. 2005 Nov;99(3)
Ospina et al. . Environ. Res. 2019 Nov (176)

Pesticides linked to PD onset



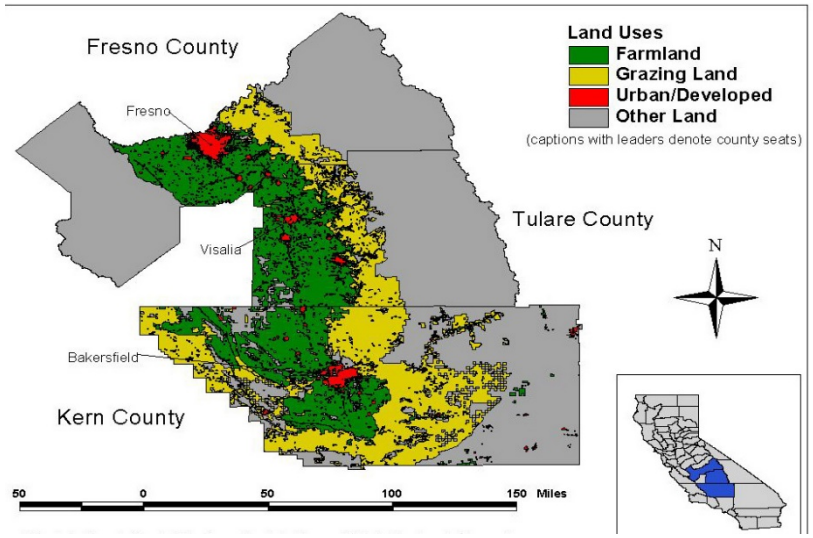
Brown et al, 2006

Meta-analyses (Priyadarshi et al, 2000, 2001):

Pesticide exposure (study n=19)
Odds Ratio (OR)= 1.9 (1.5 – 2.5)

Rural living (study n=16)
OR = 1.6 (1.2 – 2.1)

Farming (study n=18)
OR = 1.4 (1.1 – 1.9)



Parkinson's, Environment and Gene Study (PEG)

funded by
NIH/ National Institute of Environmental Health Sciences
RO1ES10544-A10, 5P01ES016732

Case-control study in three rural California counties that enrolled and interviewed:

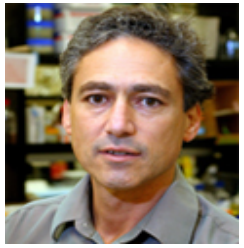
- 849 iPD newly diagnosed cases; were clinically evaluated by UCLA movement disorder neurologist 1-5 times over a 14-year period (Jan 2001- 2015)
- 1,011 population controls and 193 unaffected sibling/household controls
- Biosamples:
  - blood: DNA, RNA, plasma, serum,
  - saliva
  - urine
  - feces

Curr Envir Health Rpt
DOI 10.1007/s40572-016-0083-2

SUSCEPTIBILITY FACTORS IN ENVIRONMENTAL HEALTH (B RITZ, SECTION EI

Of Pesticides and Men: a California Story of Genes and Environment in Parkinson's Disease

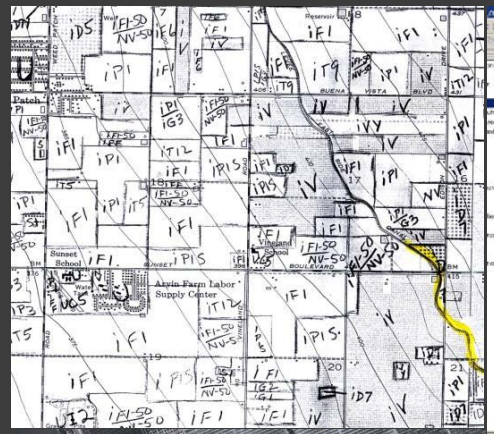
Beate R. Ritz [1,2,3] · Kimberly C. Paul [1] · Jeff M. Bronstein [3]

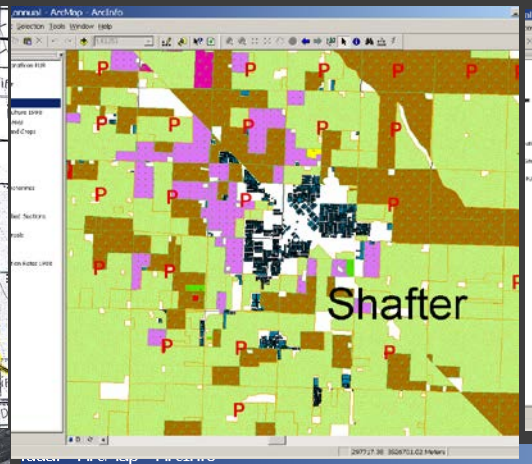


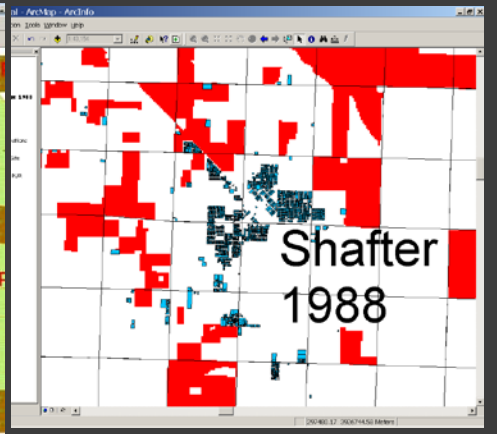
California Agricultural Pesticide Use Reports since 1974

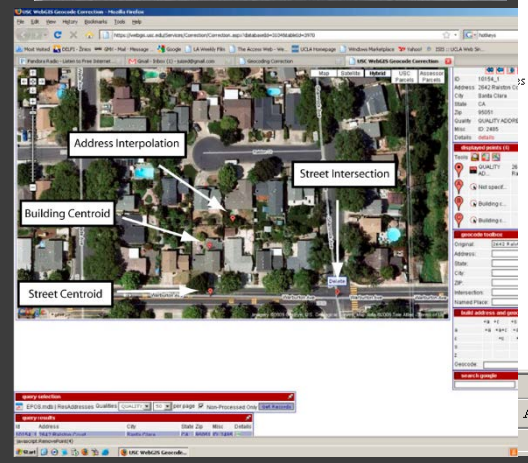
Data provided per 1 square mile land section

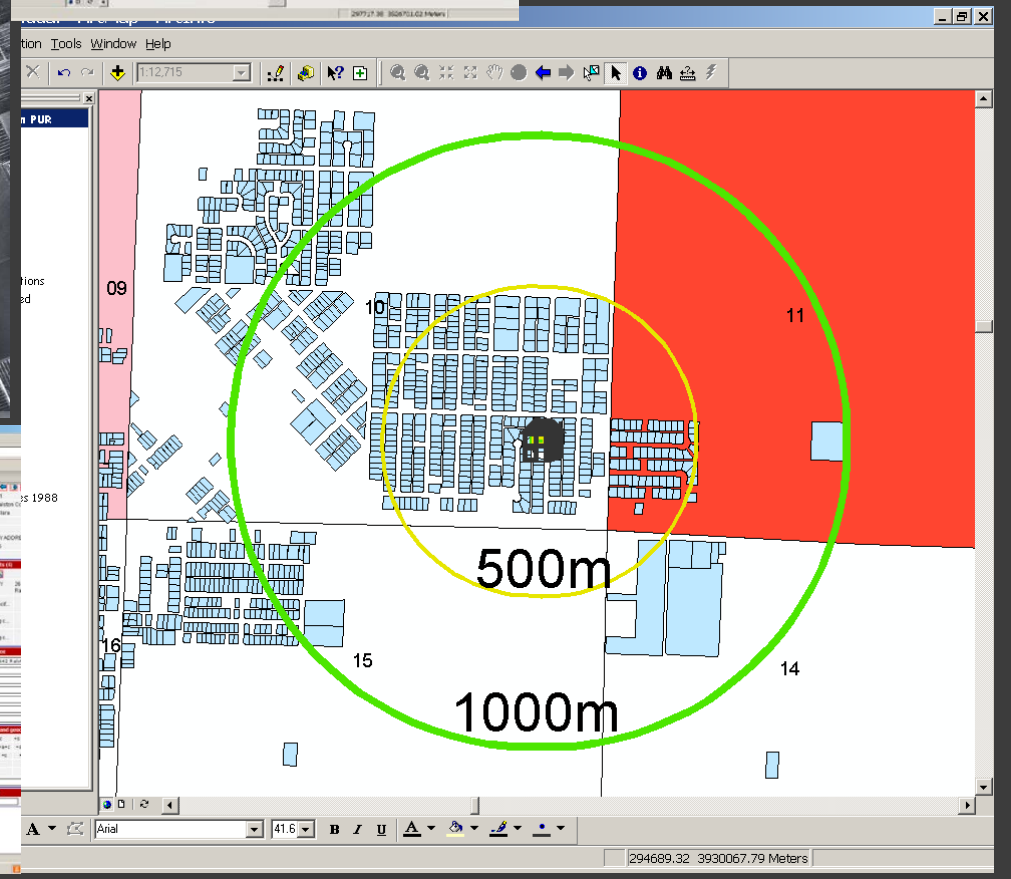
- County: Kern
- Location: 15M28S27E19
- Application date: 2/23/1989
- Commodity: 2503 (Grapes)
- Method: Ground
- Treated: 424 acres
- Product applied: 155 gallons
- Chemical: 00459 (Parathion)
- Percentage: 80%
- Active Ingredient Pounds: 1,241











