Syngenta's Paraquat Research Program
Update

US EPA Meeting

Washington DC, 6 Feb 2017

Monty Dixon, John Abbott, Charles Breckenridge & Nick Sturgess

Agenda

1. Introduction (Monty Dixon)
2. Paraquat Registration Review Status (Monty Dixon)
3. Considerations By Other Regulatory Agencies (Monty Dixon)
4. Results from Syngenta's research program
    a. Animal model (Nick Sturgess)
    b. Epidemiology (Charles Breckenridge)
5. Discussion

Paraquat Dichloride Registration Review
(Docket ID: EPA-HQ-OPP-2011-0855)

- December 21, 2012 Paraquat Registration Review Docket opened
- All DCI required studies submitted or waivers granted
- December 15, 2016 publication of Human Health Mitigation
  - Revised label statements / supplemental warning materials
  - Certified applicators
  - All products in closed systems by September 30, 2020
- Draft Risk Assessments anticipated late 2017
- Final Registration Review decision anticipated in 2018

Previous Syngenta Updates to US EPA

- May 4, 2010 Presented Syngenta's approach to emerging academic data
- February 21, 2013 Syngenta met with US EPA to present results from Syngenta's research program
- May 9, 2016 Public comments in response to EPA "Paraquat Dichloride; Proposed Interim Mitigation Decision"
  - Provided recent publication
- February 6, 2017 Syngenta meeting with US EPA to provide update

Considerations By Other Regulatory Agencies

- Australia (APVMA), Brazil (ANVISA) and US (EPA) have been regularly updated on the topic by Syngenta in Face-to-Face meetings
- Australia
  - conclusions were published on the APVMA website on 26th October 2016. The final documents can be accessed under the Publication Archive link: http://apvma.gov.au/node/12666
  - to access the documents you need to click on '**5. Assessment**' to reveal the next page and locate the three review documents, including the detailed neurotoxicity review
- Brazil
  - 4th January 2017 ANVISA public release of 2016 "Activities Report", in which they refer to the paraquat re-evaluation and state they are considering results from similar paraquat re-evaluations conducted by other regulatory agencies, i.e. APVMA and US EPA. In the report they state that they have contacted both regulatory agencies to clarify some aspects
    - precise nature of discussions is unclear
  - paraquat remains under evaluation by the ANVISA Board
    - Syngenta expectation of completion in 2017

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Evaluation of the intraperitoneal (i.p.) paraquat mouse (male C57BL/6J) model

Dr. Nick Sturgess

Historical perspective

- Over the last 15 years a number of research groups have conducted a series of studies involving i.p. dosing of paraquat (PQ) to male C57BL/6 mice
  - Originally the Di Monte group (Parkinson's Institute, Sunnyvale, CA) and the Cory-Slechta group (University of Rochester, NY & Rutgers, NJ)
  - Mona Thiruchelvam involved in a known instance of scientific fraud reported in 2012 (*Federal Register Notice Volume 77, No. 125, June 28, 2012, 38632-38633*)
  - Numerous other groups in the intervening years

- Used the C57BL/6 mouse model and i.p. dosing of PQ (1-30 mg/kg) - typically 3 weekly doses of 10 mg/kg PQ dichloride salt.

- Reported effects on up to three endpoints as markers of neurotoxicity:
  - **stereology** - loss of dopaminergic (TH^+) neurones from substantia nigra *pars compacta* (SNpc)
  - **neurochemistry** - loss of dopamine from the striatum
  - **neurobehaviour** - reduction in locomotor activity

Evaluation of the i.p. paraquat mouse model in the C57BL/6J strain

- Syngenta conducted a series of studies in an attempt to replicate the results from published studies.

- Male C57BL/6J mice were administered i.p. injections of PQ dichloride (10, 15 or 25 mg/kg - MTD) with either 1, 2 or 3 weekly doses.

  - ***Neuropathological*** markers of cell damage / death & neuro-inflammation were evaluated in the substantia nigra *pars compacta* (SNpc) & striatum at multiple time points after the last dose using selective stains (amino Cu Ag, TH, GFAP & IBA-1) - *rarely or not reported in literature studies*
  - ***Neurochemical*** evaluation of striatal dopamine & its metabolites
  - ***Stereological*** evaluation of the number of dopaminergic (TH^+) & non-dopaminergic neurons
  - *Assessments were conducted by individuals **blinded** to treatment group*

- Used MPTP (4 x 10 mg/kg i.p. at 2-hour intervals) as a positive control.

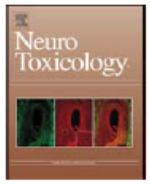
Evaluation of the i.p. paraquat mouse model

We presented these findings to EPA in 2013 and these studies have now been published (Breckenridge *et al,* 2013):

NeuroToxicology 37 (2013) 1–14

Contents lists available at SciVerse ScienceDirect

NeuroToxicology



Pharmacokinetic, neurochemical, stereological and neuropathological studies on the potential effects of paraquat in the substantia nigra pars compacta and striatum of male C57BL/6J mice

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ABSTRACT

The pharmacokinetics and neurotoxicity of paraquat dichloride (PQ) were assessed following once weekly administration to C57BL/6J male mice by intraperitoneal injection for 1, 2 or 3 weeks at doses of 10, 15 or 25 mg/kg/week. Approximately 0.3% of the administered dose was taken up by the brain and was slowly eliminated, with a half-life of approximately 3 weeks. PQ did not alter the concentration of

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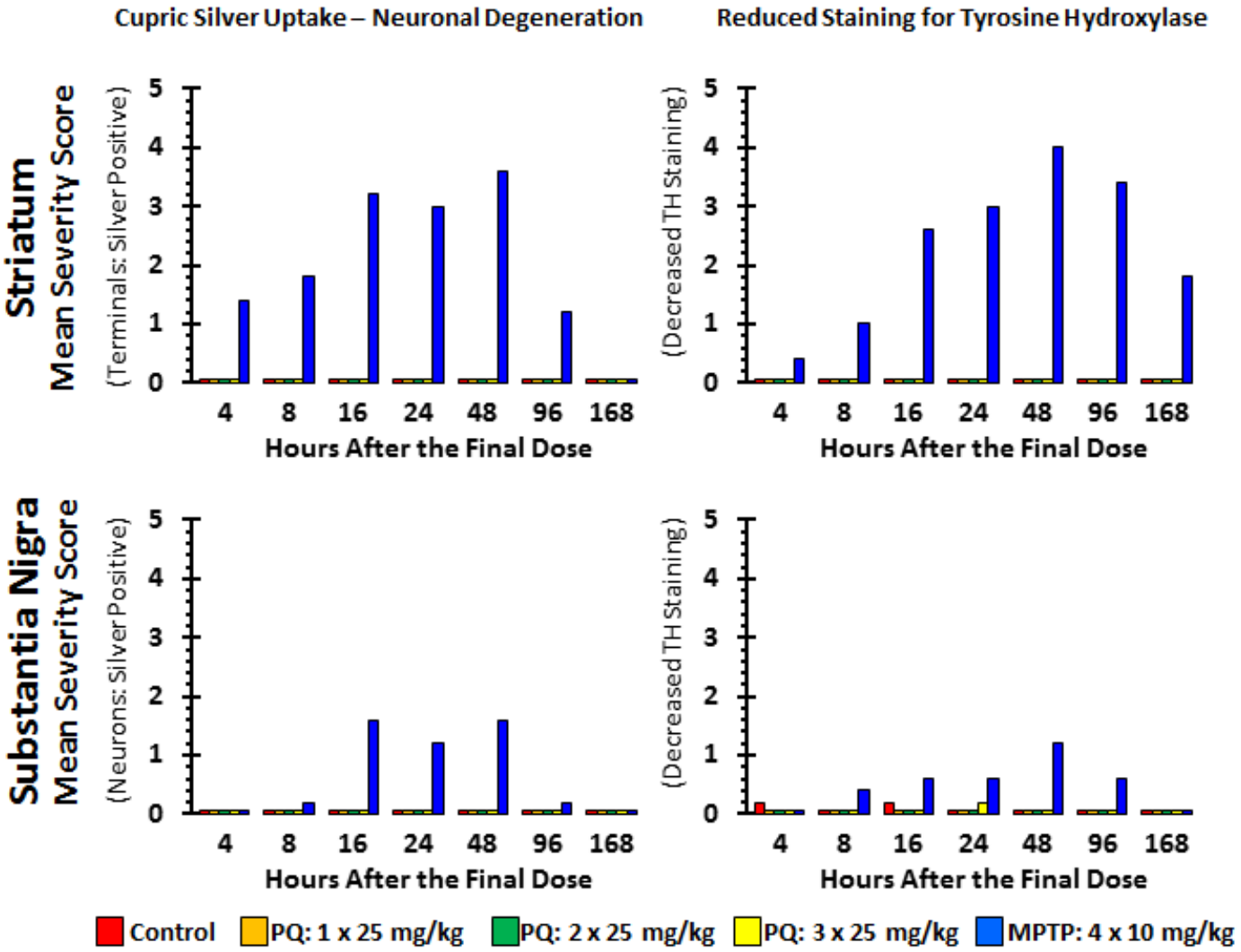
Neuropathology Assessment - Histopathology Staining

- Stains used to look for evidence of neuropathology:
  - **Amino Cu Ag stain**
    (silver-positive reflects necrotic neurones & disintegrating synaptic terminals)
  - **TH^+ - neurons immuno-labeled for Tyrosine Hydroxylase**
    (decreased staining reflects loss of dopaminergic neurons)
  - **GFAP - astrocytes immuno-labeled for glial fibrillary acidic protein**
    (increased staining reflects reactive astrocytosis)
  - **IBA-1 - microglia immuno-labeled for ionised calcium binding adaptor molecule 1** (increased staining reflects reactive microgliosis)
- Semi-quantitative grades were assigned to each section evaluated based upon the percentage of the slide displaying the finding.
- Grades: 0= normal; 1= slight; 2= minimal; 3= mild; 4= moderate; 5= severe
- All evaluations were performed by a board certified pathologist blinded to the treatment group.

Neuropathology: No evidence of PQ induced DA cell death



(Breckenridge *et al,* 2013)

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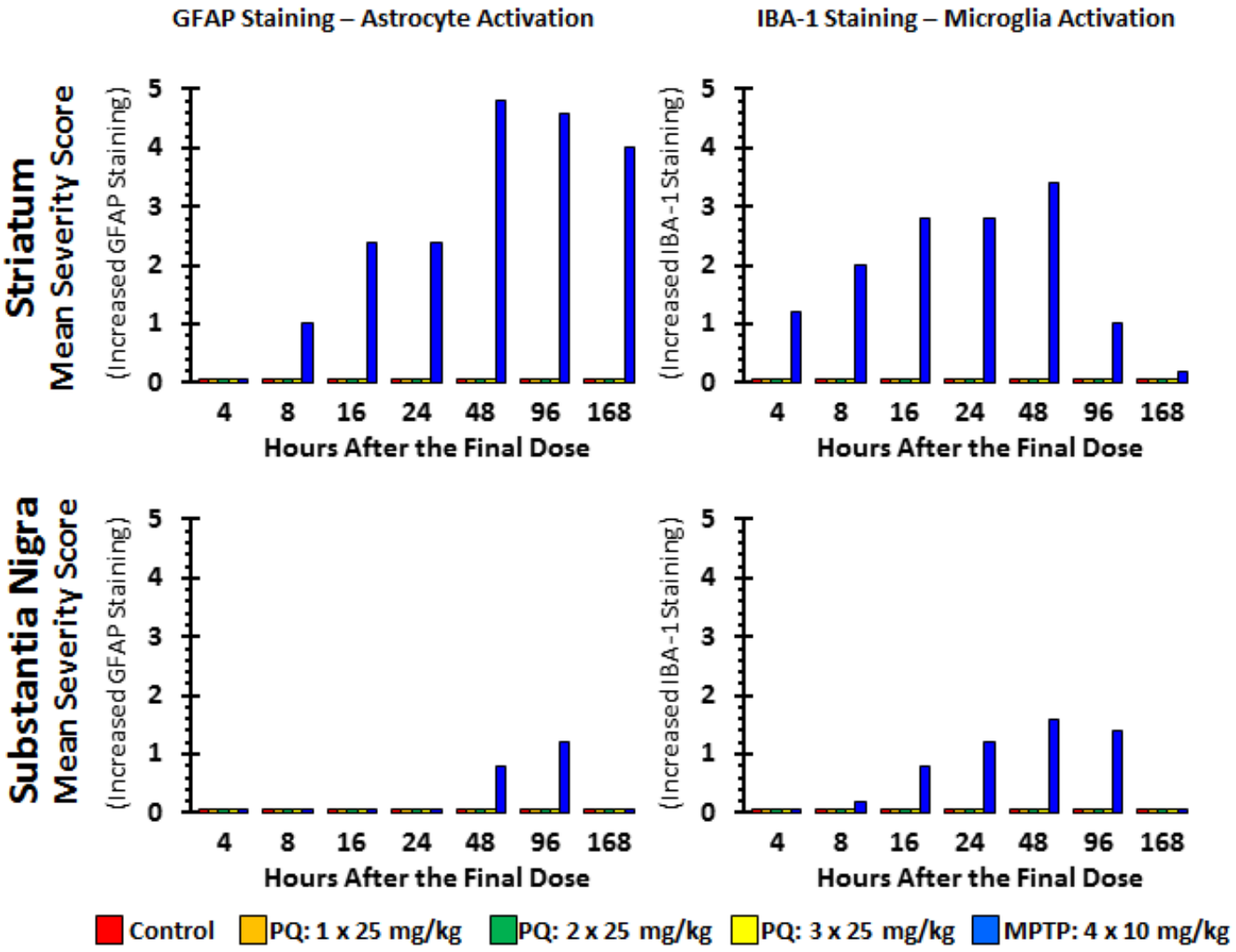
Neuropathology: No evidence of PQ induced glial activation



(Breckenridge *et al,* 2013)

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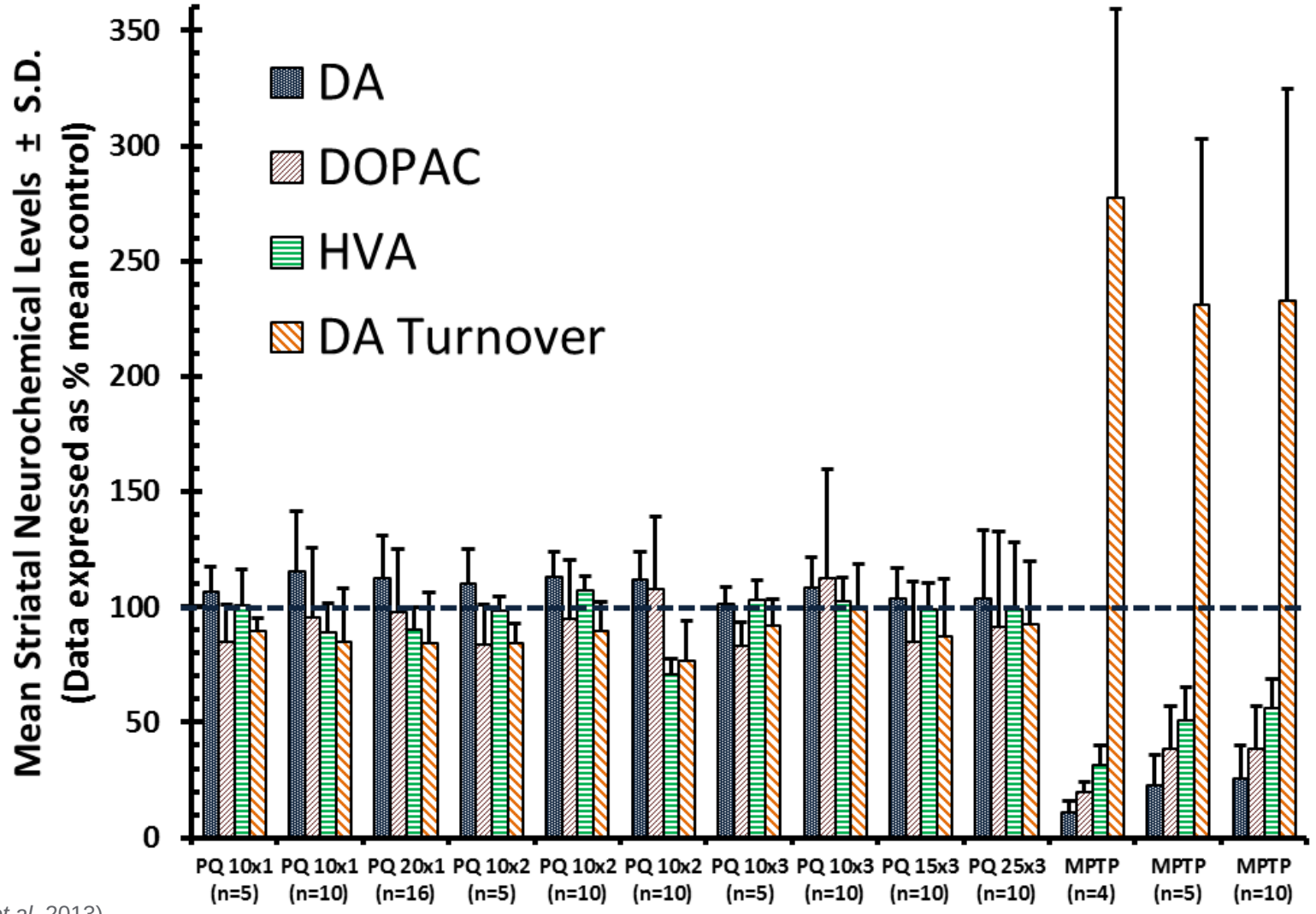
Neurochemistry: No evidence of PQ effects on striatal DA



(Breckenridge *et al,* 2013)