California Department of Pesticide Regulation

California Agricultural Pesticide Use Reporting (PUR) exists since 1974

Data provided per 1 square mile land section

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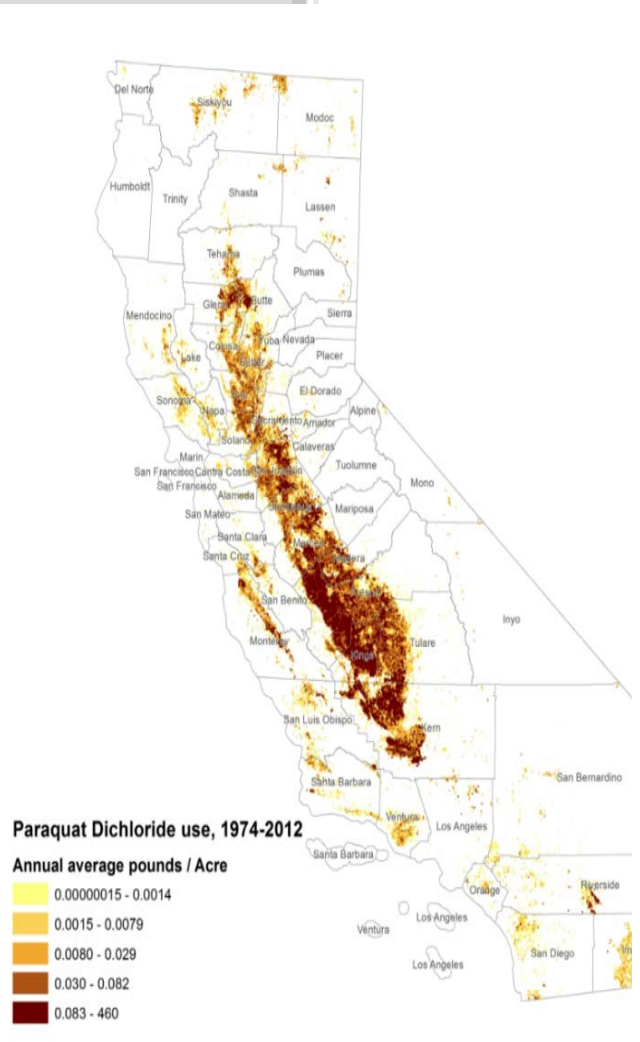
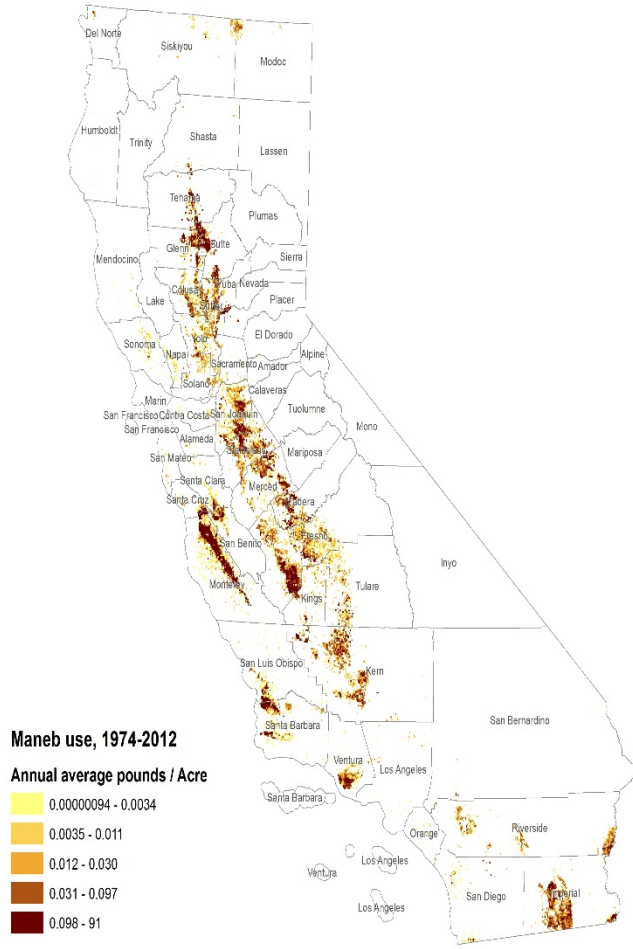
Paraquat

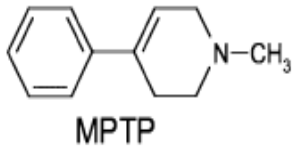


Maneb

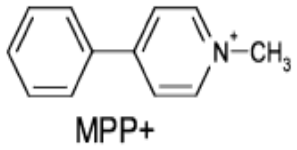




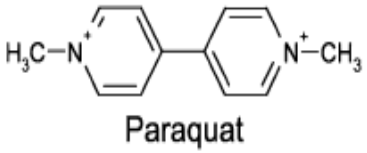
Paraquat and Parkinson's disease



MPTP

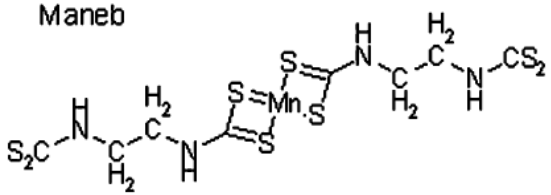


MPP+



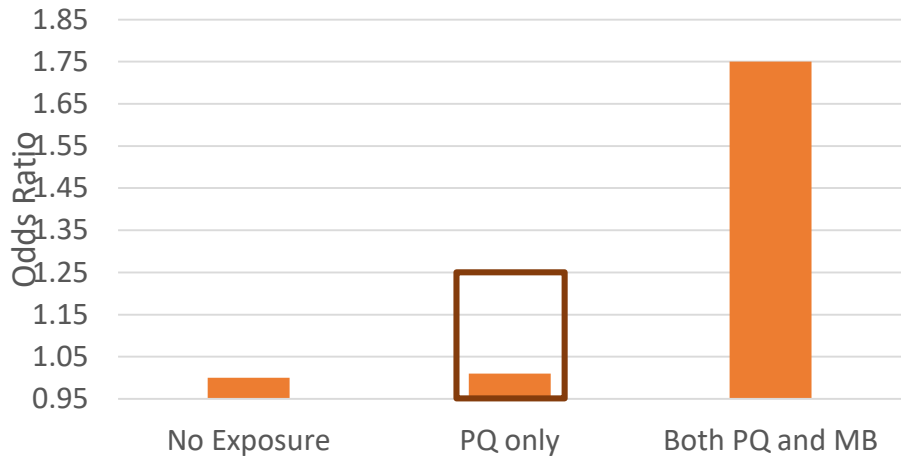
Paraquat

Maneb



Animal Co-Exposure to paraquat & maneb

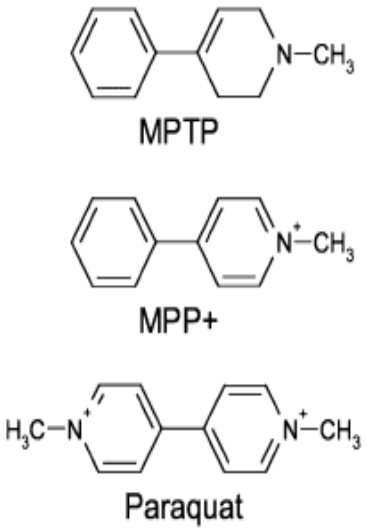
Paraquat & Maneb:
residential address
occupational address

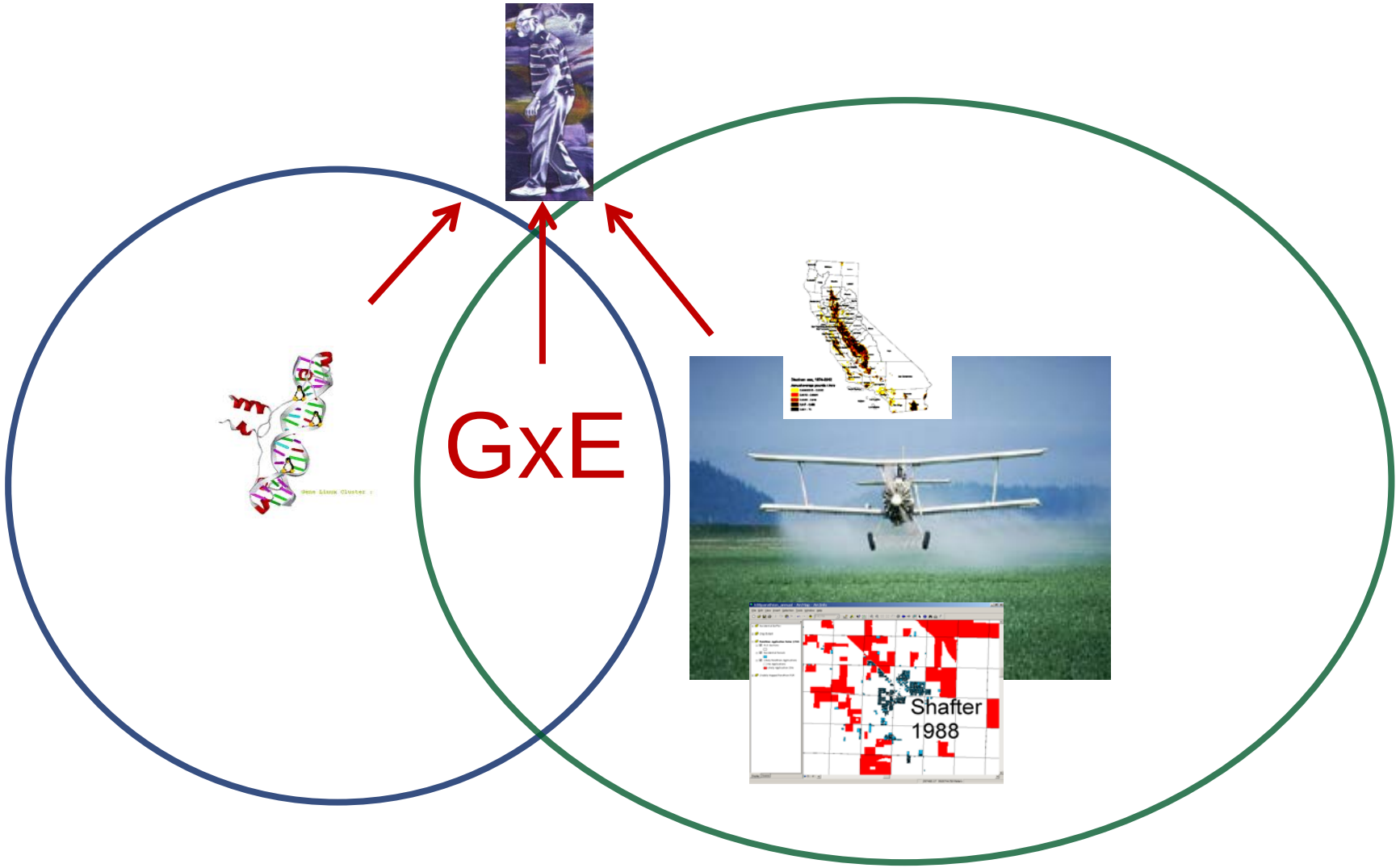


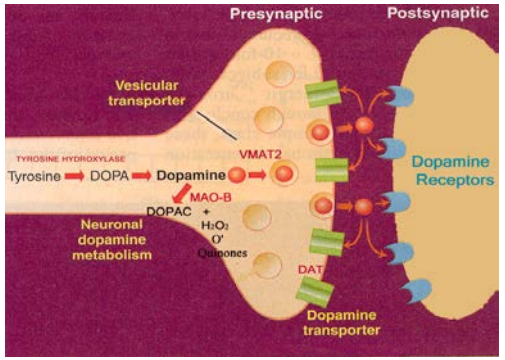
Costello et al.. Am J Epidemiol. 2009 169(8):919-26.

Thiruchelvam M, McCormack A, Richfield EK, Baggs RB, Tank AW, Di Monte DA, Cory-Slechta DA (2003) Age-related irreversible progressive nigrostriatal dopaminergic neurotoxicity in the paraquat and maneb model of the Parkinson's disease phenotype. Eur J Neurosci 18:589–600

Gene x Environment Interactions
Paraquat Herbicide

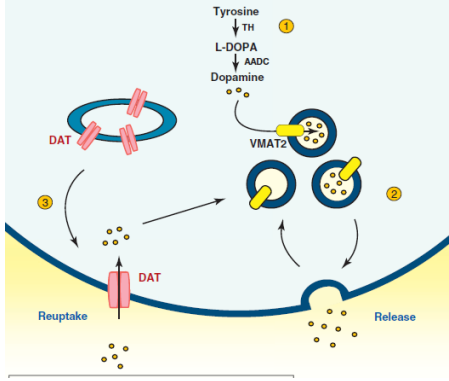


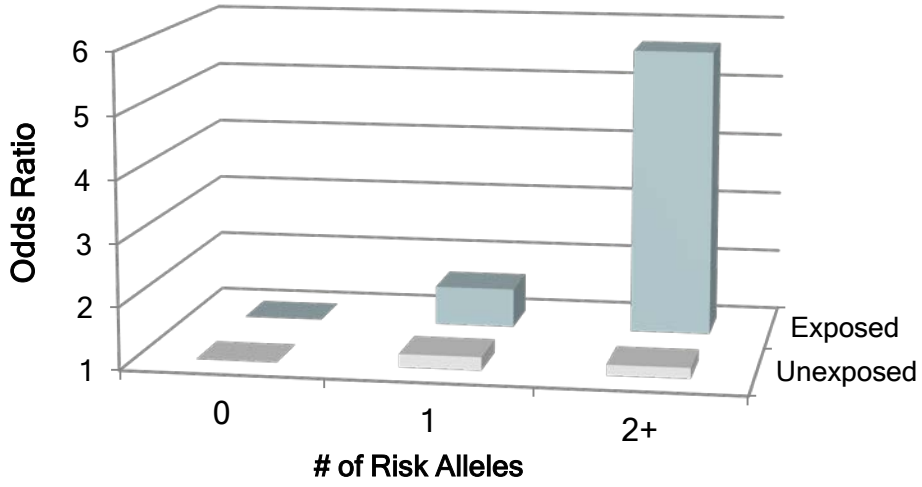




DAT1 gene variants increase PD susceptibility with paraquat/maneb pesticide exposures:

First independent replication of a GxE interaction in PD



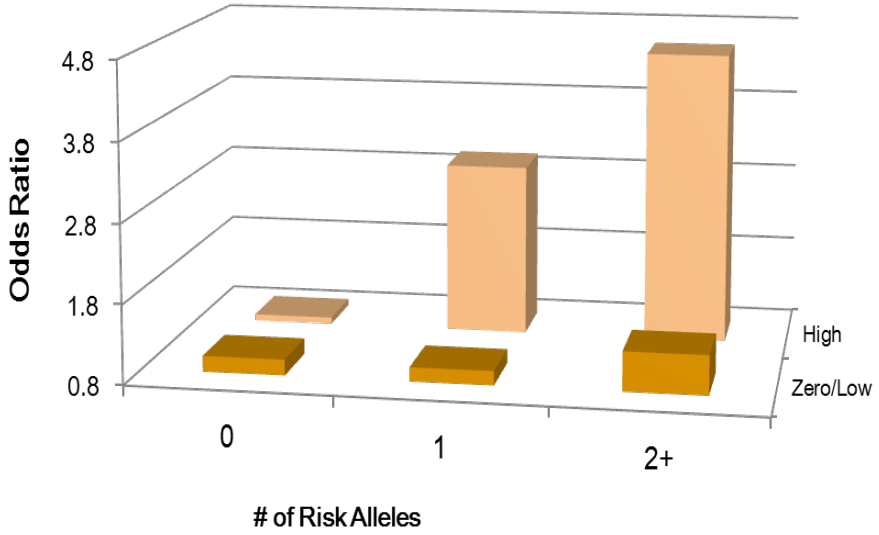


Kelada et al. HMG 2006

Human Molecular Genetics, 2006, Vol. 15, No. 20 3055–3062
doi:10.1093/hmg/ddl247
Advance Access published on September 8, 2006

5′ and 3′ region variability in the dopamine transporter gene (*SLC6A3*), pesticide exposure and Parkinson's disease risk: a hypothesis-generating study

Samir N.P. Kelada[1,*], Harvey Checkoway[1,2], Sharon L.R. Kardia[4], Christopher S. Carlson[5], Paola Costa-Mallen[1], David L. Eaton[1], Jordan Firestone[1,3], Karen M. Powers[1], Phillip D. Swanson[3], Gary M. Franklin[1,3], W.T. Longstreth Jr[2,3], Terri-Smith Weller[1], Zahra Afsharinejad[1] and Lucio G. Costa[1,6]