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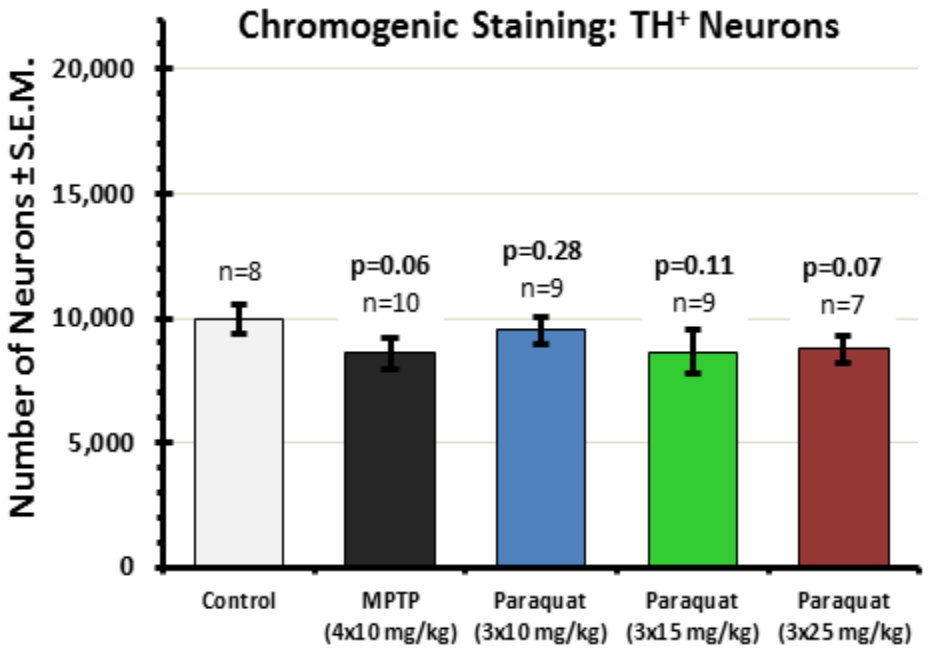
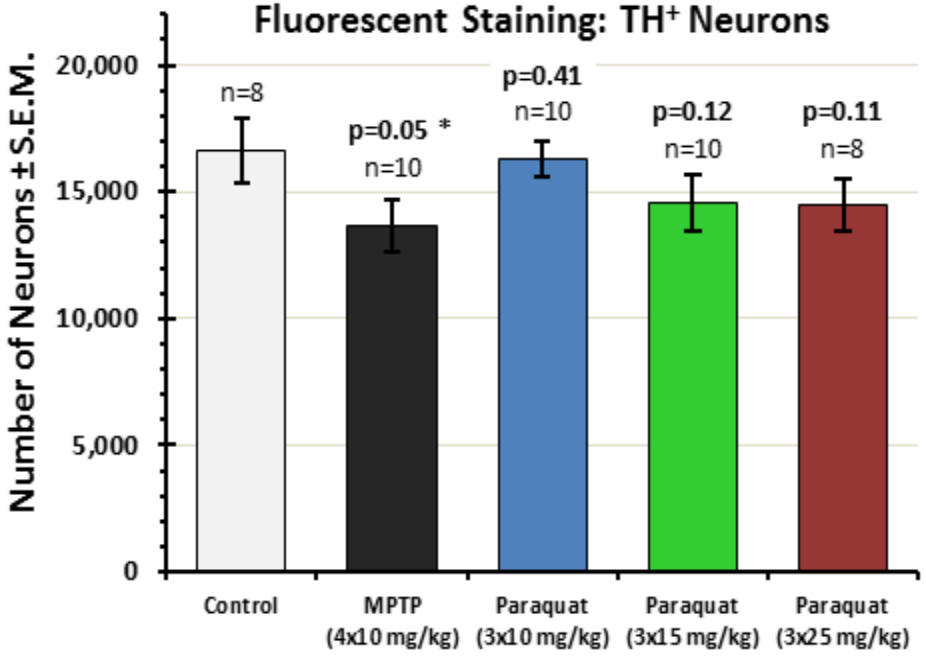
Stereology: No statistically significant effect of PQ on dopaminergic neuronal cell numbers in the SNpc





(Breckenridge *et al*, 2013)

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Evaluation of the i.p. paraquat mouse model
Summary of study findings

- ***Neuropathology***
  - **MPTP** consistently causes neuropathology changes indicative of cell death, glial cell activation and an inflammatory response
  - **PQ** at doses of up to 3 x 25 mg/kg (~MTD) does not have any effect
- ***Neurochemistry***
  - **MPTP** consistently causes a substantial loss in striatal dopamine and its metabolites
  - **PQ** at doses of up to 3 x 25 mg/kg (~MTD) has no effect on striatal dopamine or its metabolites
- ***Stereology***
  - **MPTP** causes a loss of TH^+ neurones in the SNpc
  - **Paraquat** at doses of up to 3 x 25 mg/kg (~MTD) does not cause a statistically significant loss of neurones in the SNpc. Any apparent loss of TH^+ neurons in an initial study when PQ was administered at 3 x 15 mg/kg was not reproducible.

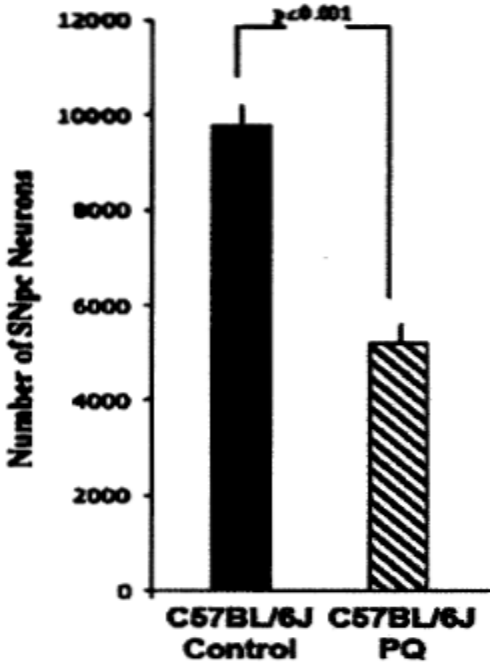
Evaluation of the i.p. paraquat mouse model - Smeyne

- In an attempt to understand the difference between Syngenta's results and those reported in the literature (dopaminergic cell loss), we conducted a collaborative program of work with ***Dr. Richard Smeyne*** (*St. Jude Children's Research Hospital, Memphis, TN; currently Thomas Jefferson University, Philadelphia, PA*)
- Smeyne previously reported a 50% loss of DA neurons following i.p. dosing of 10 mg/kg PQ twice/week for 3 weeks (Jiao *et al,* 2012).
- Collaboration involved the dosing of PQ to mice in two different labs and conducting stereological assessments (for the number of TH^+ neurons in SNpc) in different laboratories using different stereology methods.
- Also investigated the influence of additional variables including:
  - age of mice (9-week or 16-week old)
  - source / strain of mice (Jackson labs or Harlan)
  - animal husbandry (WIL Labs or SJCRH)
  - source of PQ (Syngenta or Sigma-Aldrich)
  - PQ dose level (10 or 20 mg/kg)
  - frequency of dosing (10 mg/kg twice/week or 20 mg/kg once/week)

Smeyne failure to replicate previous findings

In an initial experiment, Smeyne failed to replicate his previous published findings

Jiao *et al.*, 2012



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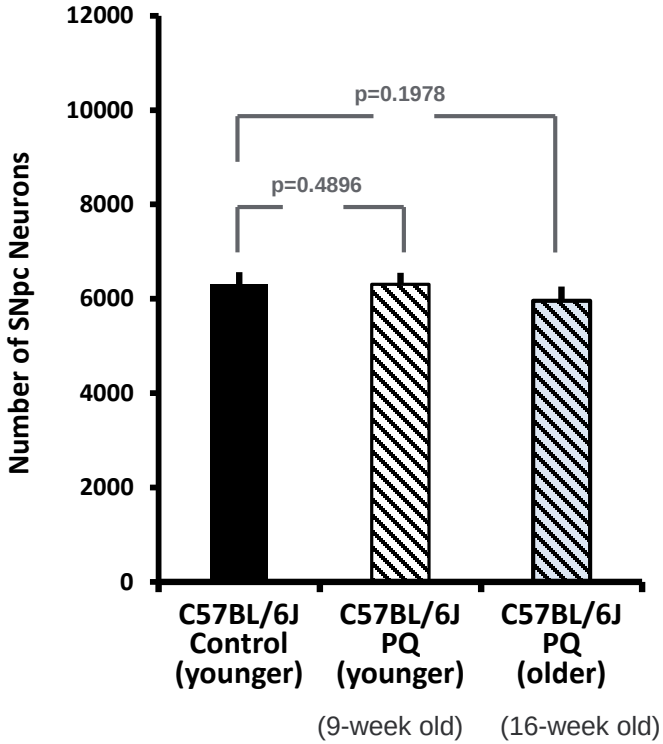

Dosing Regimen: 10 mg PQ·Cl_2/kg/dose twice per week for 3 weeks (total of 6 doses, 60 mg PQ·Cl_2/kg)

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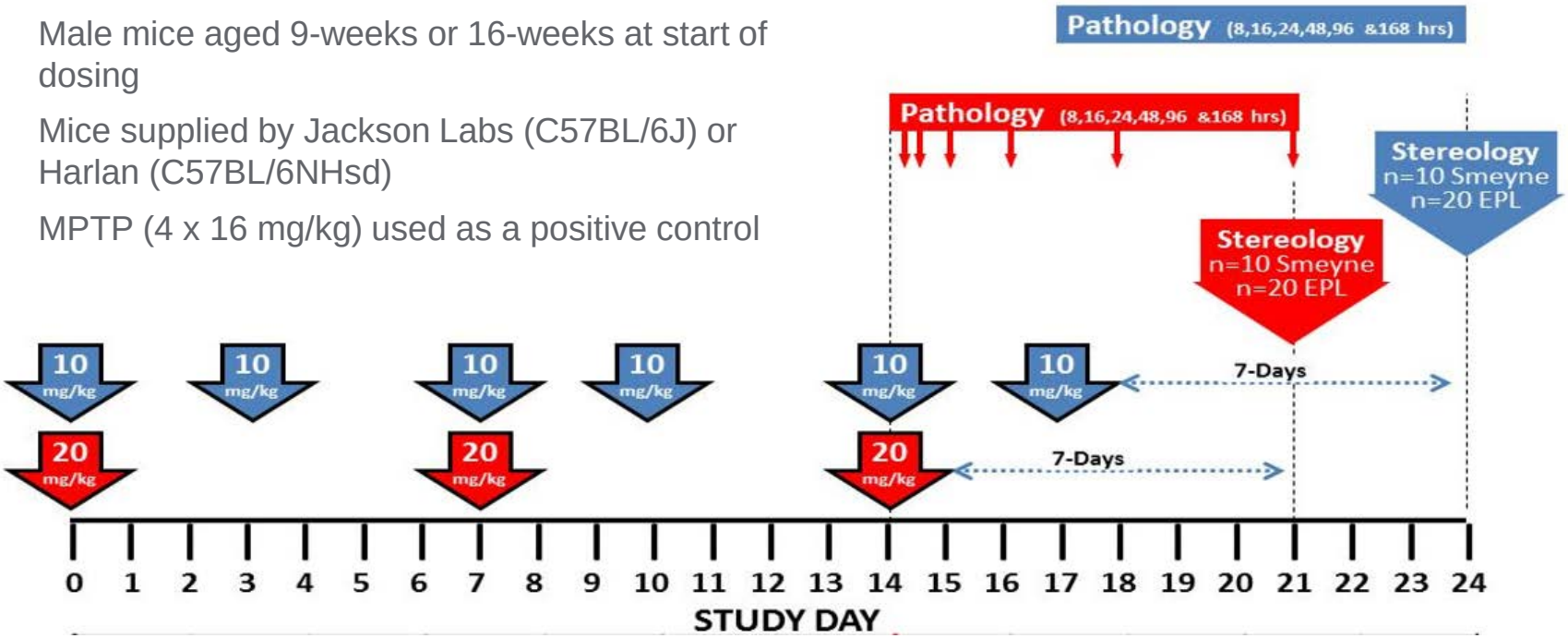
Smeyne collaboration study design

- Dosing conducted at WIL or SJCRH
- Male mice aged 9-weeks or 16-weeks at start of dosing
- Mice supplied by Jackson Labs (C57BL/6J) or Harlan (C57BL/6NHsd)
- MPTP (4 x 16 mg/kg) used as a positive control



- 2-D stereology - Smeyne (thin sections; TH^+ neurons & microglia)
- 3-D stereology - EPL (thick sections; TH^+ neurons)
- Neuropathology - Tox Path Specialists
- *Evaluations conducted by individuals **blinded** to treatment*

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Stereological assessment of the number of TH⁺ neurons (Smeyne *et al*, 2016)

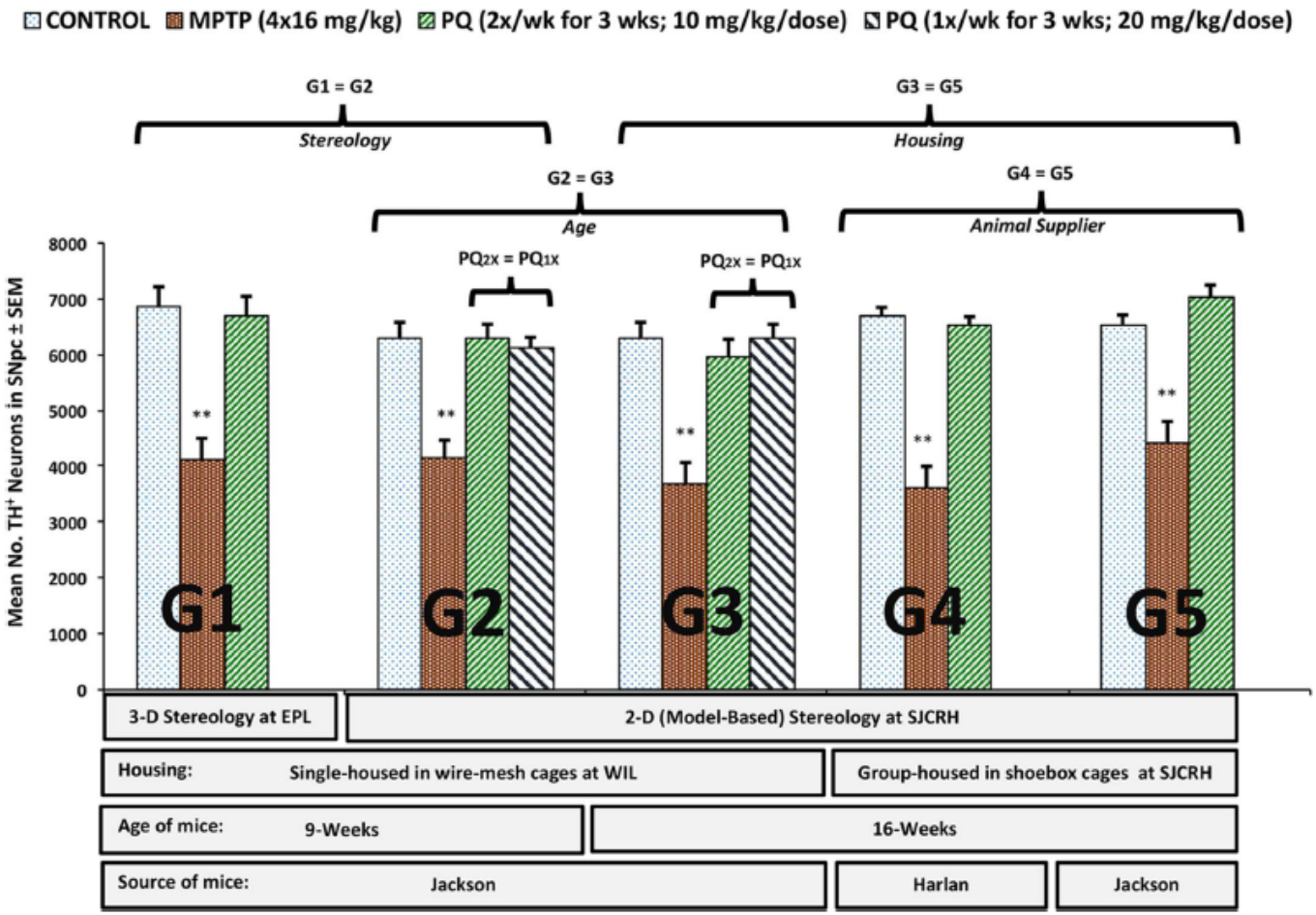

Fig 1. Stereological assessment of the mean number of TH⁺ neurons in the SNpc following PQ or MPTP treatment in C57BL/6J and C57BL/6NHsd male mice. Five different groups (G1-G5) of animals, varying in age and site of experiment were injected with saline, paraquat or MPTP and the extent of TH⁺ neuron loss was assessed by design-based or model-based stereology. ** significantly different from control mice ($p \leq 0.01$). Syngenta-sourced PQ was used to treat mice G1 to G3 mice, whereas Sigma Chemical PQ was used to treat mice in groups G4 and G5.

Factors that are likely unimportant:

- Source of mice
- Animal husbandry
- Source of PQ
- PQ dose level
- PQ dose frequency
- Stereological method

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Stereological assessment of the number of resting & active microglia (Smeyne *et al*, 2016)

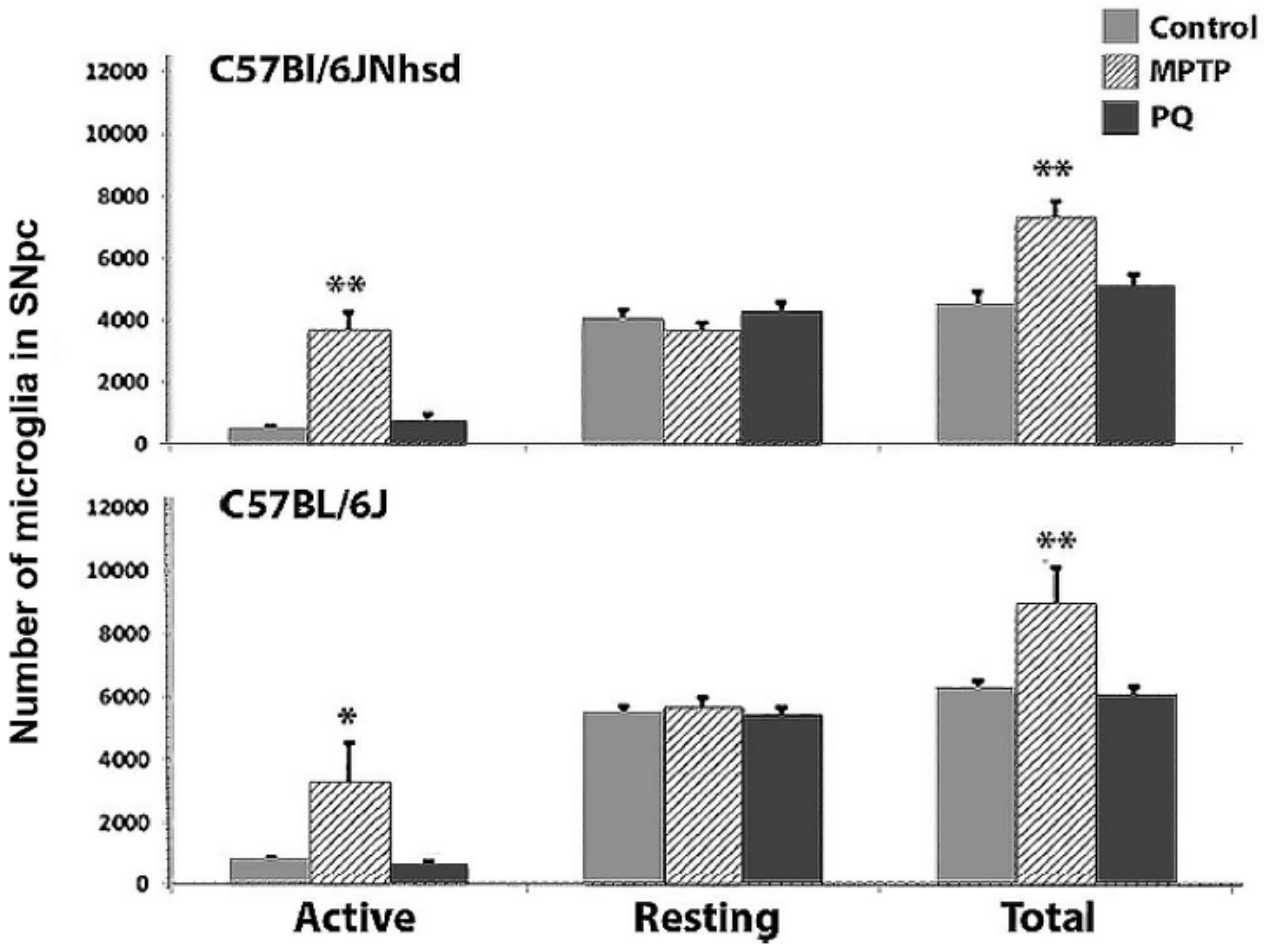

Fig 2. Stereological assessment of the mean number of resting and activated microglial cells in the SNpc of C57BL/6J or C57BL/6NHsd male mice treated with saline, PQ or MPTP. No change in resting or activated microglia number was seen in PQ-treated mice of either C57BL/6 substrain, while both C57BL/6 substrains demonstrated increased numbers of activated and total microglia 7 days following MPTP treatment. * $p \leq 0.05$, ** $p \leq 0.01$.

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Appearance of microglia in PQ- & MPTP-treated mice (Smeyne *et al*, 2016)

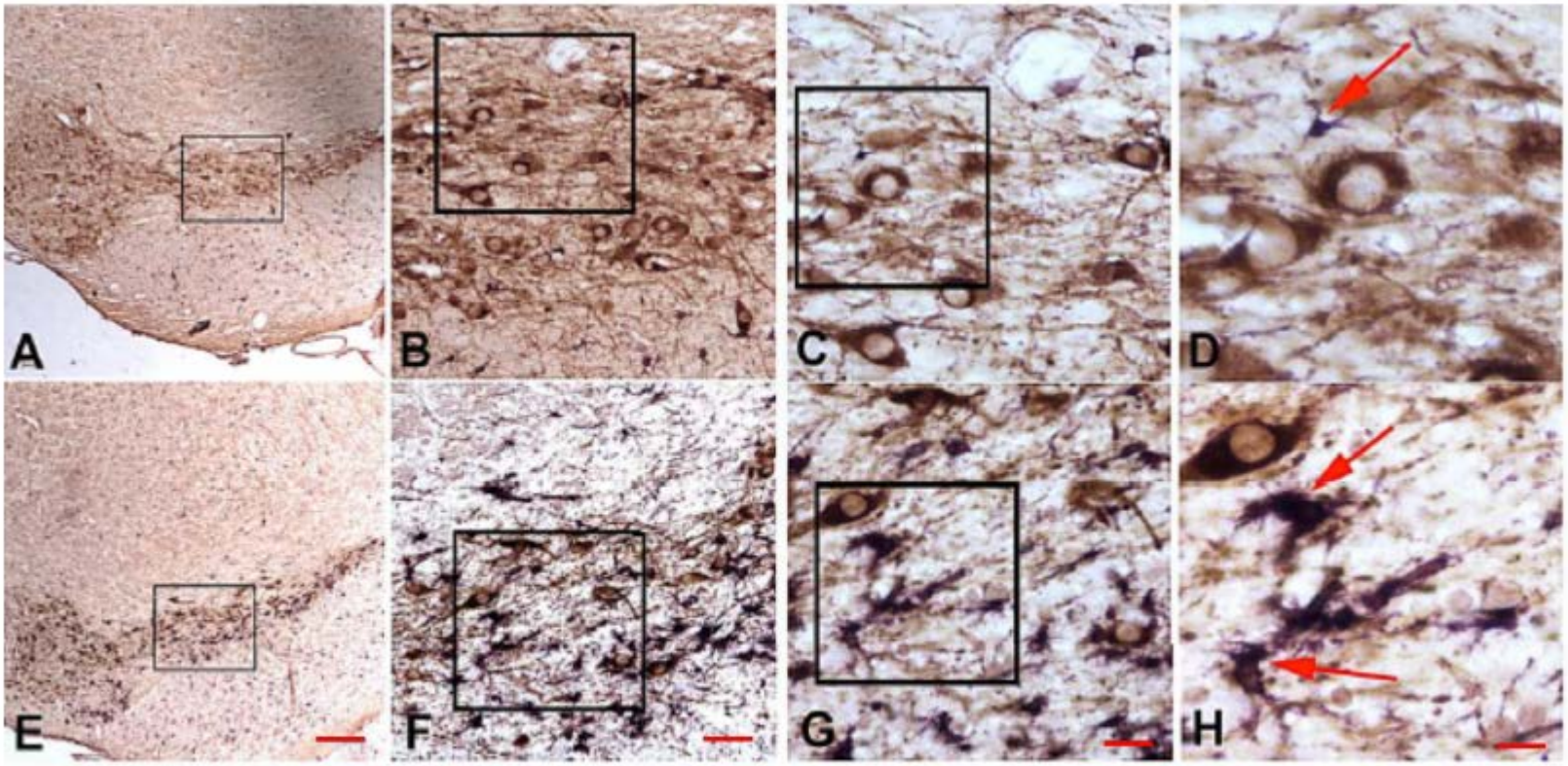

Fig 3. Appearance of microglia in PQ- and MPTP-treated mice. Representative photomicrographs of microglia (Iba-1+) cells in PQ-treated (Panels A-D) or MPTP-treated (Panel E-H) in the SNpc of C57BL/6J or C57BL/6NHsd male mice aged 9- or 16-weeks at the time of the 1st dose. The boxes in each panel indicate the region shown in the adjacent box. Red arrow in D shows an example of resting microglia; characterized by a small cell body and thin processes. Red arrows in H show the typical appearance of activated microglia seen in MPTP-treated mice where the cell body is increased in size compared to resting microglia and the processes are shortened and thickened. Scale bars A,E = 100 µm, B,F = 40 µm, C,G = 20 µm, D-H = 10 µm.

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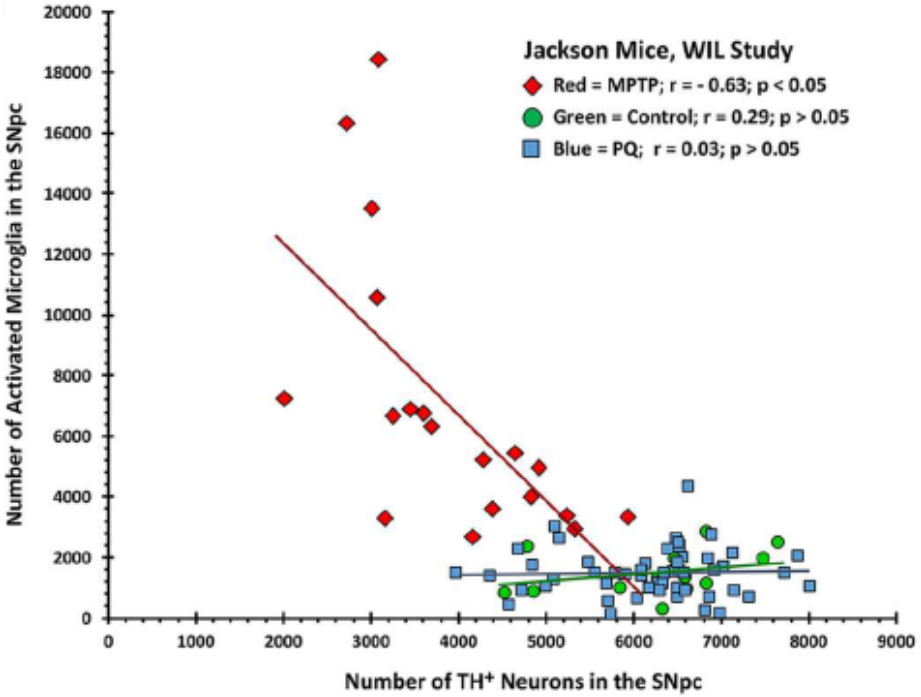
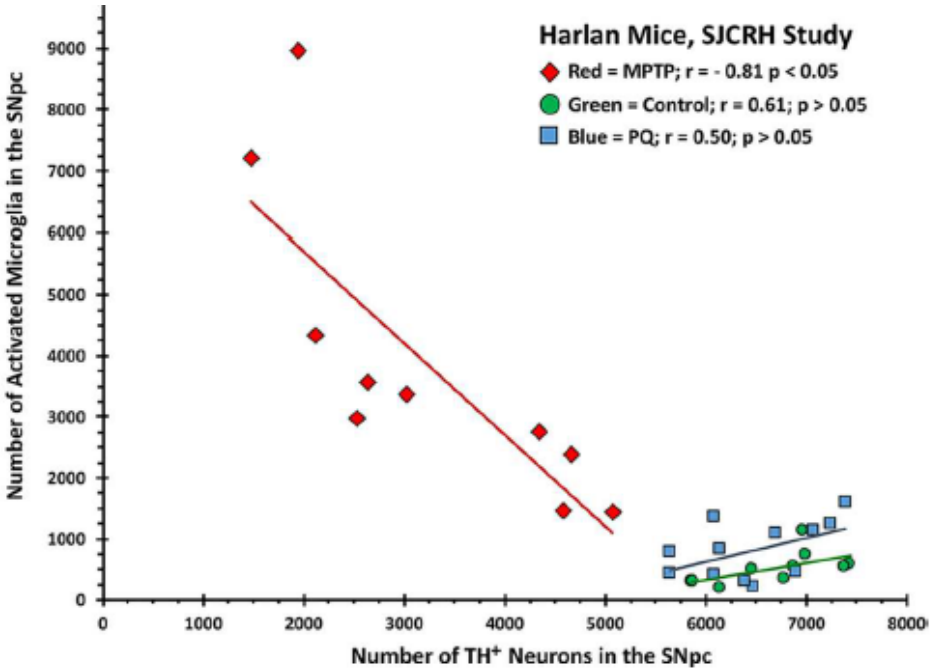
Correlation between the number of activated microglia and the number of TH$^+$ neurons in the SNpc of PQ- & MPTP-treated mice (Smeyne *et al*, 2016)





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Neuropathology severity scores for the SNpc in control, PQ- & MPTP-treated mice (Smeyne *et al*, 2016)

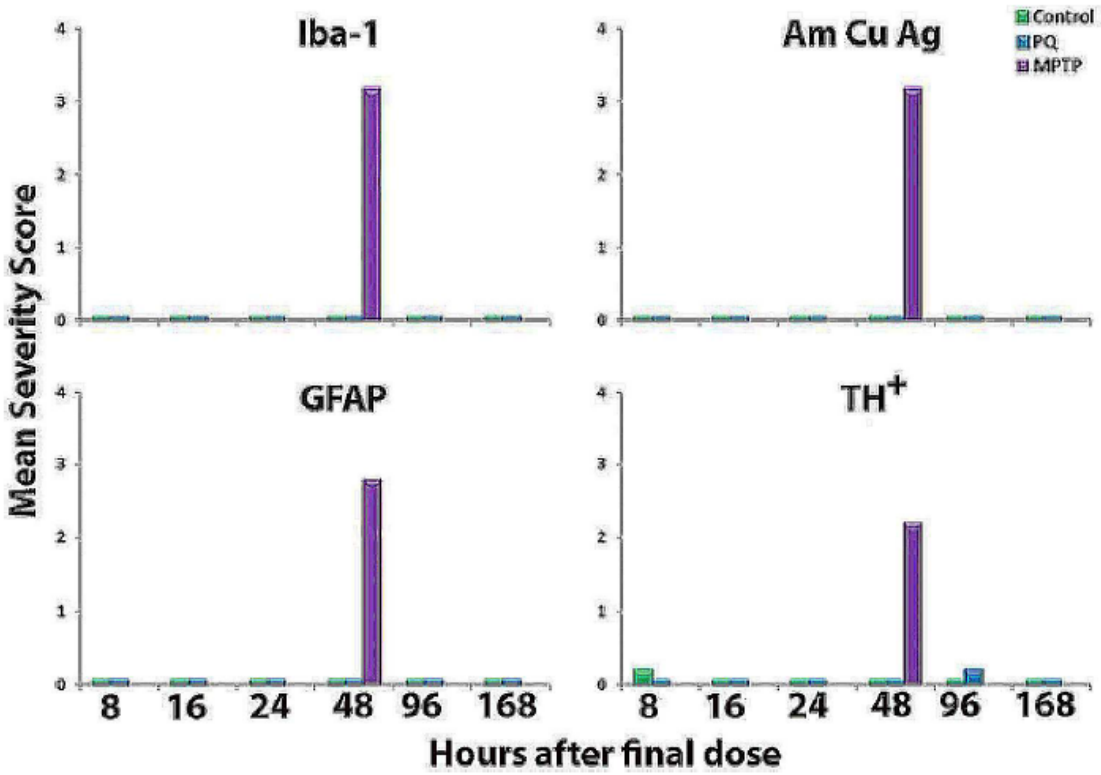

Fig 7. Mean histopathological severity scores in control, paraquat and MPTP-treated groups of C57BL/6J male mice. Mice were 16 weeks of age at the time of treatment initiation. Mice were administered 10 mg/kg/dose PQ·Cl_2 by ip injection, twice a week for 3 weeks and were sacrificed 8, 16, 24, 48, 96 or 168 hours after the last dose. Control mice were given the vehicle while MPTP-treated mice received four injections of MPTP (16 mg/kg/dose; expressed as free base) at 2-hour intervals, and then euthanized 48 hours after the final dose. Serial sections through the SNpc were evaluated qualitatively and the group mean severity grades are plotted. Grades 0 to 5 reflect increasing intensity of staining for Iba-1, AmCuGg, GFAP and decreased staining intensity of TH.

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Evaluation of the i.p. paraquat mouse model (Smeyne *et al*, 2016)

PLOS ONE

RESEARCH ARTICLE

Assessment of the Effects of MPTP and Paraquat on Dopaminergic Neurons and Microglia in the Substantia Nigra Pars Compacta of C57BL/6 Mice

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Abstract

The neurotoxicity of paraquat dichloride (PQ) was assessed in two inbred strains of 9- or 16-week old male C57BL/6 mice housed in two different laboratories and compared to the effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). PQ was administered by intraperitoneal injections; either once (20 mg/kg) or twice (10 mg/kg) weekly for 3 weeks, while MPTP-HCl was injected 4 times on a single day (20 mg/kg/dose). Brains were col-

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Conclusions from Smeyne *et al,* (2016)

- Clear inability by Smeyne to replicate his previous findings with the PQ mouse model.

- PQ administered 1x or 2x per week to 9- or 16-week old mice from two different sources, **had no effect** on the number of DA neurons or microglia as assessed by 2 groups (each blinded to treatment) using two different stereology methods.

- Neuropathology analysis showed that **PQ did not induce** neuronal cell loss or degeneration in the SNpc or striatum and there was no evidence of apoptosis, microgliosis or astrogliosis.

- Smeyne *et al,* (2016) publication includes a systematic evaluation of the literature relating to the PQ mouse model, and shows that **81%** (21/26) **of studies** where the assessment was **conducted blinded**, **did not show any effect of PQ** treatment.

Other key points relating to the evaluation of the i.p. paraquat mouse model & published literature

- Reduced TH immunostaining **alone** is not a reliable marker of DA cell death
- Neuropathology is the gold-standard for validating cell loss detected by stereology - very few studies reported in the literature use this, and those that do, demonstrate that PQ has no effect on DA neurons
- “Unbiased assessments” (e.g. stereology or neuropathology) are not unbiased unless conducted by investigators blinded to treatment
- There is poor concordance between apparent DA cell loss in the SNpc and DA neurochemistry in the striatum
- Standard Errors of the Mean (SEM) values relating to stereology results in many publications, seem biologically implausible (e.g. <1% n=5)
- Falsification of data by one investigator who has published in the field (*Federal Register Notice Volume 77, No. 125, June 28, 2012, 38632-38633:* http://www.gpo.gov/fdsys/pkg/FR-2012-06-28/html/2012-15887.htm)

Dietary administration of paraquat for 13 weeks in the C57BL/6J mouse

- The i.p. paraquat mouse model is inappropriate for human risk assessment purposes because it uses a route of administration, frequency and duration of exposure which is not relevant to human spray applicators.

- To better assess the risk to applicators and more closely mimic spray applicator exposure, continuous exposure for a prolonged period (>3weeks) would be more appropriate.

- Syngenta therefore conducted a study (13 week dietary study) to investigate the effect of PQ in the mouse using a dosing regimen more relevant to human exposure scenarios.

- We presented the initial findings from this study to EPA in 2013 and these have now been published (Minnema *et al,* 2014).

Dietary administration of paraquat for 13 weeks in the C57BL/6J mouse

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

Dietary administration of paraquat for 13 weeks does not result in a loss of dopaminergic neurons in the *substantia nigra* of C57BL/6J mice

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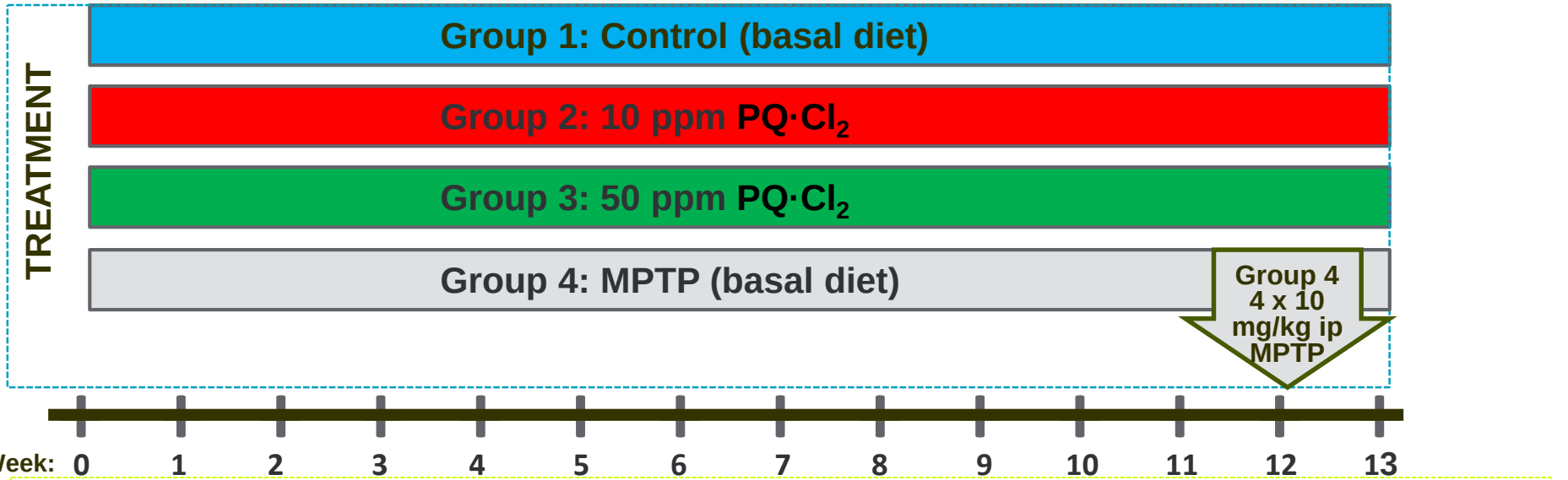
ABSTRACT

Several investigations have reported that mice administered paraquat dichloride ($PQ{\cdot}Cl_2$) by intraperitoneal injection exhibit a loss of dopaminergic neurons in the *substantia nigra pars compacta* (SNpc). In this study, male and female C57BL/6J mice were administered $PQ{\cdot}Cl_2$ in the diet at concentrations of 0 (con-

Dietary administration of paraquat for 13 weeks in the C57BL/6J mouse

- Dermal route is the most relevant route of exposure for spray applicators of PQ.
- Rodent skin poorly replicates the absorption characteristics of human skin, and prolonged exposure of rodent skin to PQ causes skin damage, excluding this route for long term toxicity studies in rodents.
- Although dietary exposure of PQ is considered negligible, the dietary route is a suitable surrogate for dermal exposure because, like dermal exposure, the dose is temporally distributed throughout the day.