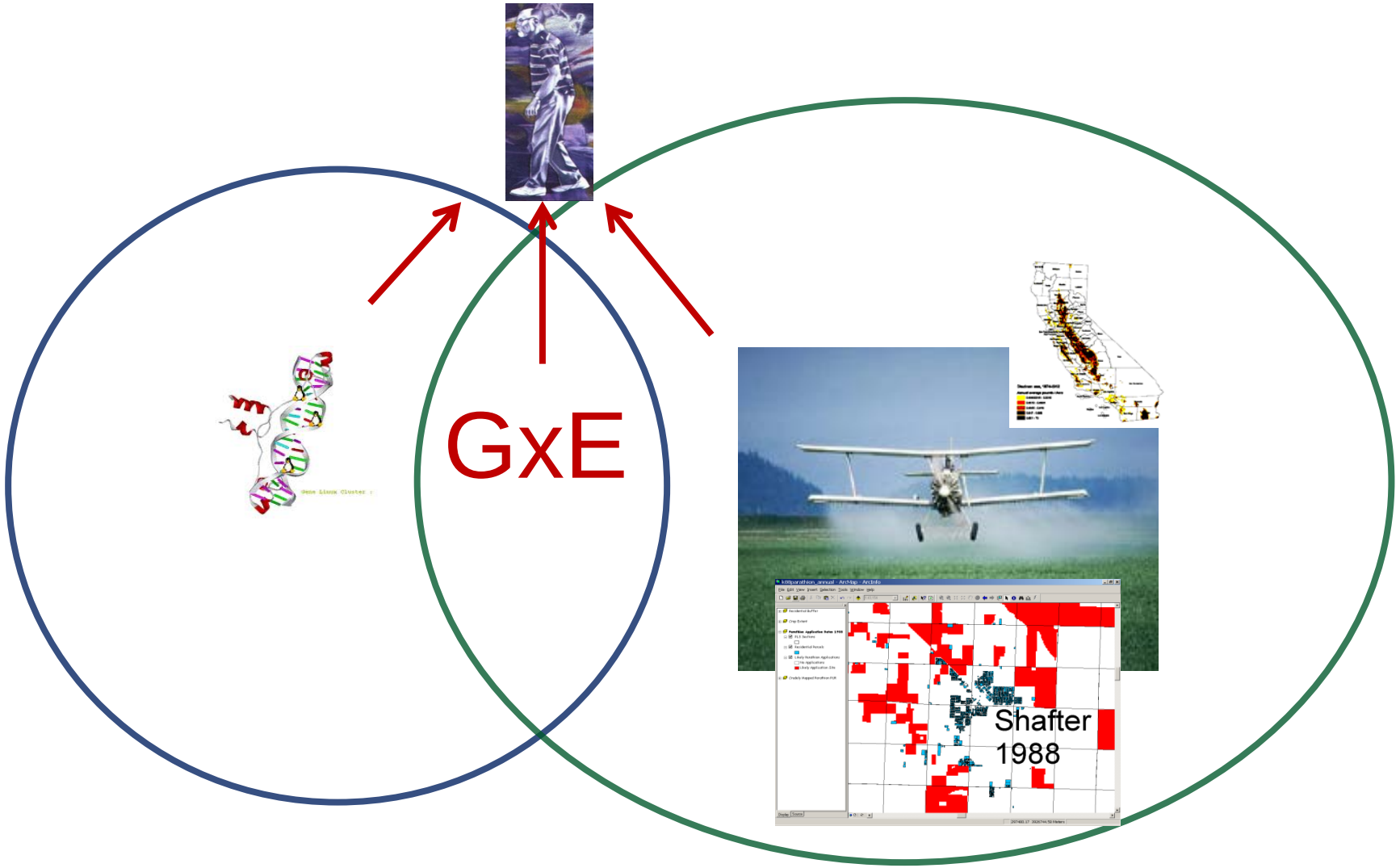


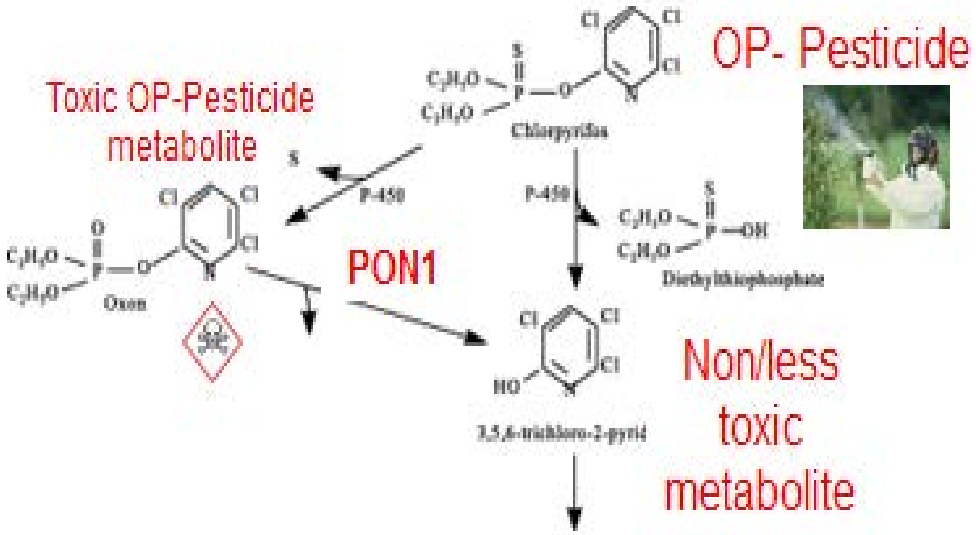
Ritz et al. EHP 2009

Dopamine Transporter Genetic Variants and Pesticides in Parkinson's Disease

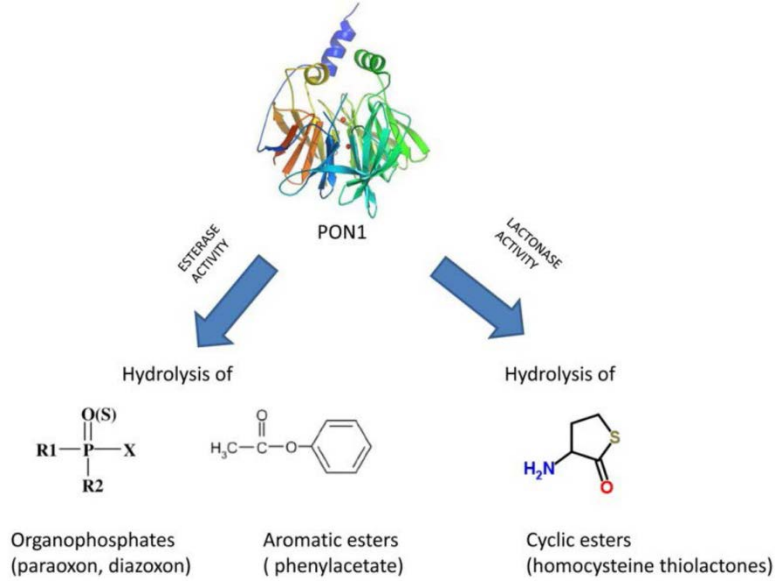
Beate R. Ritz,[1,2,3] Angelika D. Manthripragada,[1] Sadie Costello,[1,4] Sarah J. Lincoln,[5] Matthew J. Farrer,[5] Myles Cockburn,[6] and Jeff Bronstein[3]

Gene x Environment Interactions
Organophosphate pesticides





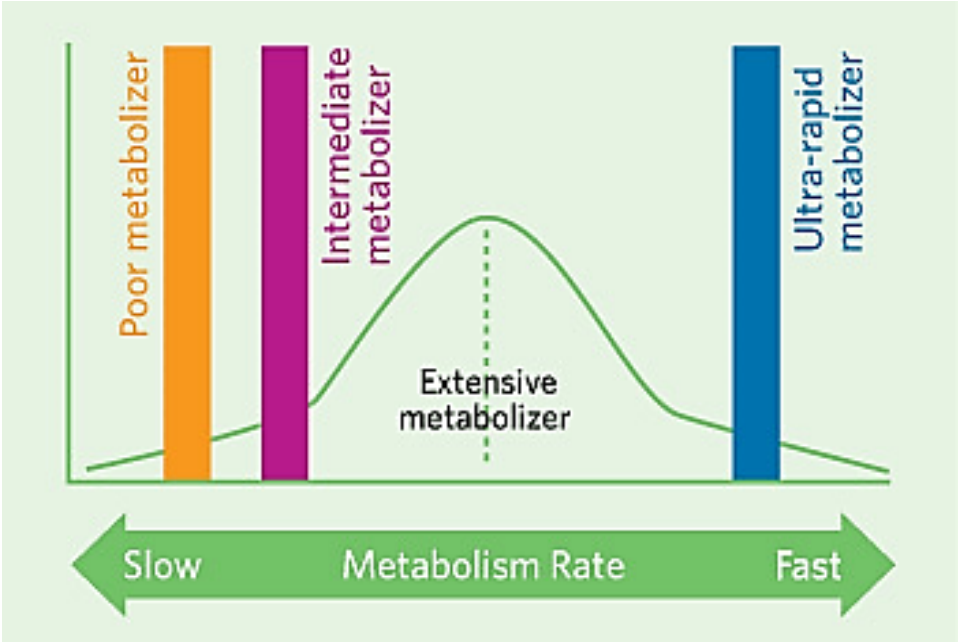
OPs are detoxified by the paraoxonase (PON1) activity of the PON1 hydrolyzing enzyme



10- to 40-fold inherited differences in PON1 enzyme activity in serum - **2** common polymorphisms in the *PON1* gene contribute to this difference

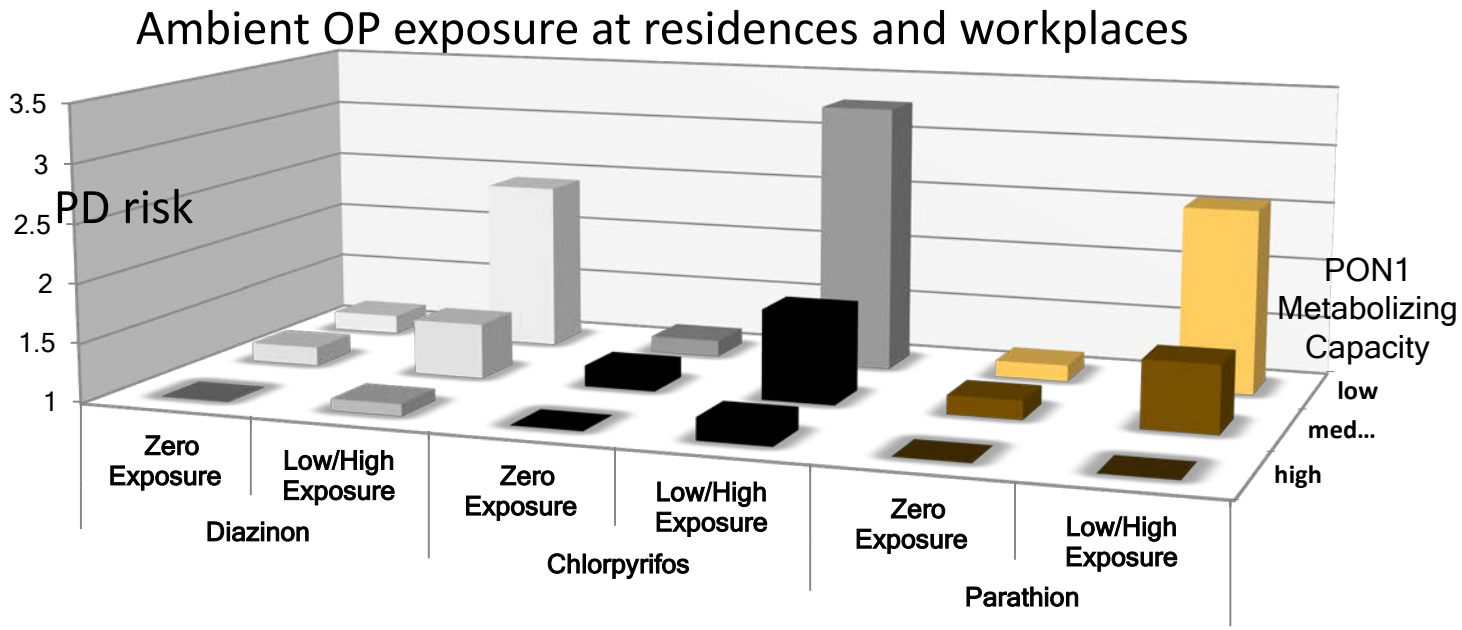
PON1 - genotypes	55-LL	55-LM	55-MM
192-QQ	15.6 ± 6.0	11.2 ± 5.0	6.35 ± 1.50
192-QR	18.1 ± 7.7	14.3 ± 2.8	-
192-RR	22.0 ± 9.4	16.4 ± 0.0	-

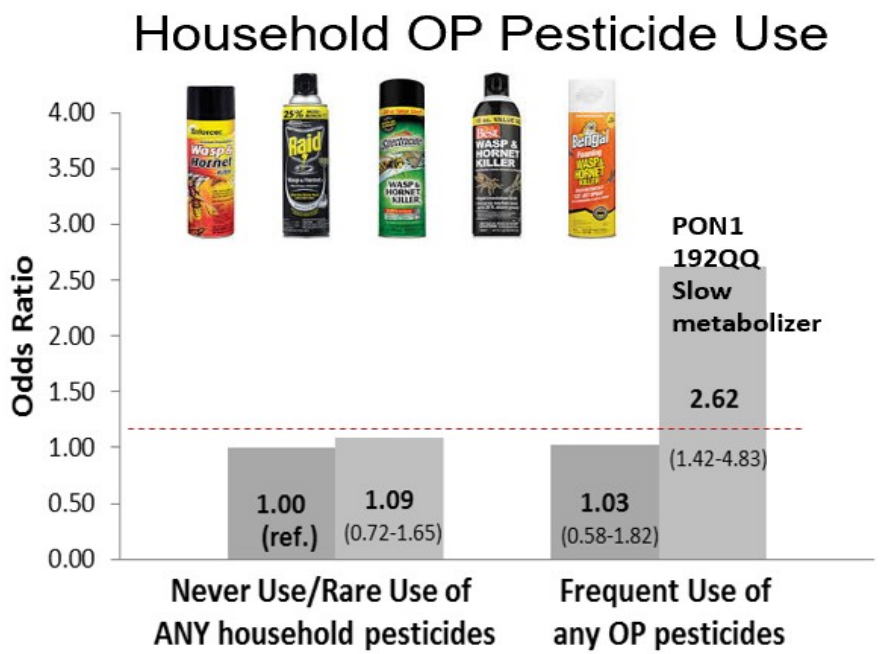
PON1 serum activities for diazoxon (50 mM) by genetic polymorphism: M55L and Q192R *[O'Leary et al. 2006]*



PD Risk by PON1 Metabolizers Status & OP-Pesticide Exposure

(genetic variants *PON1L55M & PON1Q192R*)





Functional paraoxonase 1 variants modify the risk of Parkinson's disease due to organophosphate exposure

Pei-Chen Lee [a,b], Shannon L. Rhodes [a], Janet S. Sinsheimer [c], Jeff Bronstein [d], Beate Ritz [a,d,*]

Environment International 56 (2013) 42–47

International Journal of Epidemiology 2013;1–10
doi:10.1093/ije/dyt170

Household organophosphorus pesticide use and Parkinson's disease

Shilpa Narayan,[1] Zeyan Liew,[1] Kimberly Paul,[1] Pei-Chen Lee,[1] Janet S Sinsheimer,[2] Jeff M Bronstein[3] and Beate Ritz[1,3]*

Environment International 107 (2017) 75–81

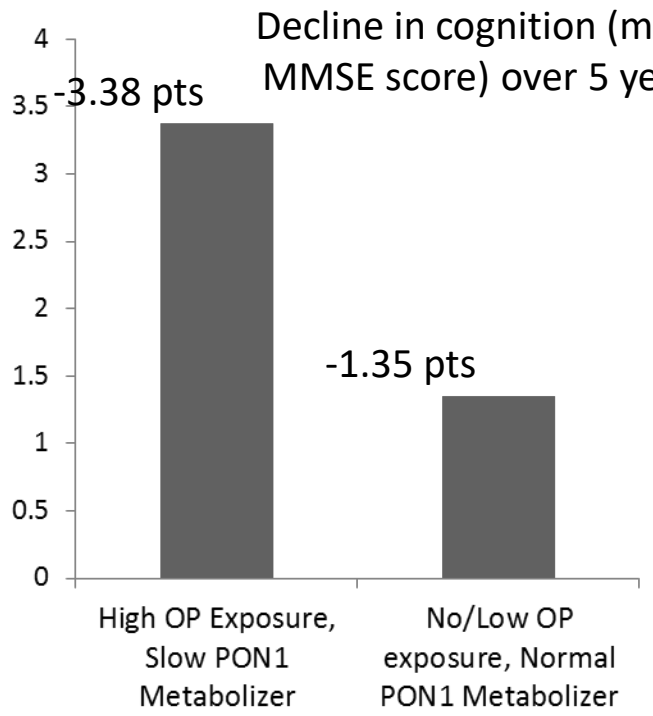
Organophosphate pesticides and *PON1* L55M in Parkinson's disease progression

Kimberly C. Paul[a], Janet S. Sinsheimer[b,c], Myles Cockburn[d], Jeff M. Bronstein[e], Yvette Bordelon[e], Beate Ritz[a,e,*]

OPs interact with *PON1 (55MM)* to speed up **cognitive decline in PD**

Repeated measure model predicting change in MMSE score (0-30) over time (age in years)

Characteristic	Outcome 1: MMSE		
	β coefficient	95% CI	*P* value
Age	− 0.27	(− 0.39, − 0.15)	< 0.0001
High OP exposure * age	− 0.06	(− 0.11, − 0.01)	0.037
Slower PON1 metabolizer * age	0.005	(− 0.04, 0.05)	0.836
High OP exposure	− 0.09	(− 0.61, 0.43)	0.730
Slower PON1 metabolizer	− 0.38	(− 0.95, 0.19)	0.190
High OP exposure * slower PON1 metabolizer	− 1.26	(− 2.43, − 0.09)	0.036



Abbreviations: MMSE = Mini-Mental State Exam; UPDRS = Unified Parkinson's Disease Rating Scale; GDS = Geriatric Depression Scale.
Models also controlled for age at PD diagnosis, age * age at PD diagnosis, sex, European ancestry, years of schooling, and age * years of schooling.

Environmental Research 160 (2018) 132–139

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

OPs are related to Cognitive Decline & Alzheimer dementia in elderly Hispanics (SALSA cohort)

Cognitive decline, mortality, and organophosphorus exposure in aging Mexican Americans

Kimberly C. Paul[a], Chenxiao Ling[a], Anne Lee[b], Tu My To[b], Myles Cockburn[c], Mary Haan[b,1], Beate Ritz[a,d,1,*]

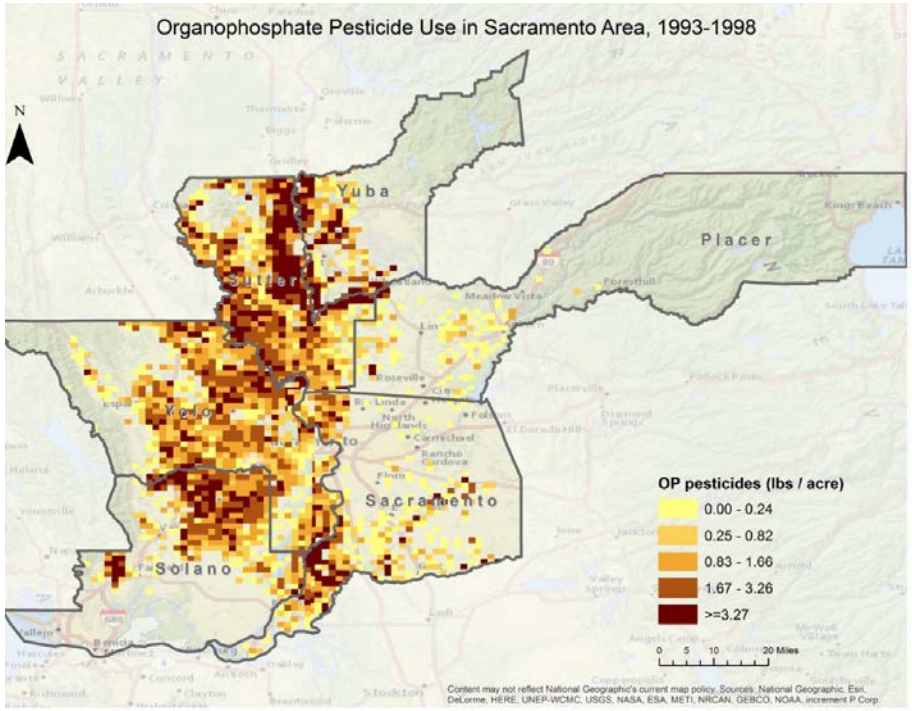


Table 2
Regression coefficients (longitudinal models: log (errors on Modified Mini Mental State Exam)) and ha and time to dementia/CIND and mortality.

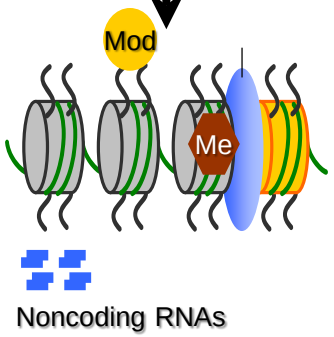
Parameters	Exposure Assessment 1: OP Count, continuous (per 1 unit, range 0–9)		
Longitudinal Model	**β**	**95% CI**	**P-value**
OP Exposure	−0.020	(−0.047, 0.007)	0.163
Time (y)	0.064	(0.046, 0.082)	< .0001
OP Exposure x time	0.006	(0.0001, 0.012)	0.030
Cox Models –Time to Event	**HR**	**95% CI**	**P-value**
Time to Dementia/CIND	1.09	(0.94, 1.25)	0.260
Time to Death	1.12	(1.03, 1.21)	0.006

Measure Methylation Changes after long term OP exposures
PEG Population-Based Study with 839 samples

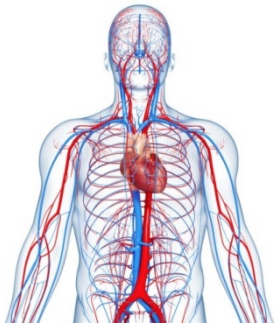
Ambient Organophosphate Pesticide Exposure Assessment

Epigenetic Changes

Exposure Related Alterations

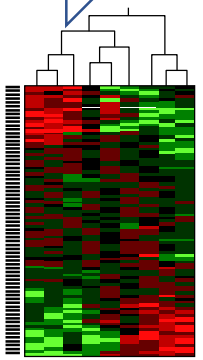


Measurement



Blood or saliva based genome-wide DNA Methylation for 839 study participants

Analysis



OP Epigenetic Profile (EWAS): Differentially Methylated CpGs

Gene Set Overrepresentation

70 CpGs (EWAS p<10e-07)

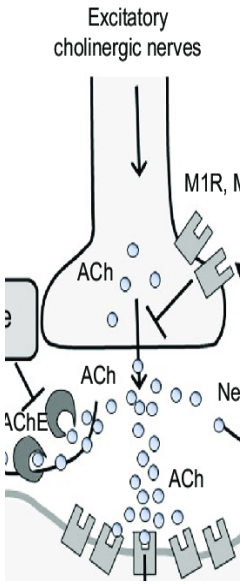
Nicotinic acetylcholine receptor signaling pathway

1077 CpGs (EWAS p<5e-04)

Muscarinic acetylcholine receptor 1&3 signaling pathway

GABA-B receptor II signaling

Endothelin signaling pathway



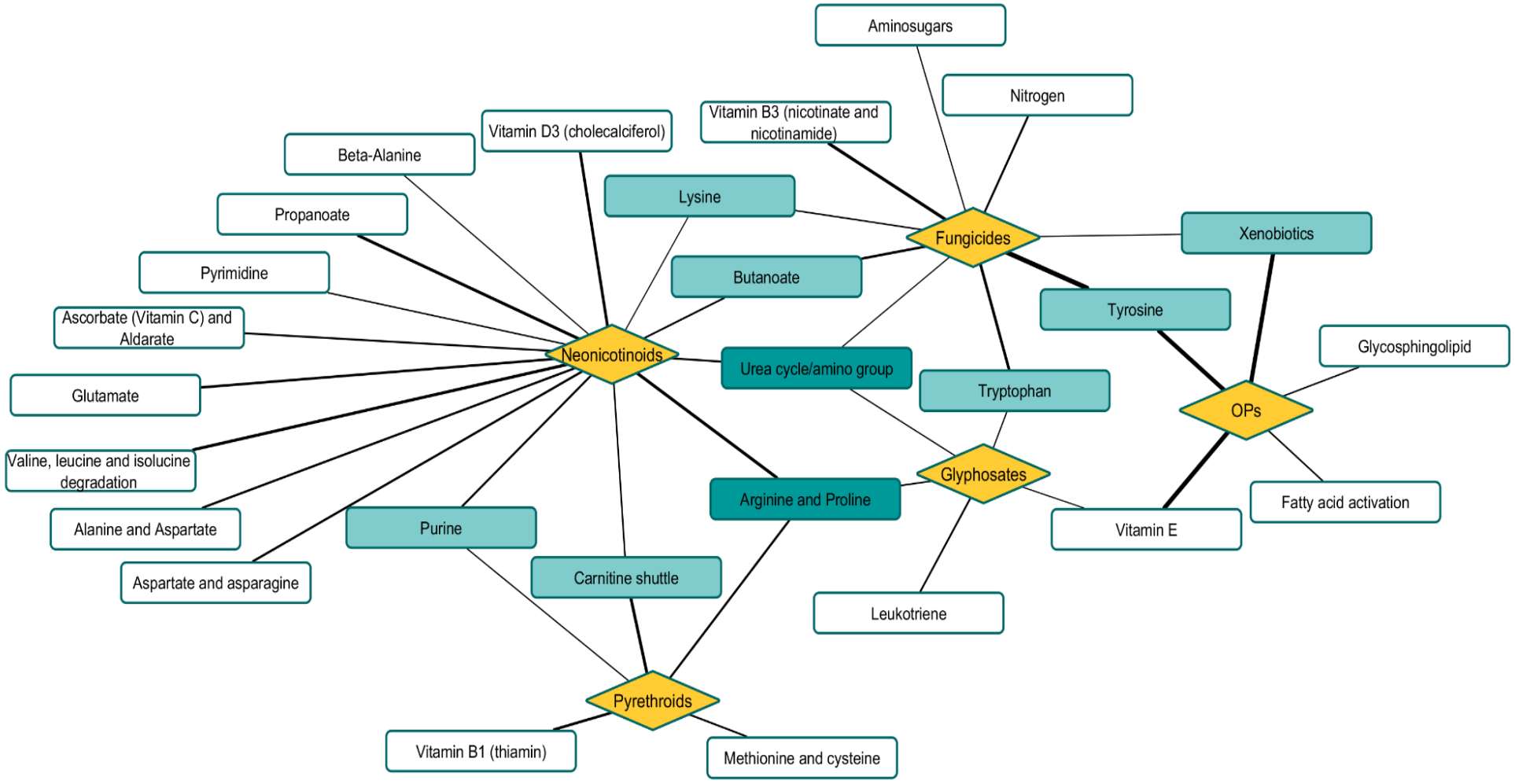
Pathway Analysis: **Pesticide Exposure Metabolomics**