Study design



ENDPOINTS

Pathology
In situ perfusion
5 mice/sex
in Groups 1 - 3

Pathology
In situ perfusion
5 mice/sex
in Groups 1 - 3

Pathology
In situ perfusion
5 mice/sex/group

Stereology
In situ perfusion
20 mice/sex/group

Striatal Neurochemistry
Collect & Freeze
6 mice/sex/group

Male & female $C_{57}Bl_{6J}$ mice

Dose groups: Control, 10 & 50 ppm paraquat dichloride salt

Positive control: MPTP (4 x10 mg/kg i.p.)

Stereology n=20 (power to detect at least a 20% reduction)

GLP & assessments conducted by individuals blinded to treatment group

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Dose selection rationale

- Two dose levels of 10 & 50 ppm $PQCl_2$ in the diet were selected.

- PQ dose levels higher than existing NOAEL's currently used by regulators in their risk assessments to protect spray applicators and consumers globally.

- Doses selected resulted in ~1.4x (low dose) and ~8x (high dose) the existing US EPA PQ NOAEL used for spray applicator exposure:
  - NOAEL = 1.25 mg PQ ion/kg/day in a rat multi-generation study

- Achieved dose levels:
  - 10 ppm 1.7 & 2.7 mg PQ ion/kg/day for males & females respectively
  - 50 ppm 10.2 & 15.6 mg PQ ion/kg/day for males & females respectively

- 50 ppm $PQCl_2$ results in a steady state PQ brain concentration after 90 days which is ~1.5 fold greater than the peak PQ brain concentration following a single i.p. dose of 10 mg/kg (Breckenridge *et al,* 2013 & Minnema *et al,* 2014).

Paraquat pharmacokinetics

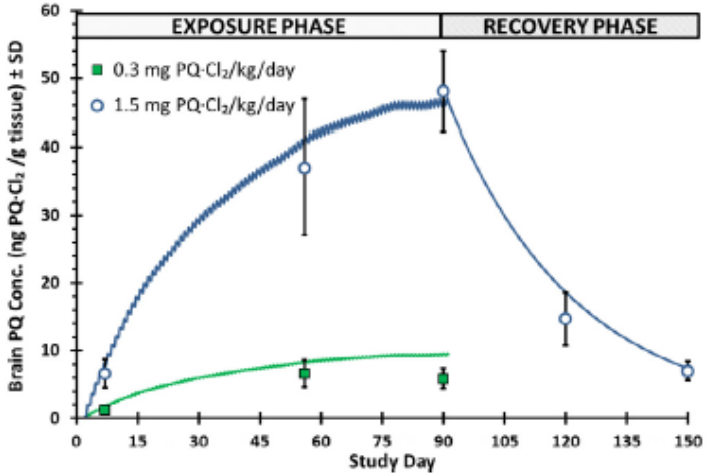

Fig. 1. Brain PQ concentrations in a 13 week kinetic study at dietary dose levels of 0.3 and 1.5 mg/kg/day. Dietary PQ-Cl_2 concentrations were adjusted weekly to maintain achieved dose levels at 0.3 and 1.5 mg/kg bw/day. Brains were collected after 7, 56, and 90 days of exposure, and after 30 and 90 days of recovery (n = 3–4 brains/interval/dose level). The curves are PBPK model predictions, which take into account the measured body weight and food consumption data from the study.

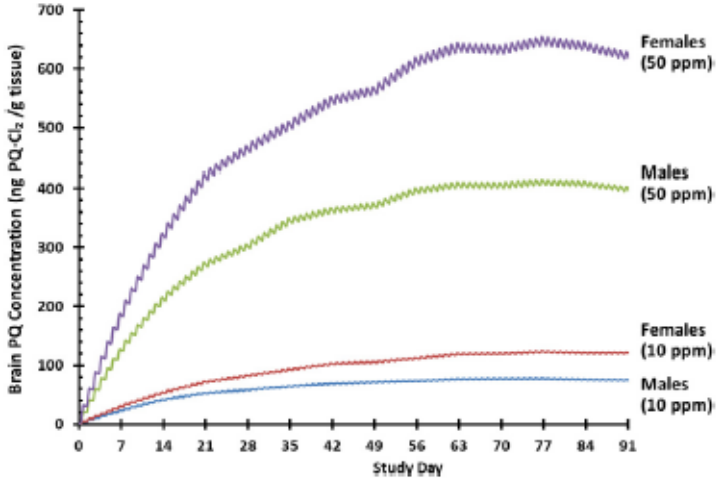

Fig. 2. Modeled PQ brain concentrations at 10 and 50 ppm over 13 weeks. Based on the modeling of the dietary kinetic study, as well as the body weight and food consumption data from the neurotoxicity study, the achieved brain PQ-Cl_2 concentrations over the exposure period were estimated for male and female mice treated with diet containing PQ-Cl_2 at 10 or 50 ppm.

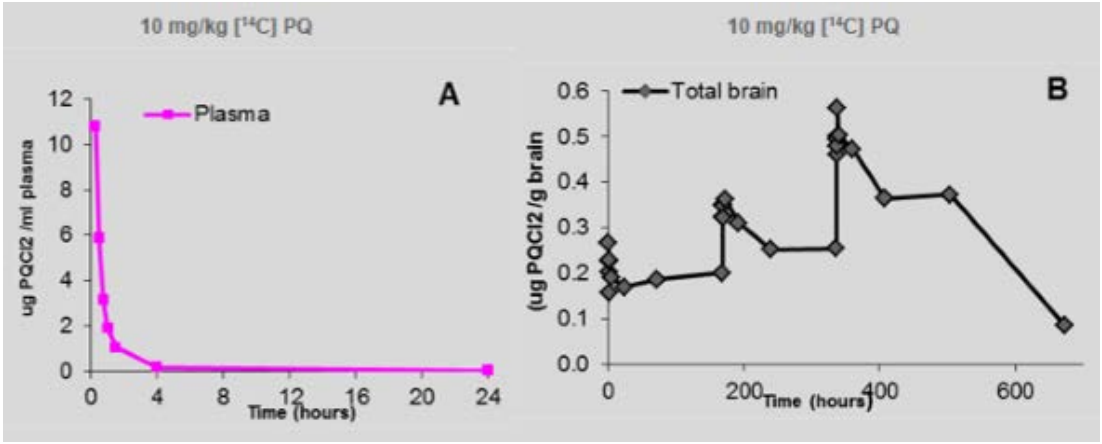

Fig. 1 & Fig. 2 taken from Minnema et al, (2014)

A & B taken from Breckenridge et al, (2013)

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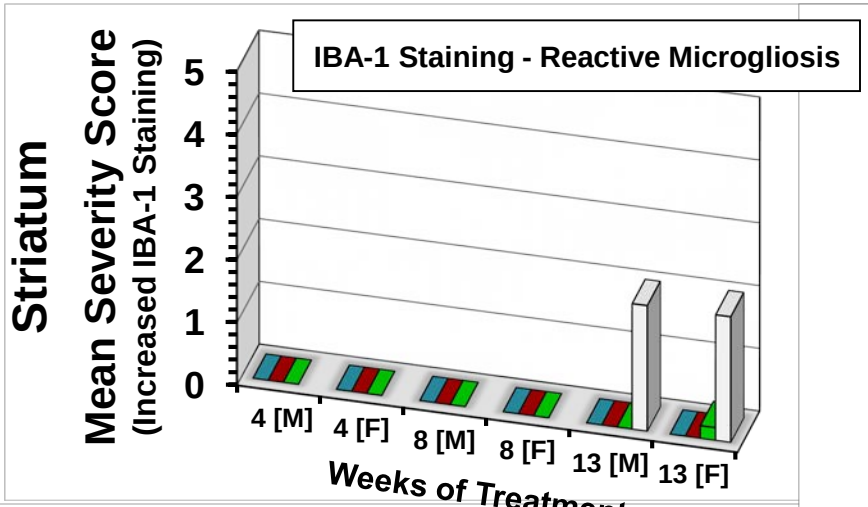
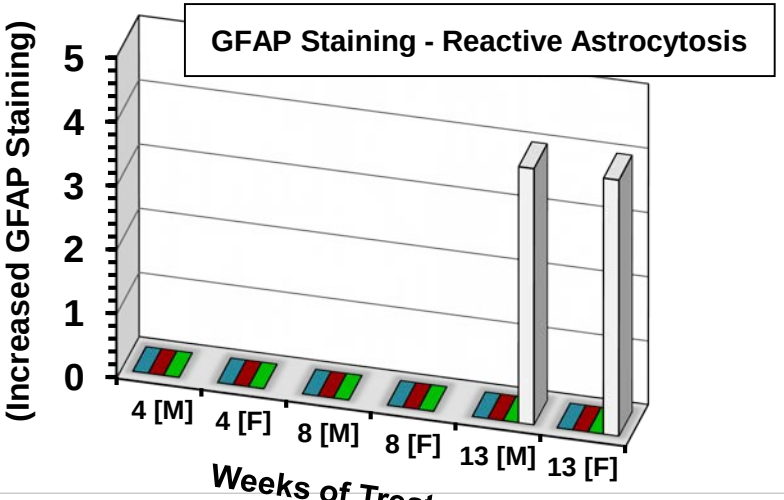
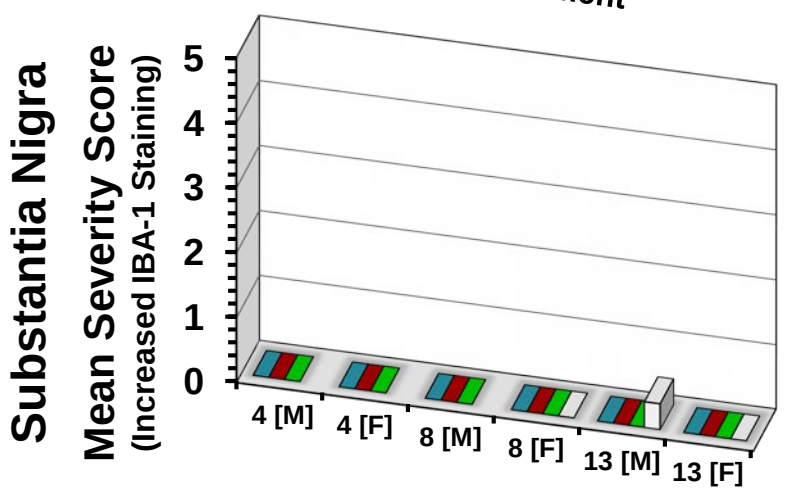
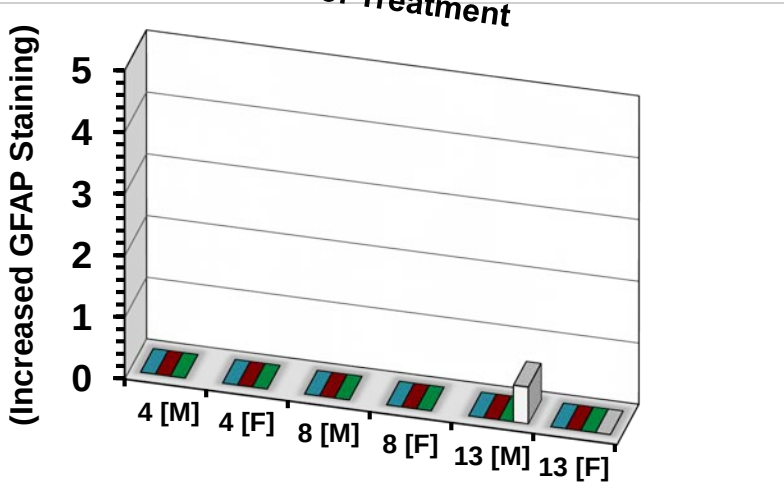
Results - Neuropathology (Minnema *et al*, 2014)

- No PQ-related changes in neuropathology (neuro-inflammation)
- MPTP findings consistent with anticipated findings 1 week after treatment









Control | 10 ppm PQ·Cl_2 | 50 ppm PQ·Cl_2 | 4x10 mg/kg MPTP

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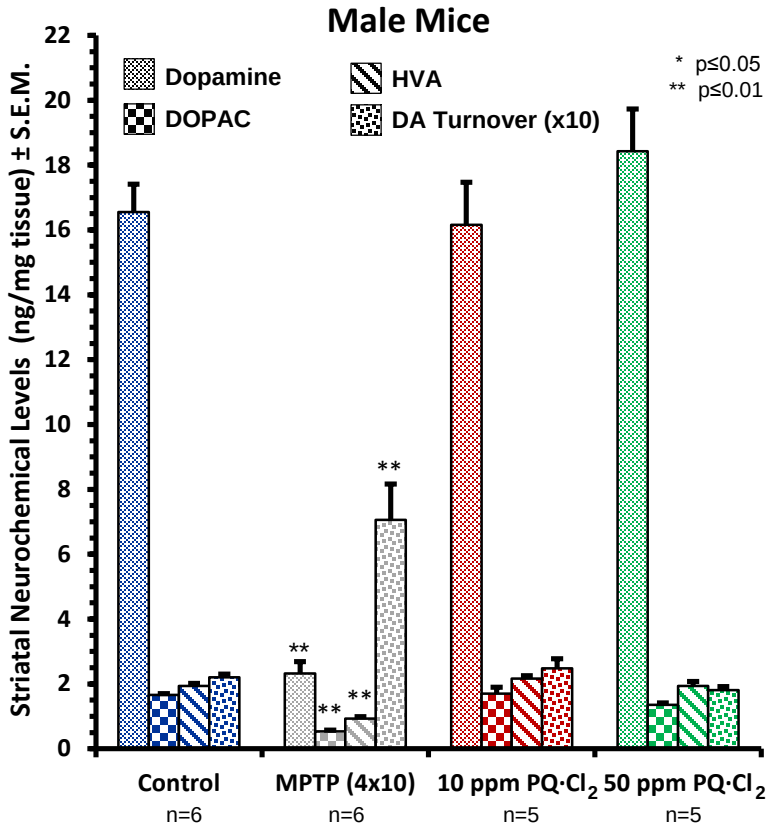
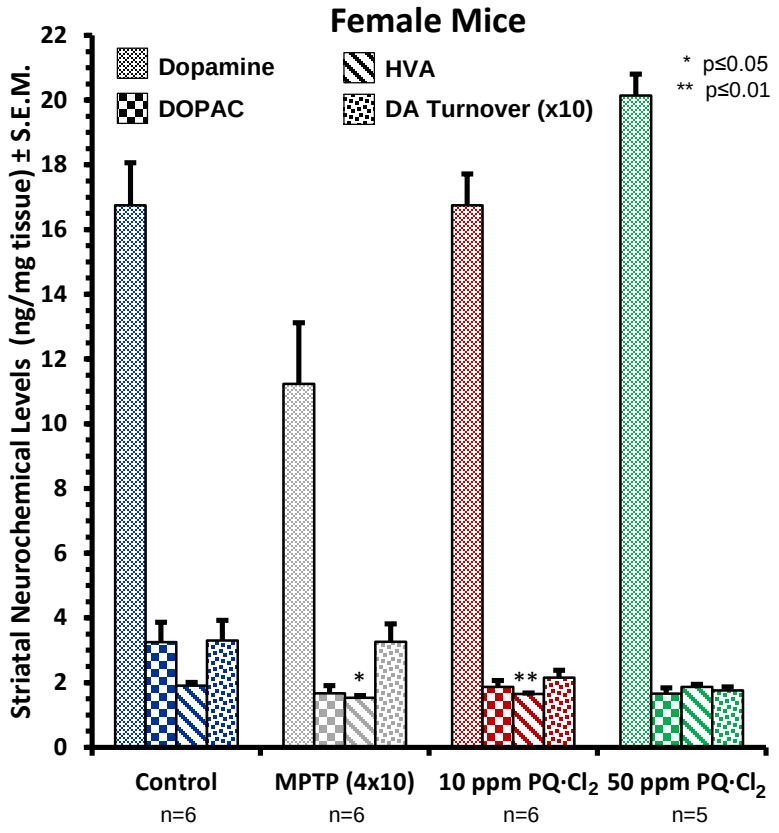
Results - Striatal neurochemistry (Minnema *et al*, 2014)

- No PQ dose-related changes in striatal neurochemistry
- MPTP findings consistent with literature (↓DA, ↓ DOPAC, ↓ HVA, and ↑ DA turnover)
- MPTP effects greater in males than in females





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Results - Stereology TH⁺ neurons (Minnema *et al*, 2014)

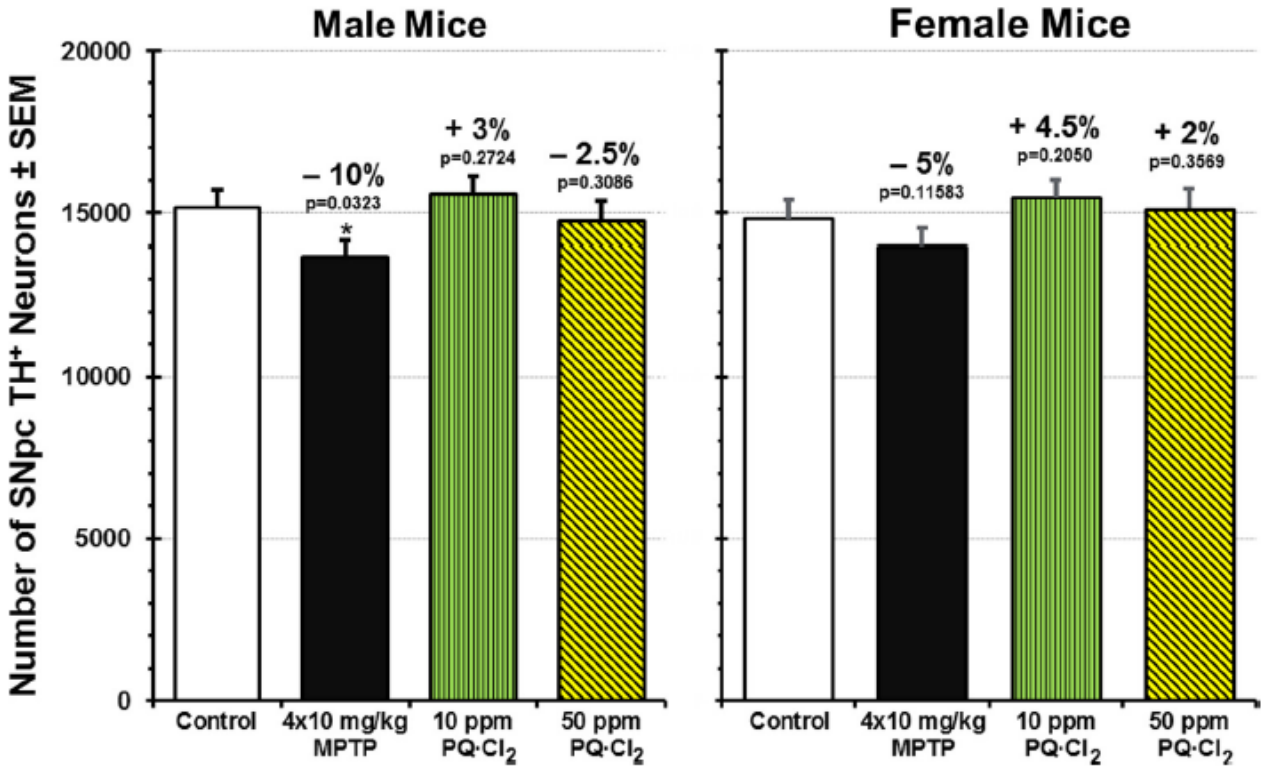

Fig. 6. Number of tyrosine hydroxylase-positive (TH^+) neurons in the *substantia nigra pars compacta* (SNpc) after 13 weeks of dietary treatment with PQ, or 7 days after the ip administration of MPTP, as determined by stereology. Sample size (n) = 20 mice/sex/group, except for 50 ppm PQ·Cl_2 males (n = 19), MPTP males (n = 17), and 50 ppm PQ·Cl_2 females (n = 18). $^*p \leqslant 0.05$.