Annotation: xMSannotator, matched to HMDB, KEGG, and LipidMaps with a mass error threshold of 10 ppm

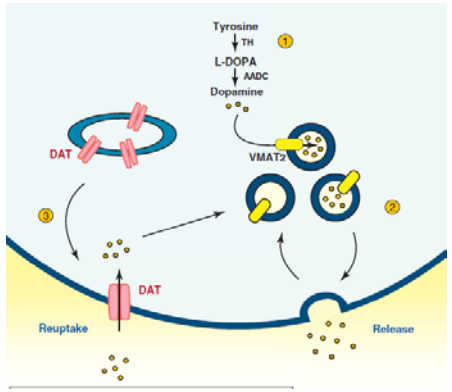
Pathway/Module analysis:

- *Mummichog*. Features previously selected by PLS-DA were included
- Adjusted p-value <= 0.05

Visualization: Cytoscape

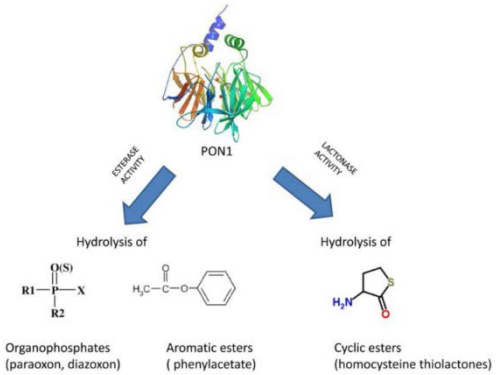


Genetic variation in many biologic pathways influence susceptibility to exposure from pesticides and increase the risk of developing neurodegenerative diseases



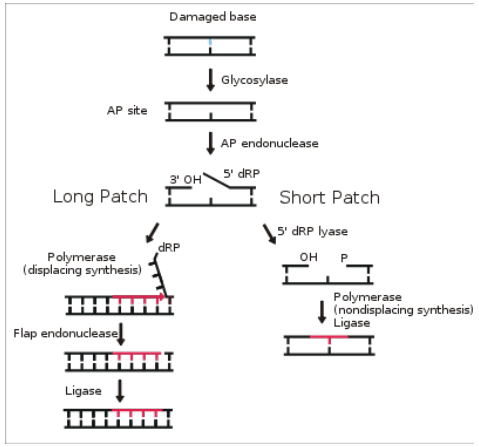
Dopamine transporter

Paraquat and DAT



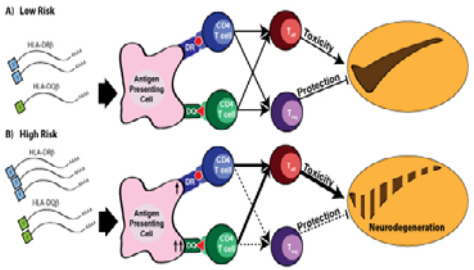
Pesticide Metabolism

Paraoxonase *(PON1)* metabolizes OPs



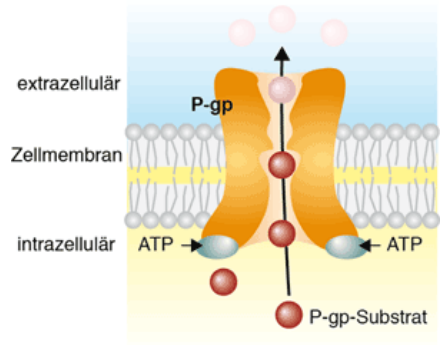
DNA Repair

Base Excision Repair *(APEX1, OGG1) and paraquat*



Immune Function

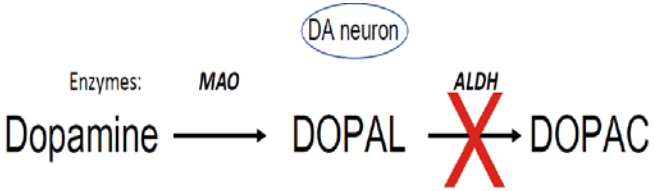
HLA-DRA variant in MHC-II locus and pyrethroids



Membrane transporter

ABCB1

and OP & OC pesticides



Dopamine Metabolism

ALDH1 variants and Dithiocarbamates (ALDH1 inhibitors)

Stronger neurotoxi-city of pesticides

OPs &PON1

DA, neurotox transport

Paraquat & DAT

UPS inhibition Fungicides & ALDH2

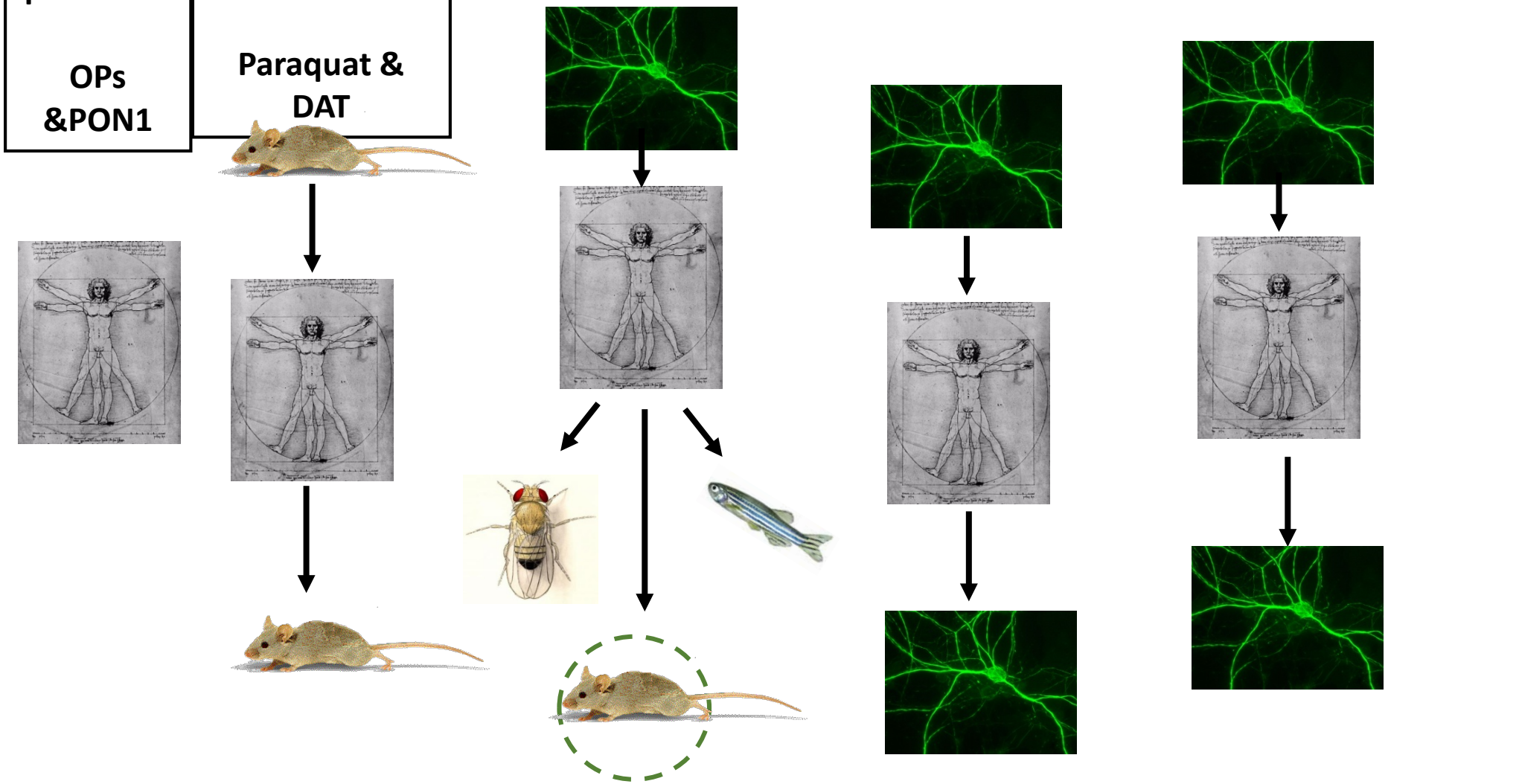
Bronstein lab UCLA

Immune response

Tansey lab Emory U

Mitochondrial DNA damage

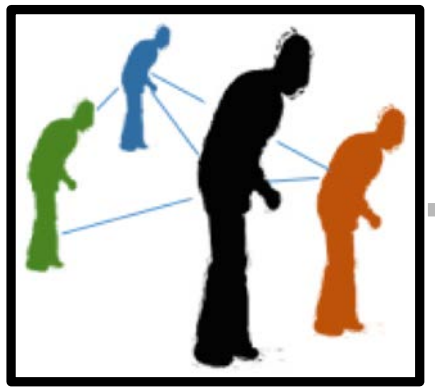
Greenamyre lab- Pittsburgh



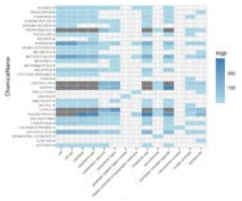
Much of this work would not have happened without a multidisciplinary Center for Neurodegeneration funded by NIEHS

Current Work: Exposomic pesticide approach meets iPS cell lines

PEG study



1



(UCLA)
Toxicant exposure ("**E**")

"GxE"