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Conclusions (Minnema *et al*, 2014)

- The dietary administration of paraquat for 13 weeks to C57BL/6J mice does not lead to the damage or loss of dopaminergic neurones from the substantia nigra:
  - no paraquat-related changes in neuropathology
  - no paraquat-related changes in striatal neurochemistry
  - no paraquat-related changes in number of TH^+ dopaminergic neurones in the SNpc measured by stereology

- These findings are consistent with our results from the i.p. PQ mouse model

- We have established a neurotoxicity NOAEL for paraquat of ≥**10.2 mg paraquat ion/kg bw/day** (highest dose tested)

- NOAEL is ~8X higher than the existing US EPA PQ NOAEL used to protect applicators (1.25 mg PQ ion/kg bw/day in a rat multi-generation study) and ~23X higher than the existing US EPA PQ chronic reference dose used to protect consumers (NOAEL = 0.45 mg PQ ion/kg bw/day in a one year dog study)

Draft EFSA PPR Panel Review
Adverse Outcome Pathway (AOP) relating to paraquat

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: dd mmmm 2016

PUBLISHED: dd mmmm yyyy

doi:10.2903/j.efsa.2016.NNNN

Investigation into experimental toxicological properties of plant protection products having a potential link to Parkinson's disease and childhood leukaemia[1]

EFSA Panel on Plant Protection Products and their Residues (PPR)[2,3]

European Food Safety Authority (EFSA)

Abstract

In 2013 EFSA published a literature review on epidemiological studies linking exposure to pesticides and human health outcome. As a follow up, the PPR Panel was requested to investigate the plausible involvement of pesticide exposure as a risk factor for Parkinson's disease (PD) and childhood leukaemia (CHL). A systematic literature review on Parkinson's disease and Childhood Leukaemia and mode of actions for pesticides was published by EFSA in 2016 and used as background documentation. The Panel used the Adverse Outcome Pathway (AOP) conceptual framework to define the biological plausibility in relation to epidemiological studies by means of identification of specific symptoms of the diseases as AO. The AOP is combining multiple information and provides knowledge of biological pathways, highlight species differences or similarities, identifies research needs and support regulatory decisions. In this context, the AOP approach could help in organizing the available experimental knowledge to assess biological plausibility by describing the link between a molecular initiating event (MIE) and the AO through a series of biologically plausible and essential key events (KEs). As the AOP is chemically agnostic, tool chemical compounds were selected to empirically support the response and temporal concordance of the key event relationships (KERs). Three qualitative and one putative AOP were developed by the Panel. Based on the results obtained, the Panel supports the use of the AOP framework to scientifically and transparently explore the biological plausibility of the association between pesticide exposure and human health outcomes, identify data gaps, define a tailored testing strategy and suggect an AOP's informed Integrated Approach for Testing and Assessment (IATA).

In a draft 2016 document, EFSA have proposed the use of Adverse Outcome Pathways (AOP's) to mechanistically explore the biological plausibility of an association between pesticide exposure (e.g. PQ) and adverse health outcomes (e.g. PD), based on their mode of action.

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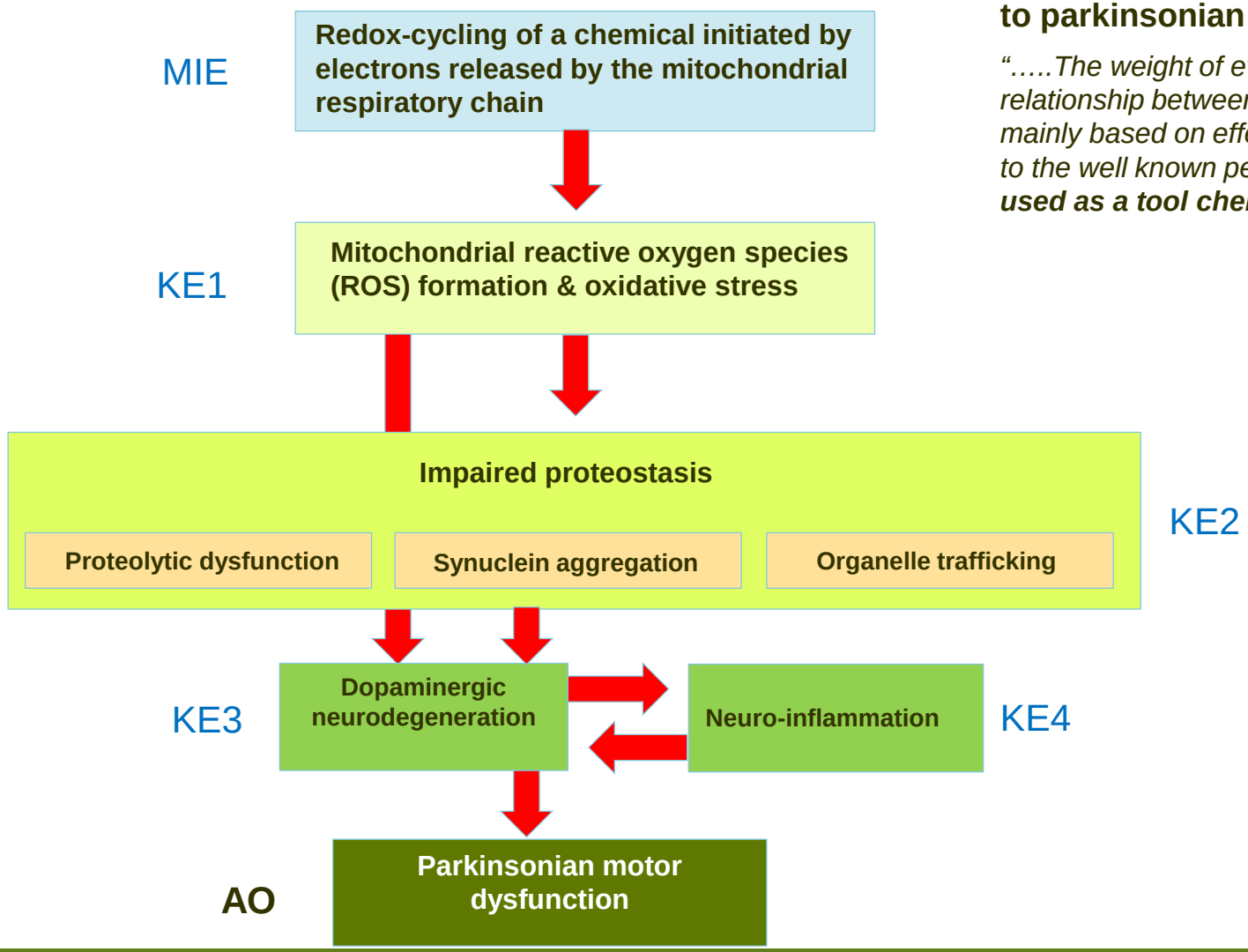
AOP 2 relates to paraquat:



AOP 2: Redox-cycling of a chemical initiated by electrons released by the mitochondrial respiratory chain leading to parkinsonian motor deficits.

"…..The weight of evidence supporting the relationship between the described key events is mainly based on effects observed after an exposure to the well known pesticide ***paraquat which will be used as a tool chemical to support this AOP****"*

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Draft EFSA PPR Panel Review - Syngenta View

- Document is currently in draft form & therefore a “work in progress”.
- Syngenta (and others) submitted comments to EFSA during the public consultation period.
- Syngenta noted that the draft document contained a number of errors, factual inaccuracies, selective quoting of the literature and missing references.
- Uncertainties & inconsistences associated with the PQ mouse model are not given sufficient weight. Data already presented demonstrate it is not a robust and reproducible model when carefully controlled blinded studies are conducted.
- *In vitro* studies cited in support of the AOP used PQ concentrations of 0.1 - >1.0 mM. This is 2 orders of magnitude greater than peak or steady state brain concentrations (2.2 µM) observed in the *in vivo* mouse model.

Draft EFSA PPR Panel Review - Syngenta View

- Clear disconnect between PQ concentrations used *in vitro* to observe effects related to ROS generation & associated neuronal cell death, and the brain PQ concentrations observed following high dose exposure *in vivo*.

- Chemical toxicity mediated by the redox-cycling AOP lacks any concordance in dose-response and incidence where it relates to PQ & PD like neurotoxicity.

- EFSA propose changes to regulatory study design, including a more in-depth evaluation of the brain in 90-day toxicity studies.

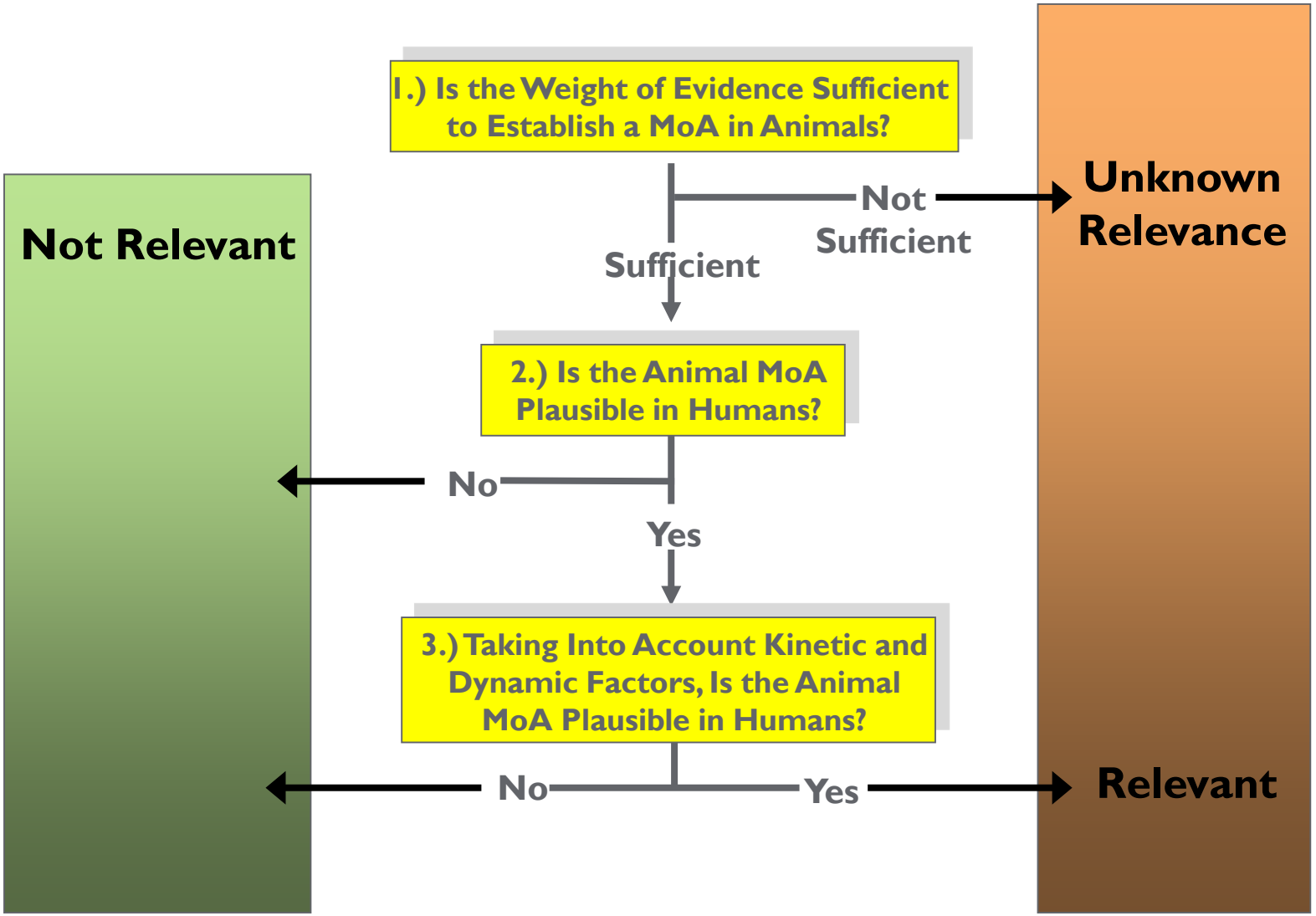
- Includes stereological assessment of the number of DA neurons in the SNpc. This is precisely what Syngenta has done in the studies described previously (Minnema *et al,* 2014), and there is no effect of PQ.

Draft EFSA PPR Panel Review - Syngenta View

- Syngenta conducted studies go beyond the EFSA recommendations with additional toxicity endpoints which include neuropathology assessments of:
  - cell loss
  - cell damage
  - neuro-inflammation
  - neurochemistry

- There is no effect of PQ on these toxicity endpoints.

The Human Relevancy Framework



Adapted from: Cohen, et al., (2004). Evaluating the Human Relevance of Chemically Induced Animal Tumors. *Toxicological Sciences* 78, 181–186.

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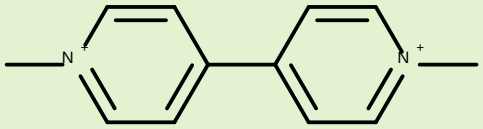
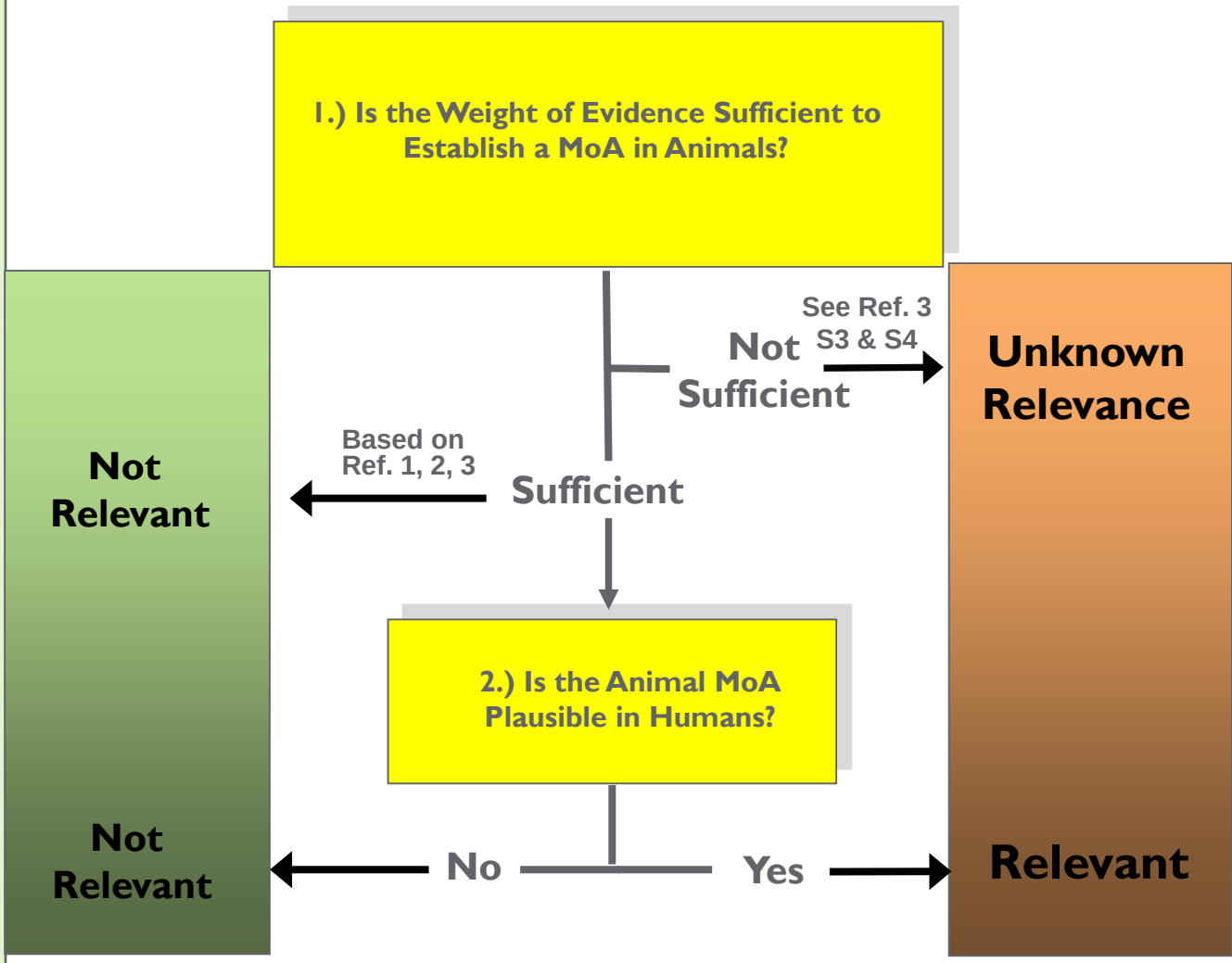
Is it Plausible that Paraquat-Induces Parkinsonism? Low