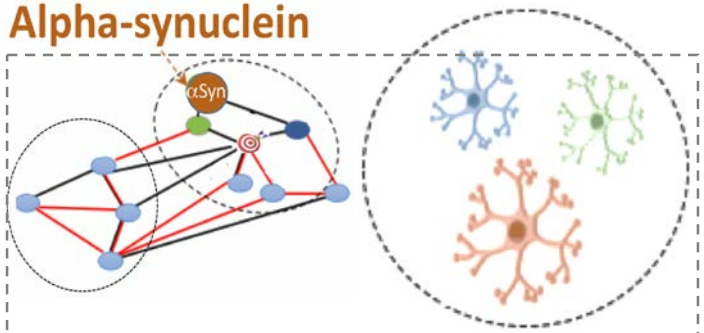
(UCLA)
Genotyping ("**G**")
(NGS Panel, Targeted Exome, GWAS)

2

3

KHURANA / Rubin (HMS/BWH)

Alpha-synuclein



4

α-Syn / GBA carrier derived cells IPS Cells:
Survival assays under exposure

Current Work: Exposomic approach to pesticide in PD

Pesticide Feature Selection

Input: PEG Data
GRAPES Pesticide Data
720 chemicals
1571 participants

Pesticide Selection:
Unsupervised + supervised

PD Association:
Meta p-value<0.05: 70
Meta p-value FDR<0.05: 36

Orthogonal PCA

PD Association:
TDB

Pesticide Biologic Enrichment:
Single Chemical & PD-Associated Chemical Set

Comparative Toxicogenomic DB:
53 Pesticides in CTDB (Meta p-value<0.05)

Single Chemicals

Associated Terms:
. Diseases: 4501 *unique diseases* (713 / 28683) associated terms/records
. Chemical-gene interactions: *12,187 unique gene symbols*, 20154 (27630)
. KEGG pathways: *1296 unique pathways*, 5702 (5702)
. GO terms: *5024 unique terms*, 27705 (27705)

Chemical Set Enrichment

TBD

Tox21 Assays Biological Enrichment:
45 Pesticides in Tox21 (Meta p-value<0.05)

Single Chemicals

TBD

Chemical Set Enrichment

6 enriched Tox21 activity assays (p <0.05):
Top: androstane receptor transcriptional factor activity (Fold enrich=2.97, p=9e-5)
39 enriched GO terms (FDR < 0.05)
Top: Progesterone biosynthetic process (Fold enrichment=21.3, FDR=6e-5)
228 total enriched terms (FDR < 0.05)
CTD Chem2Disease, 18 terms (PD fdr=0.02)
CTD Chem2Gene, 3 terms (ACHE, GSR, AR)
CTD Pathway, 10 terms (top cyto P450)
Tox21 active, 2 terms (AR, AhR)
Toxin Targets (genes), 25 terms (top ACHE, PLAUR, CYP2B6)
ToxCast active, 55 assays

*of 70 , 62 matched to CAS RN, 53 found in CTDN, 45 in Tox21

A strong scientific story is important for regulatory standard setting and public policy

Generate human effect data systematically - Regulate Pesticides like Pharmaceuticals *"Pesticidovigilance"*

How pharmaceuticals and pesticides are regulated

During early stages of discovery and testing, pharmaceuticals and pesticides are regulated in a similar way, but in the later stages and after approval, pharmaceuticals are monitored far more effectively.

	PHARMACEUTICALS	PESTICIDES
Substance discovery and development	Search for and synthesize molecules; perform biological tests and screening to determine biological activity; determine commercial prospects (e.g., potential for patents).	
Preclinical and prefield testing	Conduct safety, efficacy, and toxicological tests using in vitro and in vivo laboratory trials and computer modeling.	
Clinical and field testing	Test effectiveness, determine safety, and identify side effects using three levels of trials [phase 1 (first in human), 2, and 3] with increasing patient numbers.	Test effectiveness, toxicology, fate, and behavior in the environment using laboratory and field trials. No equivalent to the phased trials used in pharmaceutical testing.
Registration and approval	Submit development study results on efficacy, effectiveness, safety, toxicology, fate, and behavior to regulatory body for license approval.	
Postapproval marketing, use, and monitoring	Once licensed, the substance can be used in accordance with the product label. Long-term monitoring starts (phase 4) to determine unexpected effects in different categories of people and the population more generally (using national and global reporting systems and pharmacovigilance regulations).	Once licensed, the substance can be used in accordance with the product label (MRL monitoring checks compliance with product label to protect human health). Ad hoc reporting of incidents or effects. No post-approval or long-term monitoring of effects and no equivalent to pharmacovigilance.

BRIDGE – MEDICINES AND PESTICIDES

Toward pesticidovigilance

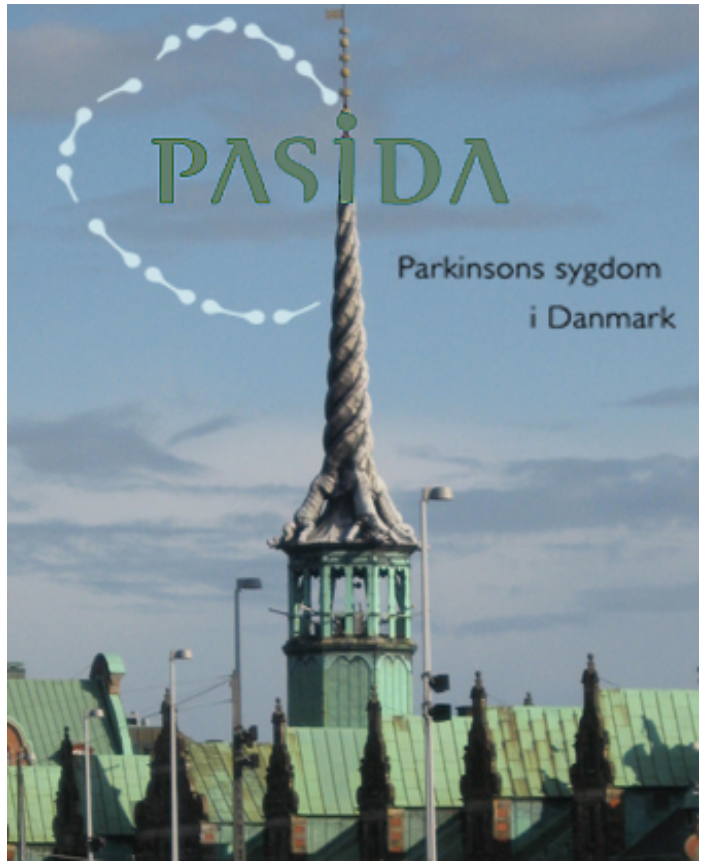
Can lessons from pharmaceutical monitoring help to improve pesticide regulation?

By **Alice M. Milner**[1] *and* **Ian L. Boyd**[2]

scales, pesticides can harm the environment (*1*), but there is a trade-off between this ef-

Important insights into how pesticides can be better regulated come from the regulation and monitoring of pharmaceuticals. In particular, antibiotics provide some intriguing parallels with pesticides. Society depends on pesticides in a similar way to how it relies on antibiotics. Both have been manufactured and supplied to market demand with little care taken to consider whether this is sensible. Both are often used prophylactically or as therapies

PASIDA
Danish Parkinson's Disease Study and Air Pollution



Funded by NIEHS - ES013717

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***Pei Chen Lee,* Taipei**

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Jorgen Olsen, Johnni Hansen, Christina Funch Lassen, Mette Sørensen,
Danish Cancer Society

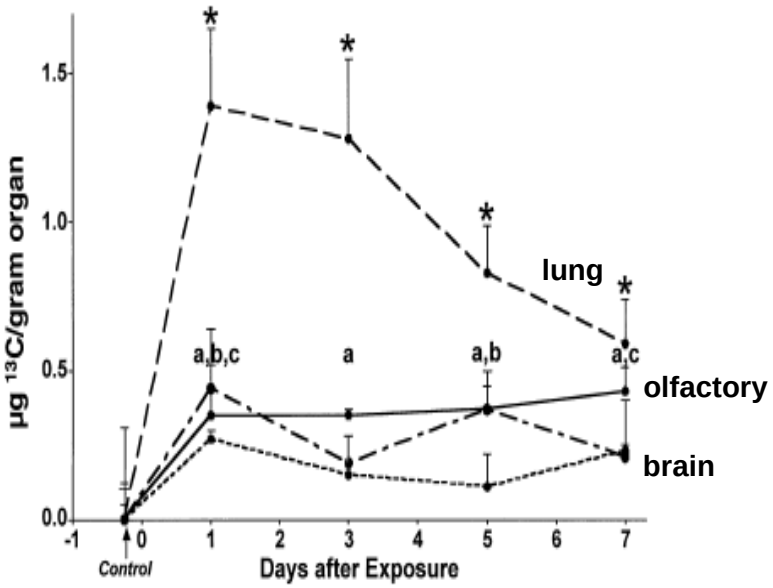
Matthias Ketzel , EHS Roskilde Denmark

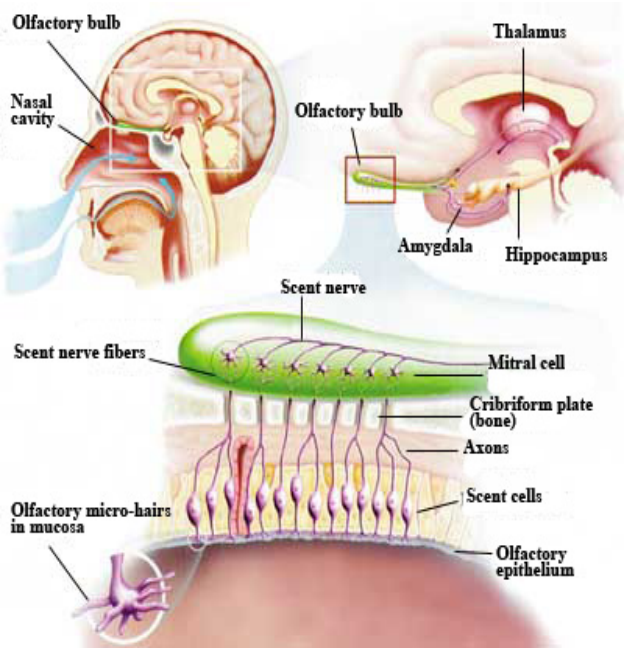
Lene Wermuth*, *Odense University Hospital

Inhalation Tox Animal Model Translocation of Inhaled Ultrafine Particles to the Brain