Paraquat



- Hydrophilic, divalent cation
- Not metabolized
- Limited uptake of PQ by brain
  - 0.04% of an oral dose
  - 0.0009% of a dermal dose
- PQ is not transported into the brain by the dopamine transporter (DAT)
- PQ does not kill DA neurons after:
  - 13 weeks of exposure
  - in a sensitive mouse strain (C57BL/6J males)
  - exposed to a maximum tolerated dose. (Minnema et al., 2014)



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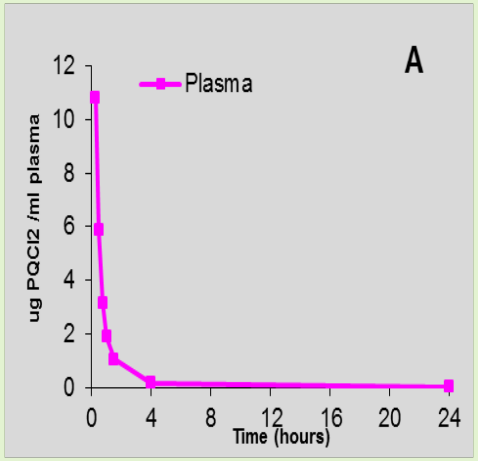
Is it Plausible that Paraquat-Induces Parkinsonism? Low

Paraquat Pharmacokinetics

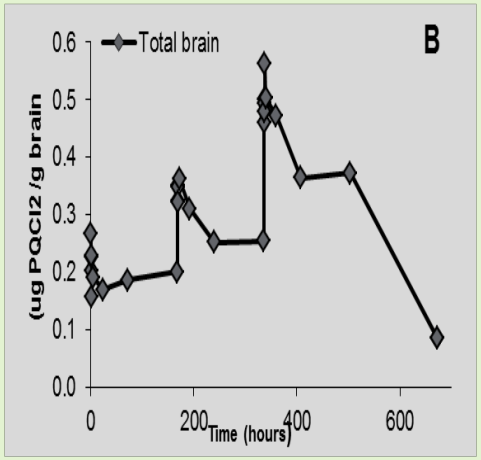
IP Route of Administration: 10 mg/kg [^{14}C]PQ; (Breckenridge et al., 2013)

- Rapid clearance from blood (~4 hours; Panel A)
- ~ 0.3% of the administered dose found in the brain (Panel B).
- PQ does not concentrate in the midbrain where the SNpc is located.
- After a single dose, peak brain concentration 50-fold lower than peak plasma concentration.
- Terminal half-life of elimination from brain is 21- 24 days.
- Steady state brain concentration after 90 days (416 ng/g; Panel C) is 1.5 fold greater than peak concentration (268 ng/g) after single 10 mg/kg dose (Panel B)

10 mg/kg [^{14}C] PQ ip.
(Breckenridge et al., 2013)



10 mg/kg [^{14}C] PQ ip.



PBPK-Modeled Brain Concentration (diet)
Minnema et al., 2014

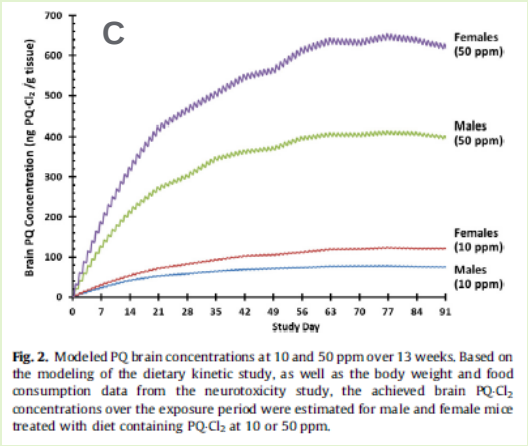
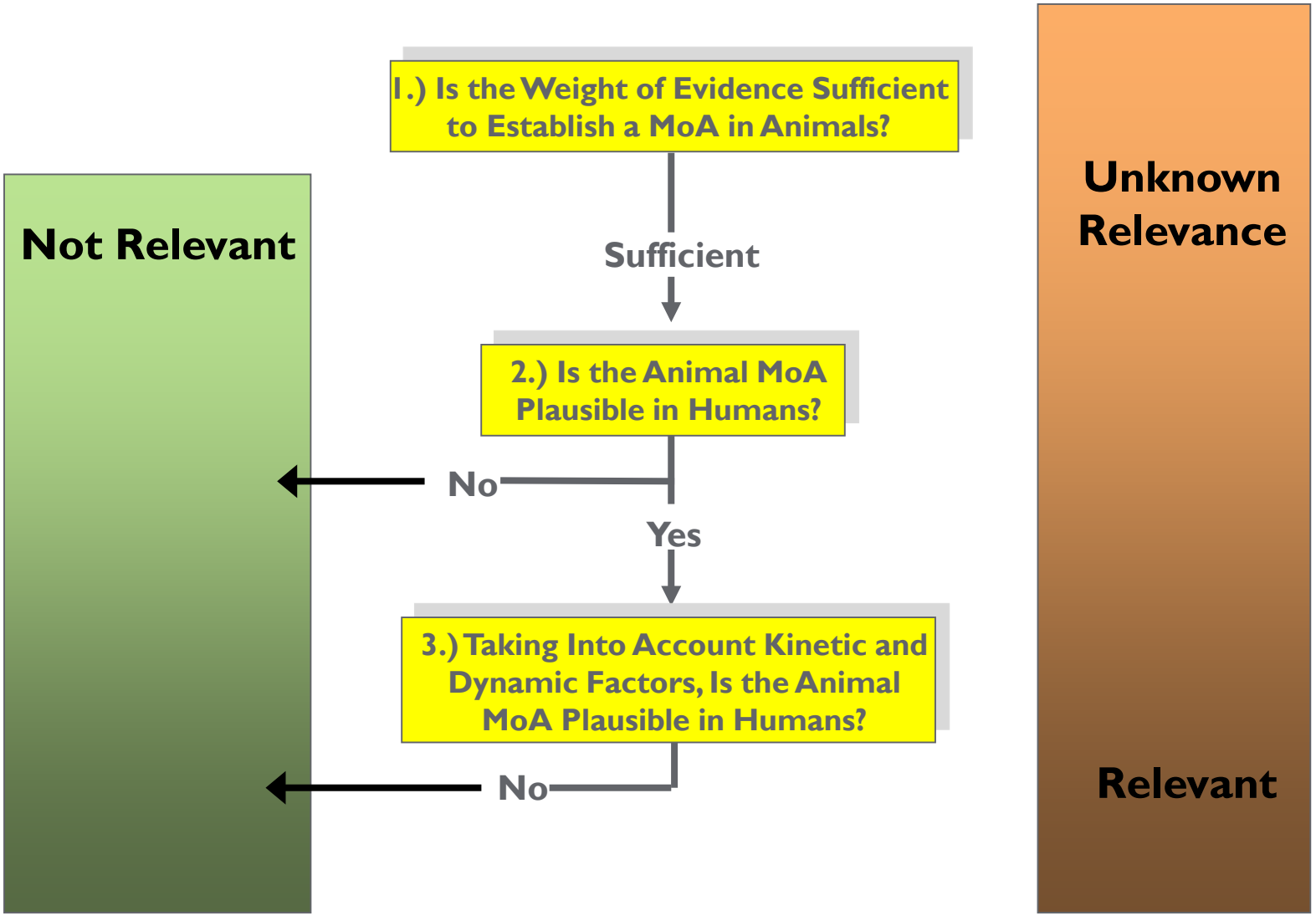


Fig. 2. Modeled PQ brain concentrations at 10 and 50 ppm over 13 weeks. Based on the modeling of the dietary kinetic study, as well as the body weight and food consumption data from the neurotoxicity study, the achieved brain PQ-Cl_2 concentrations over the exposure period were estimated for male and female mice treated with diet containing PQ-Cl_2 at 10 or 50 ppm.

Dermal Route of Administration (Wester et al.,1984).

- ~ 0.3% of the dermal dose entered the blood of which 0.3% enters the brain
- ≈ 0.0009% of the dermal dose expected to enter the brain

The Human Relevancy Framework



Adapted from: Cohen, et al., (2004). Evaluating the Human Relevance of Chemically Induced Animal Tumors. *Toxicological Sciences* 78, 181–186.

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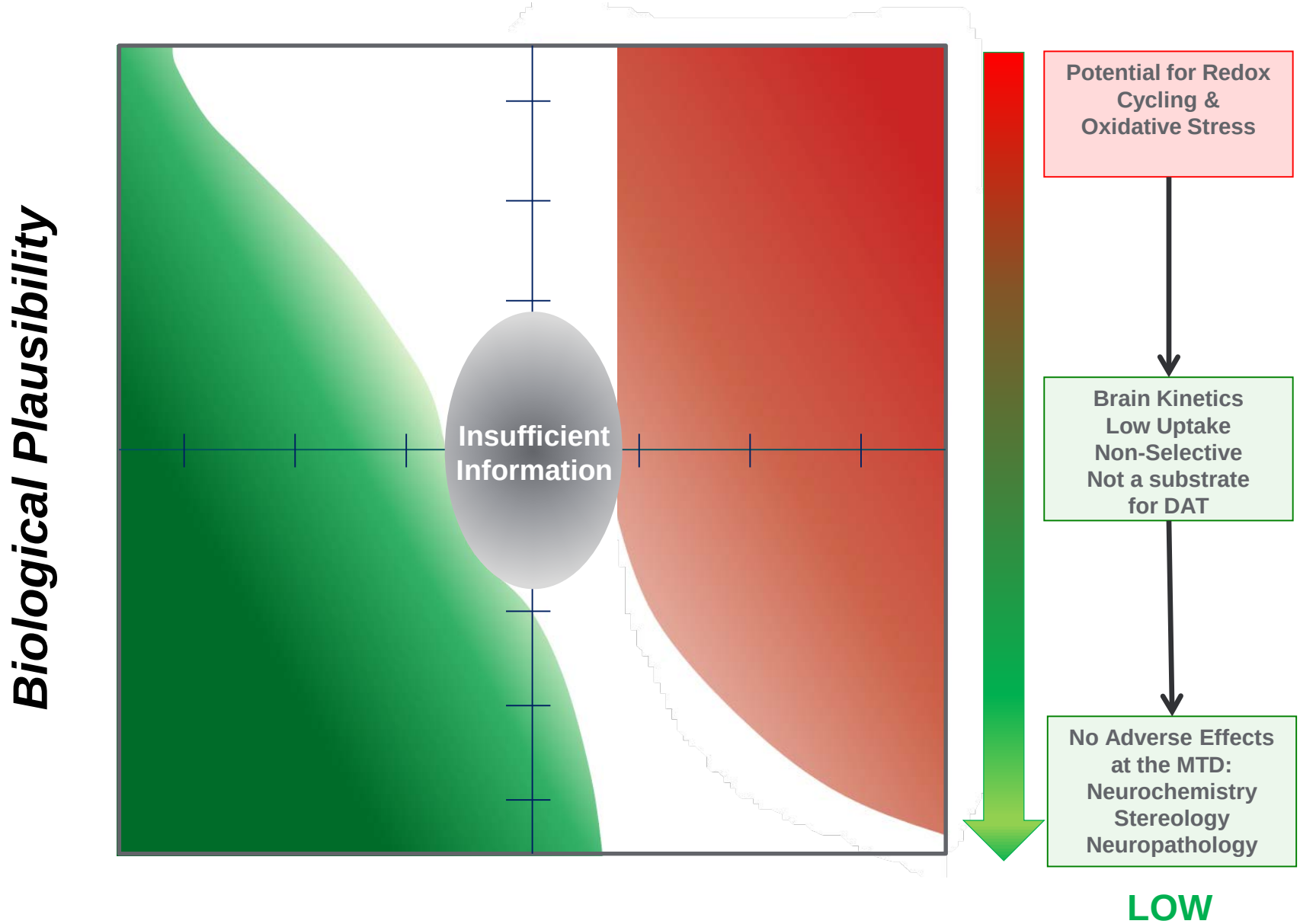
BIOLOGICAL PLAUSIBILITY - PARAQUAT



Adami et al., (2011). Toxicology and Epidemiology: Improving the science with a framework for combining toxicological and epidemiological evidence to establish causal inference. *Toxicological Sciences* 122, 223–234.

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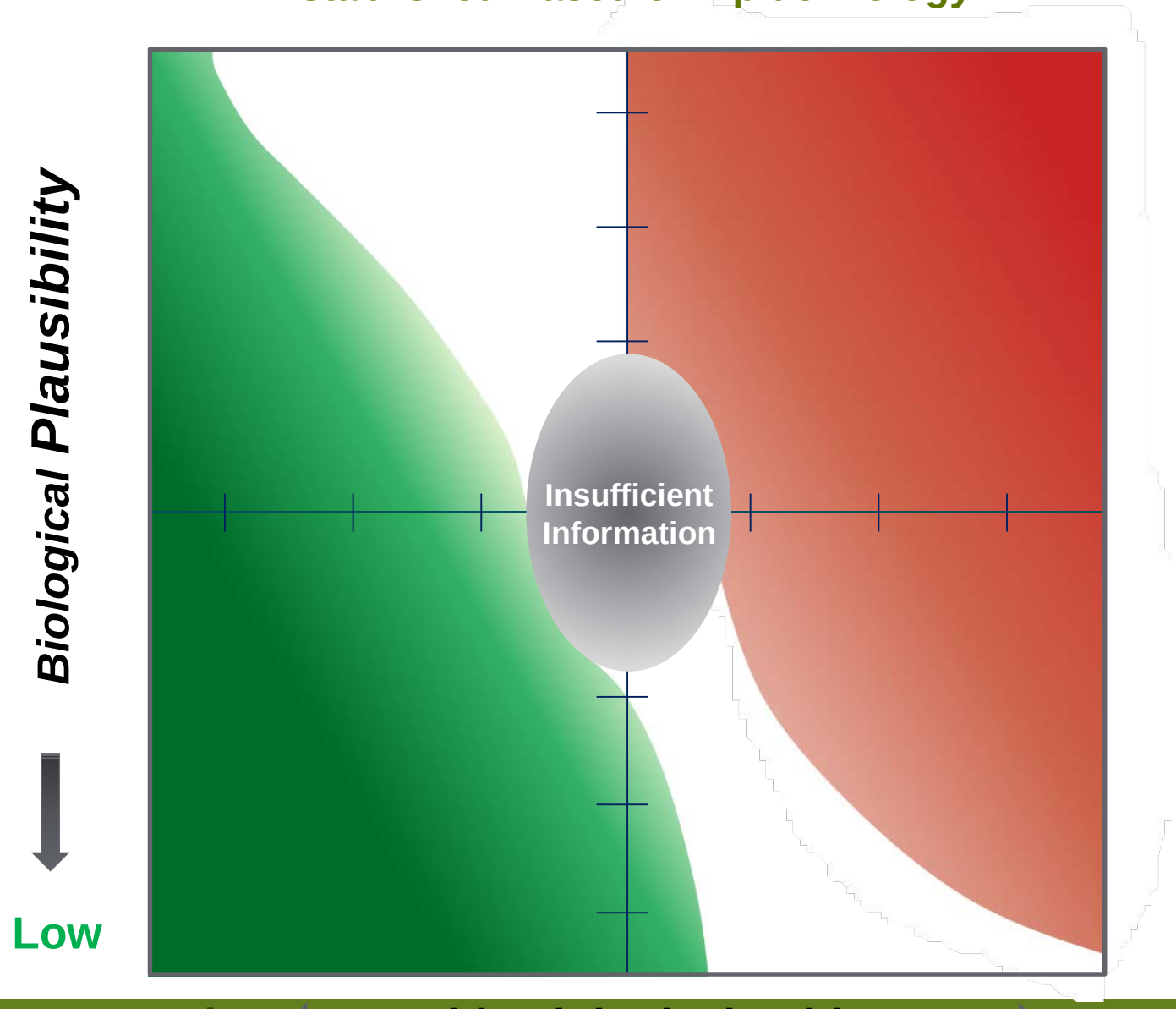
Has a Causal Relationship Between Paraquat Use and Parkinson's Disease been Established Based on Epidemiology



Epidemiological Studies or Reviews Sponsored by Syngenta

Risk Factors for Parkinson's Disease

Wirdefeldt, K., Adami, H-O., Cole, P., Trichopoulos, D. and Mandel., J. (2011). Epidemiology and etiology of Parkinson's disease: A review of the evidence. *Eur. J. Epidemiol.* 26, S1-S58.

Effects of Known Human Exposure to Paraquat

Brent, J. and Schaeffer, T.H. (2011). Systematic review of Parkinsonian syndromes in short and long-term survivors of paraquat poisoning. *JOEM* 53, 1332-1336.

Tomenson, JA, Campbell C. (2011). Mortality from Parkinson's disease and other causes among a workforce manufacturing paraquat: a retrospective cohort study. *BMJ Open.* 2011; 2:e000283. Epub.

Systematic Reviews/Meta-Analysis

Berry, C., C. La Vecchia and Nicotera, P. (2010). Paraquat and Parkinson's disease. *Cell Death Differ.* **17**(7): 1115-1125.

Mandel, J.S., Adami, H-O., and Cole, P. (2012) Paraquat and Parkinson's disease: An overview of the epidemiology and a review of two recent studies. *Reg Tox Pharmacol.* 62,385–392.

Breckenridge, C.B., Berry, C., Chang, E. Sielken Jr., R.L. and Mandel, J.S. (2016). Association between Parkinson's disease and cigarette smoking, rural living, well water consumption, farming and pesticide use. PLOS One. 11(4): e0151841. doi:10.1371/journal.pone.0151841

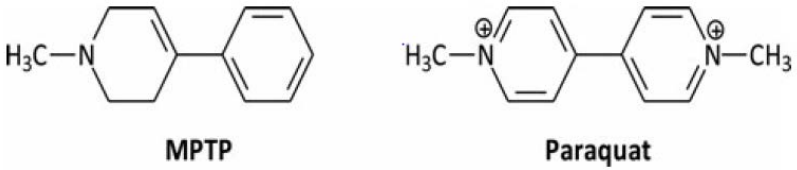
Methodological Reviews

Chang, E.T., Adami, H-O., Bailey, W.H., Boffetta, P., Krieger, R.I., Suresh H. Moolgavkar, S.H. and Jack S. Mandel, J.S., (2014) Validity of geographically modeled environmental exposure estimates. Crit. Rev. Toxicol., 44, 450-466.

Systematic review of parkinsonian syndromes in short- and long-term survivors of paraquat poisoning (Brent & Schaeffer, 2011)

Question

Does exposure to high doses of paraquat result in parkinsonism similar to that observed in individuals exposed to the structurally-similar human neurotoxin MPTP?