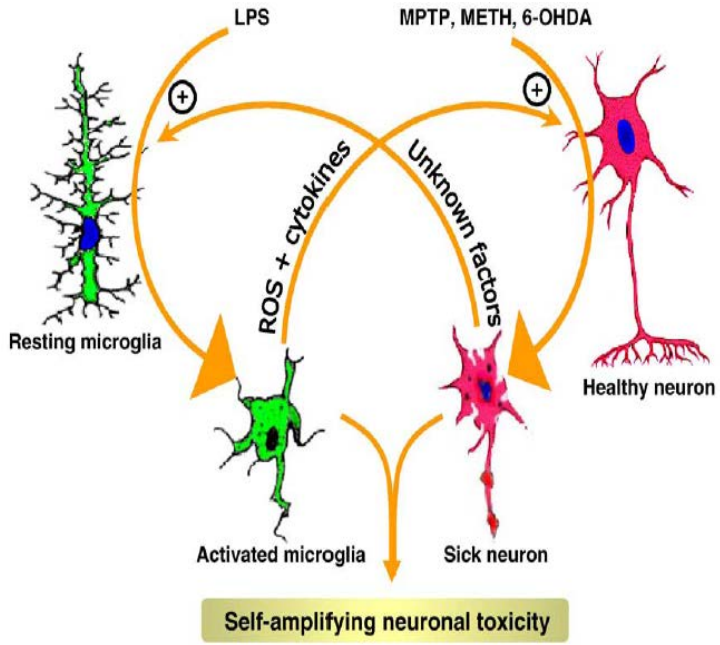
Oberdoerster et al. 2004

Insoluble nano-size ^{13}C access brain *via* olfactory nerve



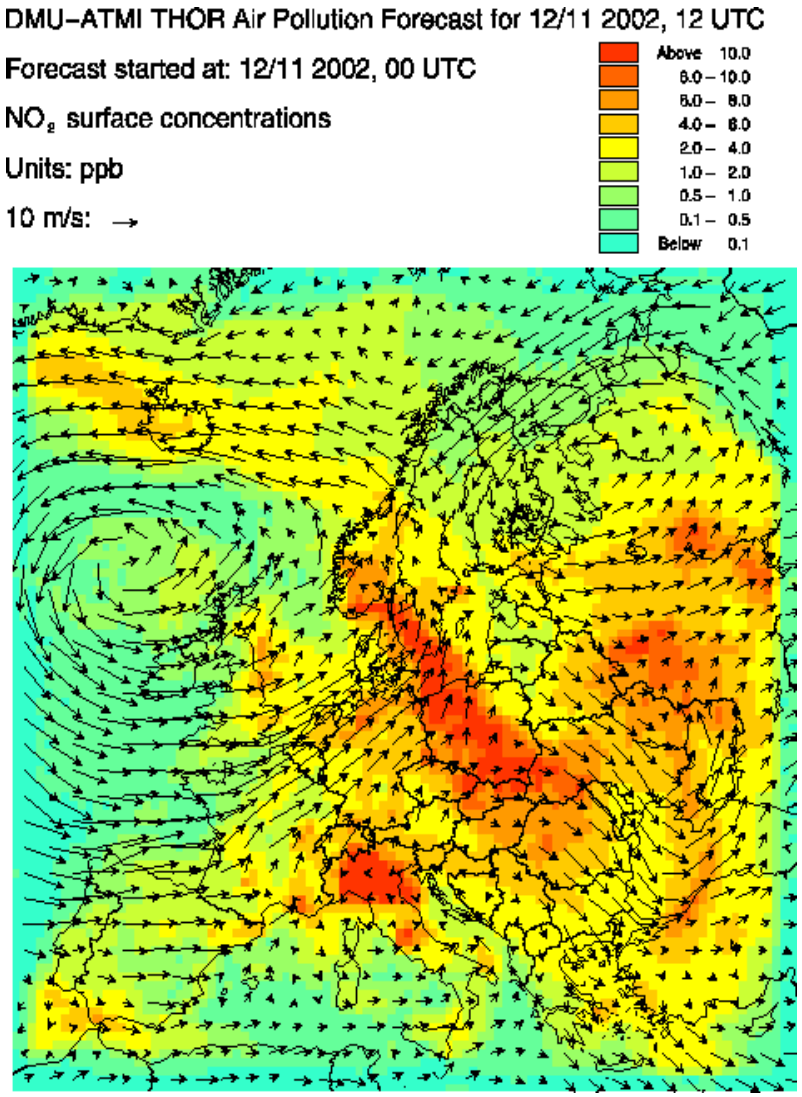


Hald & Lotharius. Exp Neurol 193(2):279-90



Neuron-microglia activation in Parkinson's

Air Pollution Modelling in PASIDA



picture from AARHUS University, Thor Air pollution modeling

Traffic generated air pollution exposure based on geocoded addresses starting in 1971:

Sum of:

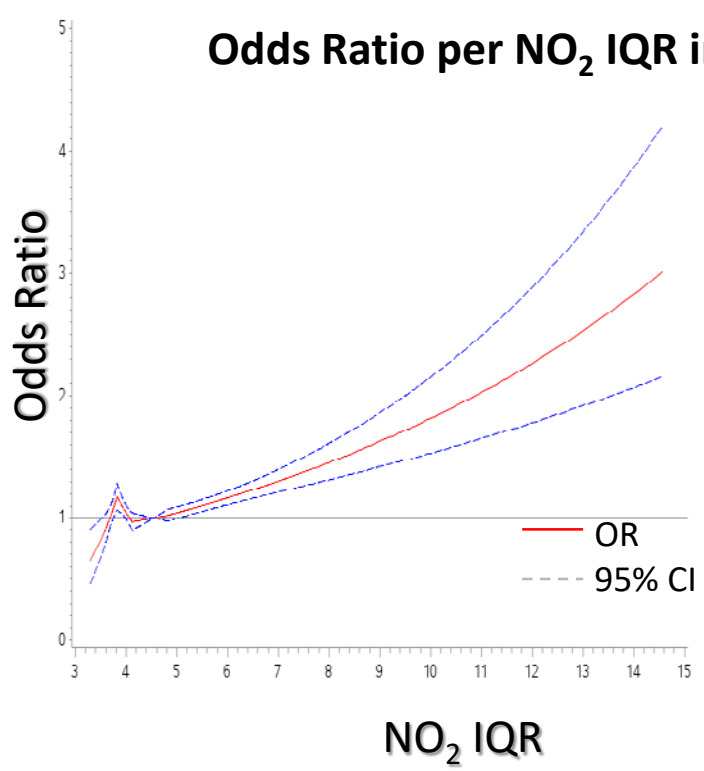
1. **local air pollution** from street traffic, calculated from traffic intensity and type, emission factors for the car fleet, street and building geometry and meteorology;

2. **urban background**, calculated from data on urban vehicle emission density, city dimensions and building heights;

3. **regional background**, estimated from trends at rural monitoring stations and from national vehicle emissions.



Traffic Related Air Pollution and PD Risk



	Cases/ctrls	aOR (95% CI)
All - NO_2 (µg/m3)*	1696/1800	1.09 (1.03 – 1.16)
Copenhagen and Suburbs	349/473	**1.16 (1.05 – 1.27)**
Provincial cities	926/903	**1.16 (1.05 – 1.29)**
Countryside	367/373	0.99 (0.83 – 1.19)

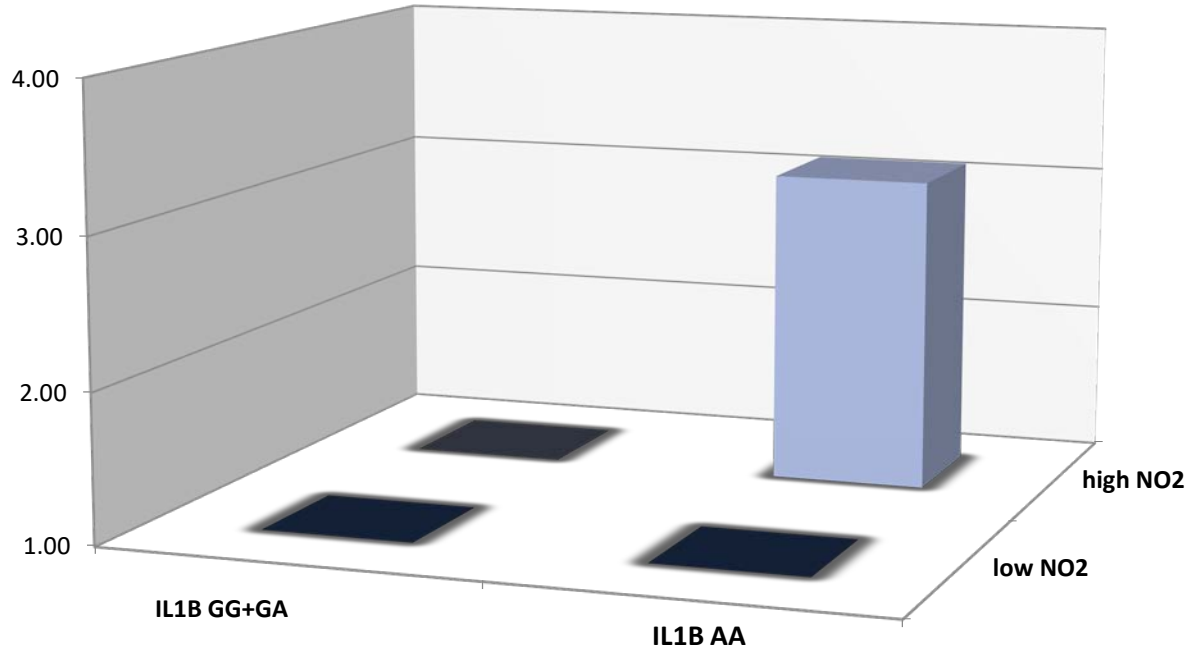
*per 1 IQR (2.97 µg/m^3) increase

Adjusted for: for age at first cardinal symptom , gender, education, smoking status (never, ex-smoker, current smoker), pack-years of smoking, family history of PD, year of birth, and job history

Traffic Air Pollution: Incident PD and Inflammatory Gene Variants in PASIDA

Hallmarks of chronic inflammation in PD

- marked increase of cytokines (tumor necrosis factor α (TNFα) and interleukin-1 (IL-1beta)) found in brain and CSF of Parkinson's patients



Logistic regression model adjusted for age at first cardinal symptom , gender, education, smoking status (never, ex-smoker, current smoker), pack-years of smoking, family history of PD, and year of birth, and job history

Standard Setting for Neurotoxic Environmental Exposures and Public Policy are Needed to Reduce Neurodegenerative Diseases in Aging Populations

Many Thanks to our Funders

Individual donations to our program from Parkinson's patients and their families

National Institute of Environmental Health Sciences
Your Environment. Your Health.

PARKINSONALLIANCE

AMERICAN PARKINSON DISEASE ASSOCIATION
Strength in optimism. Hope in progress.