Study Objective

Evaluate whether individuals exposed to high, near lethal doses of paraquat display a parkinsonian syndrome

Method

Identification of Potentially Eligible Studies

Obtained/translated all case reports of PQ exposure published in peer reviewed journals (17 languages) before August, 2010.

Inclusion/Exclusion Criteria

- Paraquat exposure was documented (Table 1).
- Patients assessed for evidence of parkinsonian symptoms
  - Tremor, rigidity, bradykinesis, postural instability

TABLE 1. Criteria for Fulfilling the Case Definition of Paraquat Poisoning

Cases were considered to have paraquat poisoning if they met either of the following sets of criteria:

1. Have laboratory confirmation of paraquat exposure, and
2. Have corrosive skin or mucosal injury or syndromes of renal or pulmonary injury consistent with paraquat toxicity.

OR

1. Have a history of paraquat exposure, and
2. Have at least two of the following: corrosive skin or mucosal injury, renal injury consistent with paraquat toxicity, or pulmonary injury consistent with paraquat toxicity.

Cases were excluded if laboratory studies done on presentation failed to detect the presence of paraquat.

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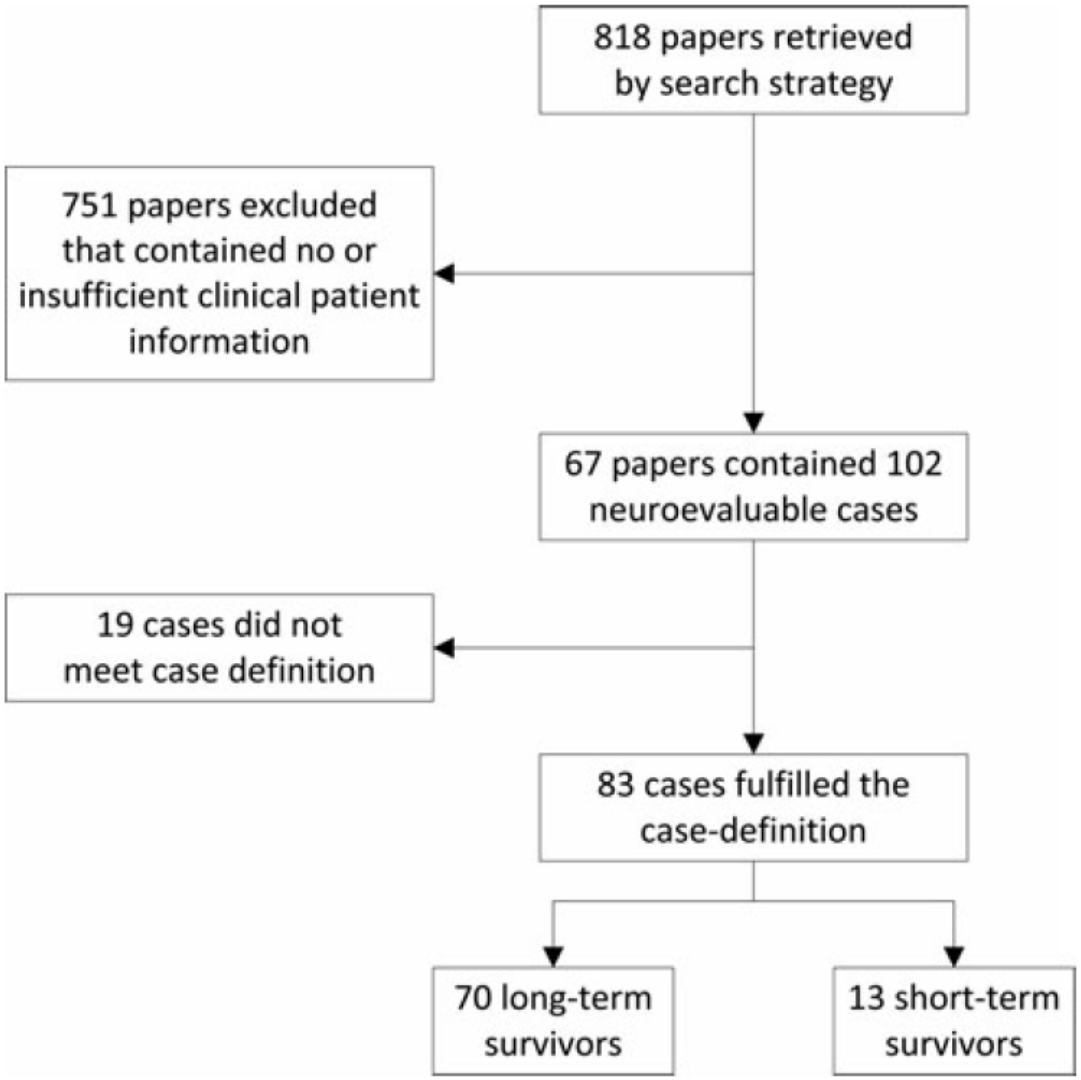
Systematic review of parkinsonian syndrome in short- and long-term survivors of paraquat poisoning (Brent & Schaeffer, 2011)

Publications Meeting Selection Criteria



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Systematic review of parkinsonian syndrome in short- and long-term survivors of paraquat poisoning (Brent & Schaeffer, 2011)

Clinical Outcomes in Short- and Long-Term Survivors

TABLE 3. Clinical Features of 70 Long-term Survivors Meeting the Case Definition of Paraquat Poisoning

Characteristic	Number (%)
Documented suicide attempt	30 (43)
Pulmonary syndrome	36 (51)
Renal syndrome	55 (79)
Skin or mucus membrane corrosive injury	52 (74)
Laboratory confirmation	54 (77)
Exposed by oral ingestion	64 (91)
Exposed dermally	5 (7)
Exposed by inhalation	1 (1.4)
Exposed intravenously	1 (1.4)
Unknown route of exposure	3 (4.3)
Tremor	0 (0)
Rigidity	0 (0)
Bradykinesia	0 (0)
Postural instability	0 (0)

TABLE 4. Clinical Features of 13 Patients Meeting the Case Definition of Paraquat Poisoning and Surviving 15 to 30 Days

Characteristic	Number (%)
Suicide attempt	5 (38)
Pulmonary syndrome	13 (100)
Renal syndrome	12 (92)
Skin or mucus membrane corrosive injury	6 (46)
Laboratory confirmation	12 (92)
Exposed by oral ingestion	13 (100)
Tremor	0 (0)
Rigidity	0 (0)
Bradykinesia	0 (0)
Postural instability	0 (0)

Conclusion: Unlike MPTP, acute exposure to high, near-lethal doses of paraquat does not result in parkinsonism.

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Mortality from Parkinson's disease and other causes among a workforce manufacturing paraquat: a retrospective cohort study (Tomenson & Campbell, 2011)

Question

Does exposure to paraquat in a cohort of manufacturing workers result in higher mortality from Parkinson's disease as reported on death certificates as compared with national and local morality rates?

Method

Cohort comprised 926 male workers who were engaged in the manufacturing of paraquat at ICI's Widnes plants in northeastern England from 1961 to 1995 (Table 1)

Mortality in the cohort was followed up to June 30, 2009 (Table 2)

Standardized mortality rates (SMR; 95%CI) calculated for cohort members that had PD indicated on their death certificate.

Reference groups: Local and England & Wales mortality records

Table 1 Plants where male subjects were employed

Plants	N (%)
HTS only	79 (8.5)
HTS and MAG	17 (1.8)
HTS, MAG and LTS	27 (2.9)
HTS and LTS	18 (1.9)
HTS, LTS and AC	1 (0.1)
MAG only	79 (8.5)
MAG and LTS	147 (15.9)
MAG, LTS and AC	10 (1.1)
LTS only	462 (49.9)
LTS and AC	75 (8.1)
AC only	11 (1.2)
Total	926 (100.0)

AC, ammonia cyanide; HTS, high-temperature sodium; LTS, low-temperature sodium; MAG, magnesium.

Table 2 Vital status on 30 June 2009

Vital status	Males
Alive	616
Dead	292
Emigrated or joined armed forces	10
Lost to follow-up	8
Person years of follow-up	28963
Total	926

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Mortality from Parkinson's disease and other causes among a workforce manufacturing paraquat: a retrospective cohort study (Tomenson & Campbell, 2011)

Results

Cause and Period	Observed	Expected	SMR	95% CI	Reference Mortality
Underlying cause 1960-1992 Mentioned cause 1993-2008	1 1	1.8 3.3	55 31	1-309 1-171	England and Wales
Underlying cause 1960-1992 Mentioned cause 1993-2008	1 1	1.6 3.2	61* 32	2-340 1-176	Local

*** p < 0.05; SMR significantly less than 100.**

Conclusion

There was no evidence of an increased risk of Parkinson's disease assessed by death certificate in a cohort of paraquat manufacturing workers

Association between Parkinson's disease and cigarette smoking, rural living, well water consumption, farming and pesticide use: Systematic review and meta-analysis.[1]

Study Objectives:

- Bradford Hill's viewpoints were used to conduct a weight-of-the evidence assessment of the association between Parkinson's disease (PD) and rural living, well water consumption, farming and pesticide use, including PQ.
- The results were compared to an assessment based upon meta-analysis.
- For comparison, we also evaluated the association between PD and cigarette smoking as a "positive control" because a strong inverse association has been consistently described in the literature.

Methods

Standard methods for the conduct (Cochrane, 2008[2]) and reporting of meta-analysis and systematic reviews (Moher et al., 2009[3]) were used.

Identification of Eligible Studies

A systematic search was conducted to identify all eligible studies. Estimated RR's and 95% CI's were extracted; used most highly adjusted estimates or used/calculated crude RRs and 95% Cis if not reported.

Only one RR per study was included; overlapping RRs were excluded from meta-analysis.

Study Quality

Each study was categorized independently by two epidemiologists as a

- Tier 1 Study: Incidence (newly diagnosed) cases, with clinical confirmation exposure assessed at the individual level.
- Tier 2 Study: All other studies.

Sensitivity Analysis

For each study, the source of the exposed and unexposed population, the method used to assess exposure and whether there were adjustments for potential confounders (Age, gender, smoking)

Association between Parkinson's disease and cigarette smoking, rural living, well water consumption, farming and pesticide use: Systematic review and meta-analysis.

Methods (Cont'd)

Study Heterogeneity: Assessed by I^2 (the percentage of total variability arising from between-study variability)

Within-Study Variance: Assessed by calculating the variance **(σ^2)** about the study mean

Between-Study Variance: Assessed by calculating T^2 = Total variance – Within-study variance

Assessment of an Association

Fixed Effects Model: Studies were weighted inversely proportion to σ^2.

Random Effects Model: Study were weighted inversely proportion to $\sigma^2 + T^2$

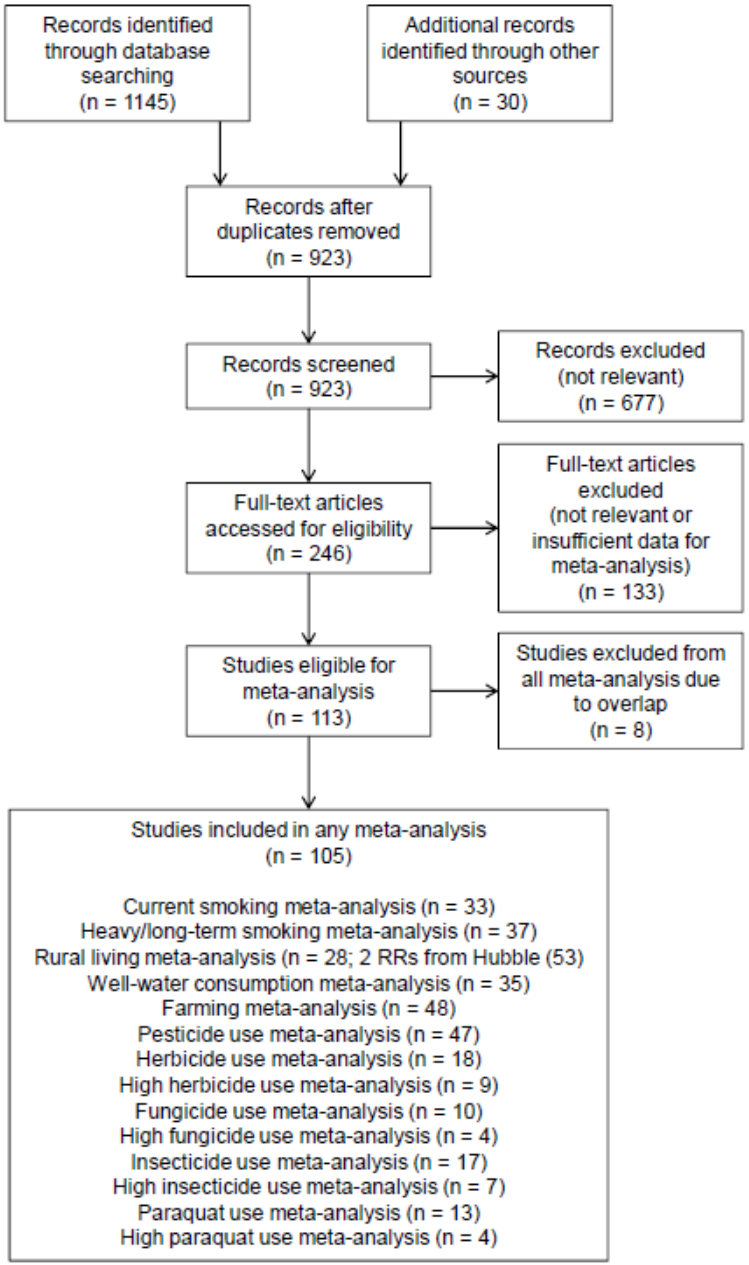
Publication Bias

Funnel Plots and Egger Statistics: Asymmetry of RRs (Funnel plots) were analyzed statistically using Egger's statistic.

Correction for Publication Bias: RRs and 95%CI were calculated before and after adjustment for publication bias (Trim and Fill procedure of Duval and Tweedie, (2000)

Study Identification, Inclusion & Exclusion Criteria

Summary of Literature Search and Study Eligibility: Inclusion and Exclusion



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Association between Parkinson's disease and cigarette smoking, rural living, well water consumption, farming and pesticide use

RESULTS

Tier 1 vs. Tier 2 Studies: Overall, only 20% of the 316 RRs evaluated were from Tier 1 studies (Breckenridge et al., Table 2).

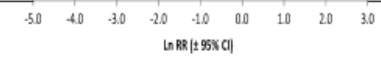
Control for Known Risk Factors: Overall, 52% of the 316 RRs evaluated adjusted for all three known risk factors (age, gender and cigarette smoking) whereas 48% did not.

Study Heterogeneity: For all scenarios assessed, except fungicides, there was statistically significant heterogeneity between studies (Breckenridge et al., 2016; Table 3).

Publication Bias: There was limited statistical evidence of publication bias. Correction for asymmetry in the distribution of RRs had little to no impact on the meta-analysis RRs.

Association Between Heavy Cigarette Smoking and Parkinson's Disease

Year	Author	Tier	RR	LCL	UCL	Exposure
1994	Grandinetti [96]	1	0.77	0.66	0.90	Per 10 pack-years
2000	Benedetti [60]	1	0.69	0.32	1.48	> 30 pack-years smoked
2001	Hernán [61]	1	0.30	0.20	0.60	15+ cig./day, pooled HPFS & NHS
2002	Checkoway [62]	1	0.40	0.20	0.80	40+ pack-years smoked
2005	Wirdefeldt [64]	1	0.52	0.29	0.95	> 120 cig/week, both sexes, external
2007	Thacker [65]	1	0.55	0.35	0.86	45+ pack-yrs vs. never smoke, M&F
2008	Tan [66]	1	0.18	0.07	0.45	Current smoker, 13+ cigarettes/day
2009	Costello [68]	1	0.54	0.38	0.78	> 19 pack-yrs of cig. smoking
2009	Ritz [70]*	1	0.61	0.35	1.05	40+ pack-years of smoking
2010	Chen [71]	1	0.57	0.44	0.74	50+ pack-years of smoking, M&F
2012	Liu [74]	1	0.68	0.55	0.83	≥ 30 yrs, past smokers
1990	Sasco [97]	2	0.27	0.08	0.96	Current Smoking ≥ 40 yrs
1990	Sasco [97]*	2	0.71	0.30	1.60	40+ cigarettes/day
1993	Butterfield [98]	2	0.32	0.15	0.67	Packs/day 15 yrs before, model 2
1993	Wang [99]	2	0.50	0.23	1.09	Smoking > 20/day
1994	Mayeux [75]	2	0.60	0.30	1.20	> 30 pack-years
1994	Morano [100]	2	0.23	0.04	1.20	> 20 cigarettes/day, males
1997	Hellenbrand [77]	2	0.30	0.20	0.60	> 40 pack-years, vs. neighbors
1997	Liou [59]	2	0.43	0.20	0.90	20+ yrs of smoking cigarettes
1999	Gorell [101]*	2	0.08	0.01	0.62	> 30 pack-years, current smoker
1999	Fall [80]	2	0.31	0.11	0.78	24-123 pack-years cigarettes
1999	Taylor [102]	2	0.82	0.66	0.90	Per 10 pack-years
2000	Vanacore [103]	2	0.32	0.15	0.66	> 30 pack-years
2001	Paganini-Hill [82]	2	0.42	0.22	0.80	Current smoking, 1+ pack/day
2001	Behari [104]	2	0.74	0.46	1.19	Smoking > 20 years
2003	Dong [84]	2	0.35	0.18	0.70	20+ years smoked
2003	Tan [105]	2	0.38	0.20	0.72	Smoking 3 packs/day for 10 years
2003	Pals [106]	2	0.44	0.23	0.82	Lifetime max daily # cig. (per 20 cig.)
2003	Ragonese [85]	2	0.72	0.29	1.01	> 30 pack-years
2003	Baldereschi [107]	2	0.60	0.32	1.12	20+ pack-years
2004	Gorell [108]	2	0.42	0.25	0.71	> 30 pack-years
2005	Galanaud [87]*	2	0.40	0.20	0.70	> 17.4 pack-years
2005	Scott [86]*	2	0.31	0.14	0.72	> 18.75 pack-yrs, truncated at ref.
2006	Evans [109]	2	0.33	0.12	0.92	40.1-50 pack-years of cig. smoking
2006	Ma [110]	2	3.41	1.20	7.74	> 30 pack-years
2007	Hancock [88]	2	0.35	0.20	0.62	> 48 pack-yrs, truncated at ref. age
2007	Ritz [111]*	2	0.60	0.46	0.80	60+ pack-years, both sexes
2007	Kamel [18]	2	1.00	0.40	2.30	> 30 pack-yrs, incident PD
2007	Kamel [18]*	2	0.30	0.10	0.90	> 30 pack-yrs, prevalent PD
2008	Powers [90]	2	0.44	0.31	0.64	40+ pack-years
2008	Petersen [91]	2	0.53	0.26	1.08	Smoking for 30 yrs or more
2009	Elbaz [55]	2	0.40	0.20	0.70	Ever cig. smoke, pack-yrs > 17
2010	Tanaka [93]	2	0.28	0.15	0.49	30+ pack-years of smoking
2010	Nicoletti [95]	2	0.39	0.26	0.60	35+ years of smoking
Meta (Tier 1, Random Effects)			0.55	0.45	0.67	
Meta (Tier 2, Random Effects)			0.47	0.39	0.57	
Meta (Tiers 1&2, Random Effects)			0.49	0.43	0.57	



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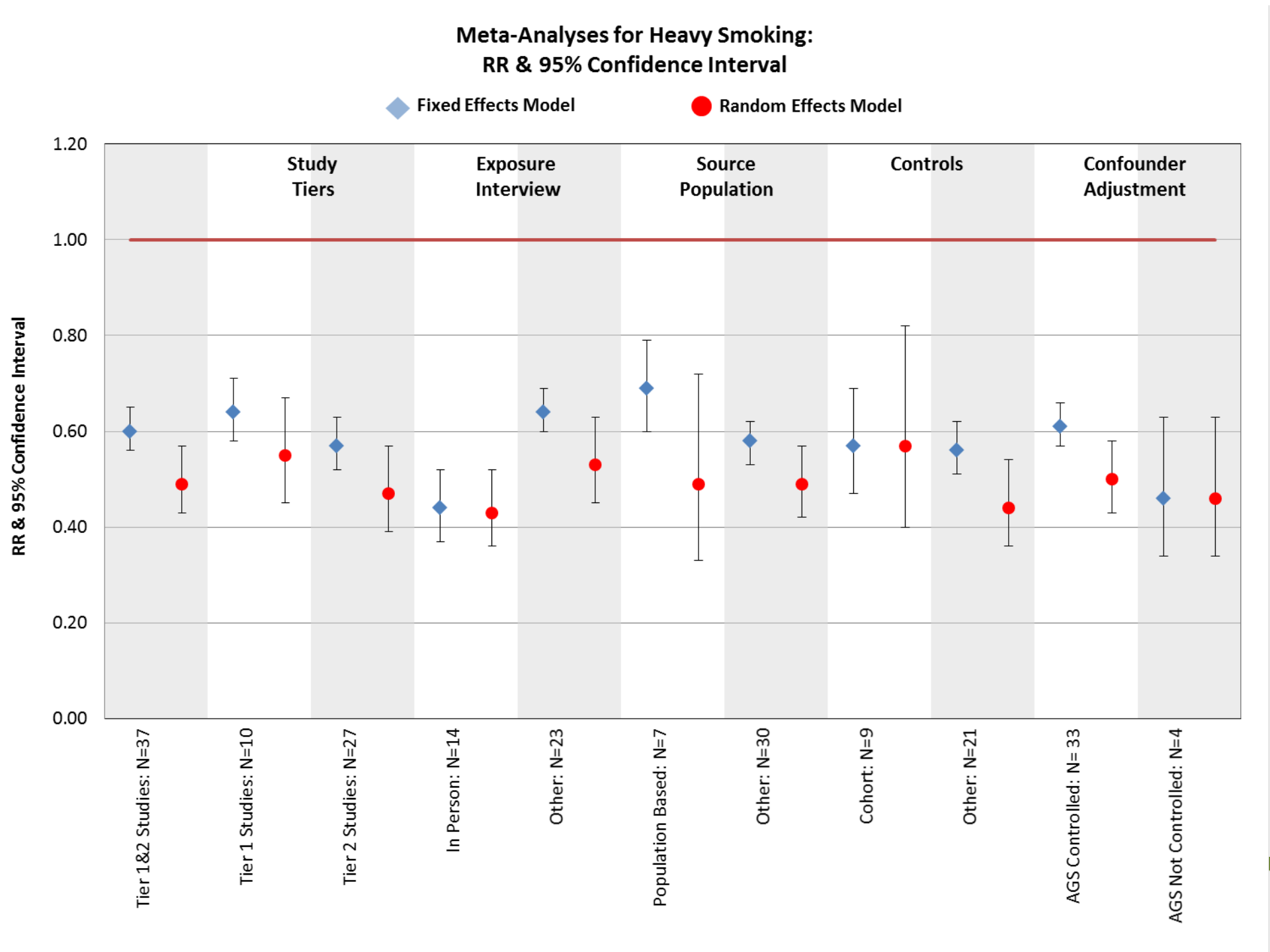
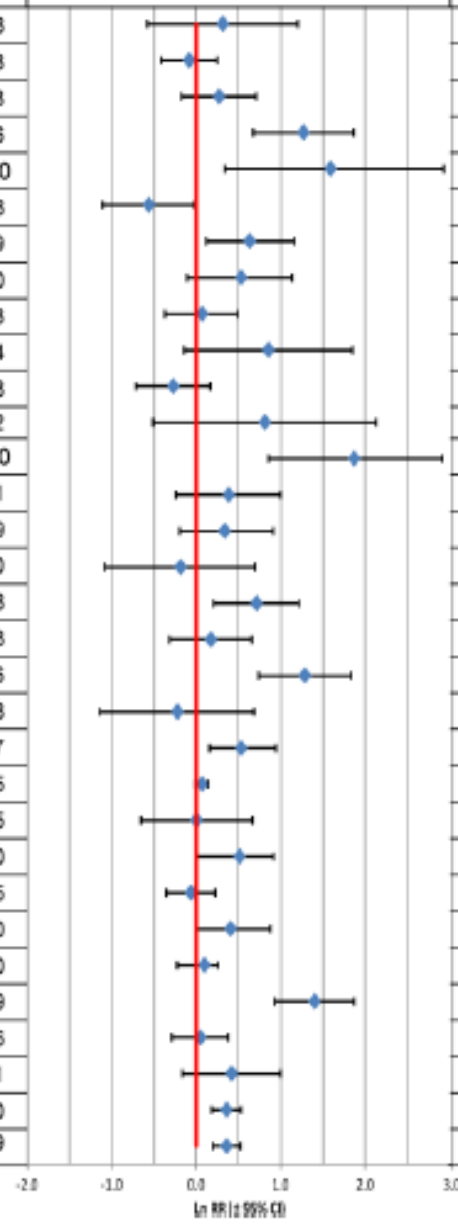
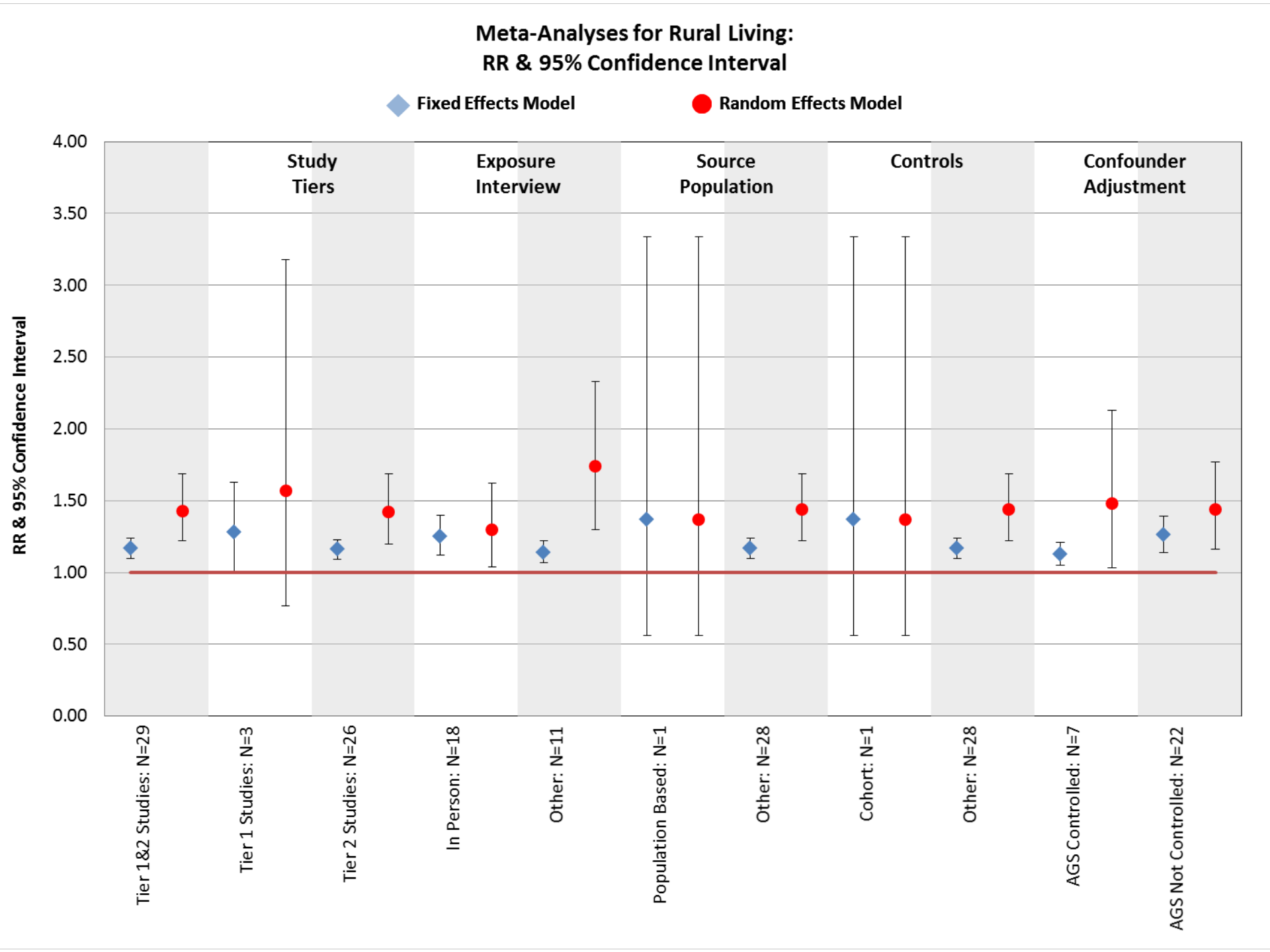

Meta-Analyses for Heavy Smoking:
RR & 95% Confidence Interval
Fixed Effects Model
Random Effects Model
Study Tiers
Exposure Interview
Source Population
Controls
Confounder Adjustment
RR & 95% Confidence Interval
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Tier 1&2 Studies: N=37
Tier 1 Studies: N=10
Tier 2 Studies: N=27
In Person: N=14
Other: N=23
Population Based: N=7
Other: N=30
Cohort: N=9
Other: N=21
AGS Controlled: N= 33
AGS Not Controlled: N=4

Association Between Rural Living and Parkinson's Disease

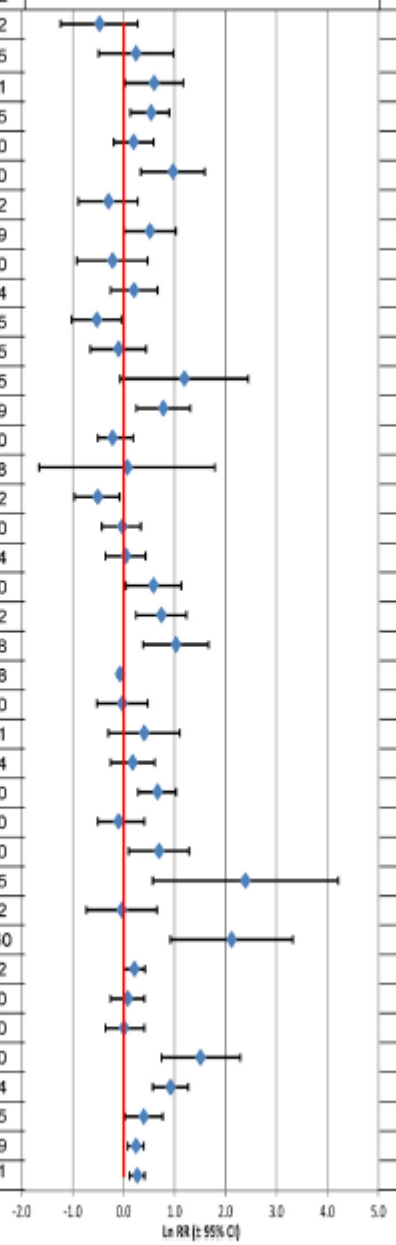
Year	Author	Tier	RR	LCL	UCL	Exposure
2003	Baldi [56]	1	1.37	0.56	3.33	Rural residency, overall
2005	Wirdefeldt [64]	1	0.92	0.66	1.28	Rural area, both sexes, external
2005	Firestone [114]	1	1.31	0.84	2.03	Home based - agriculture region
2010	Vlajinac [115]	1	3.56	1.96	6.46	Rural living any time
1989	Ho [116]	2	4.90	1.40	18.20	Rural living > 40 yrs
1989	Tanner [117]	2	0.57	0.33	0.98	Village residence
1990	Koller [118]	2	1.88	1.13	3.19	Rural residence
1991	Stern [119]	2	1.70	0.90	3.10	Rural living ≥ 1 year vs. never
1992	Jiménez-J. [120]	2	1.07	0.69	1.63	Ever lived in town with pop. < 2,000
1993	Butterfield [98]	2	2.35	0.87	6.34	Rural residence at diagnosis, model 3
1993	Wang [99]	2	0.76	0.49	1.18	Rural areas
1993	Hubble [54]	2	2.25	0.60	8.42	Urban Study, current residence - Rural
1993	Hubble [54]	2	6.49	2.35	17.90	Rural Study, current residence - Rural
1994	Morano [100]	2	1.47	0.79	2.71	Rural living (towns with pop. < 2,000)
1995	Martyn [76]	2	1.40	0.82	2.49	First home in village vs. large town
1996	Seidler [121]	2	0.83	0.34	2.00	Low ave. pop. density, neighbors
1997	Liou [59]	2	2.04	1.23	3.38	Living in rural residence
1998	Gorell [122]	2	1.19	0.73	1.93	Lived in rural area
1998	De Palma [123]	2	3.62	2.09	6.26	Living in a rural area ≥ 10 yrs
1998	Marder [124]	2	0.80	0.32	1.98	Rural living
1998	McCann [125]	2	1.70	1.17	2.57	Rural residency
1999	Taylor [102]	2	1.07	0.99	1.15	Years of rural living
1999	Werneck [126]	2	1.00	0.52	1.95	Rural living ≥ 15 yrs
2000	Preux [127]	2	1.67	1.00	2.50	Non-urban area (≤ 2,000 pop.)
2001	Behari [104]	2	0.94	0.70	1.25	Rural residency > 10 yrs
2002	Zorzon [128]	2	1.50	1.00	2.40	Rural living
2005	Wright [129]	2	1.10	0.80	1.30	Lived in town (< 1,500 pop.) in first 40
2010	Sanyal [130]	2	4.05	2.53	6.49	Rural living
2011	Das [58]	2	1.05	0.75	1.46	Rural living place
Meta (Tier 1, Random Effects)			1.52	0.85	2.71	
Meta (Tier 2, Random Effects)			1.43	1.20	1.70	
Meta (Tiers 1&2, Random Effects)			1.43	1.22	1.69	




Meta-Analyses for Rural Living:
RR & 95% Confidence Interval
Fixed Effects Model
Random Effects Model
RR & 95% Confidence Interval
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Study Tiers
Exposure Interview
Source Population
Controls
Confounder Adjustment
Tier 1&2 Studies: N=29
Tier 1 Studies: N=3
Tier 2 Studies: N=26
In Person: N=18
Other: N=11
Population Based: N=1
Other: N=28
Cohort: N=1
Other: N=28
AGS Controlled: N=7
AGS Not Controlled: N=22

Association Between Well Water Consumption and Parkinson's Disease

Year	Author	Tier	RR	LCL	UCL	Exposure
2004	Park [131]	1	0.62	0.29	1.32	Well, healthy controls
2004	Park [131]*	1	1.27	0.61	2.65	Well, neurological controls
2005	Firestone [114]	1	1.81	1.02	3.21	Well, lifelong
2005	Park [63]	1	1.71	1.14	2.45	Well, crude, neurological controls
2009	Gatto [69]	1	1.21	0.82	1.80	Well, ever or lifetime
2010	Vlajinac [115]	1	2.62	1.40	4.90	Well, ever
1989	Tanner [117]	2	0.74	0.41	1.32	Well
1990	Koller [118]	2	1.67	1.01	2.79	Well
1991	Stern [119]	2	0.80	0.40	1.60	Well ≥ 1 year vs. never
1992	Jiménez-J. [120]	2	1.22	0.77	1.94	Well (≥ 1 yr)
1993	Wang [99]	2	0.59	0.36	0.95	Well
1994	Hertzman [132]	2	0.90	0.52	1.55	Well, both sexes
1994	Morano [100]	2	3.28	0.93	11.5	Well, ≥ 1 yr
1996	De Michele [133]	2	2.17	1.28	3.69	Well
1996	Seidler [121]	2	0.80	0.60	1.20	Well, neighbors
1997	Liou [59]	2	1.07	0.19	5.98	Well
1998	McCann [125]	2	0.60	0.38	0.92	Well, bore, or spring water
1998	Gorell [122]	2	0.97	0.65	1.40	Well, ever
1998	Chan [79]	2	1.04	0.70	1.54	Well
1998	Marder [124]	2	1.79	1.04	3.10	Unfiltered water
1998	De Palma [123]*	2	2.09	1.27	3.42	Well ≥ 10 yrs
1998	Smargiassi [134]	2	2.78	1.46	5.28	Well ≥ 10 yrs
1999	Taylor [102]	2	0.93	0.88	0.98	Well; years of consumption
1999	Kuopio [81]	2	0.97	0.59	1.60	Pooled, calc. with comp. meta
1999	Werneck [126]	2	1.49	0.74	3.01	Well ≥ 15 yrs
2000	Preux [127]	2	1.19	0.77	1.84	Well only
2001	Behari [104]	2	1.94	1.33	2.80	Well-water drinking > 10 yrs
2001	Engel [135]	2	0.90	0.60	1.50	Well
2002	Zorzon [128]	2	2.00	1.10	3.60	Well
2002	Tsai [83]	2	10.9	1.77	67.5	Well, young-onset PD
2003	Dong [84]	2	0.96	0.48	1.92	Well
2005	Wright [129]	2	8.30	2.50	27.60	Well, any childhood expos.
2007	Dick [136]	2	1.23	1.00	1.52	River or well
2008	Hancock [137]	2	1.08	0.77	1.50	Well, child or adult
2009	Elbaz [55]	2	1.00	0.70	1.50	Well, ever
2010	Sanyal [130]	2	4.50	2.10	9.90	Well
2011	Das [58]	2	2.50	1.77	3.54	Well
Meta (Tier 1, Random Effects)			1.48	1.02	2.15	
Meta (Tier 2, Random Effects)			1.27	1.08	1.49	
Meta (Tiers 1&2, Random Effects)			1.30	1.12	1.51	



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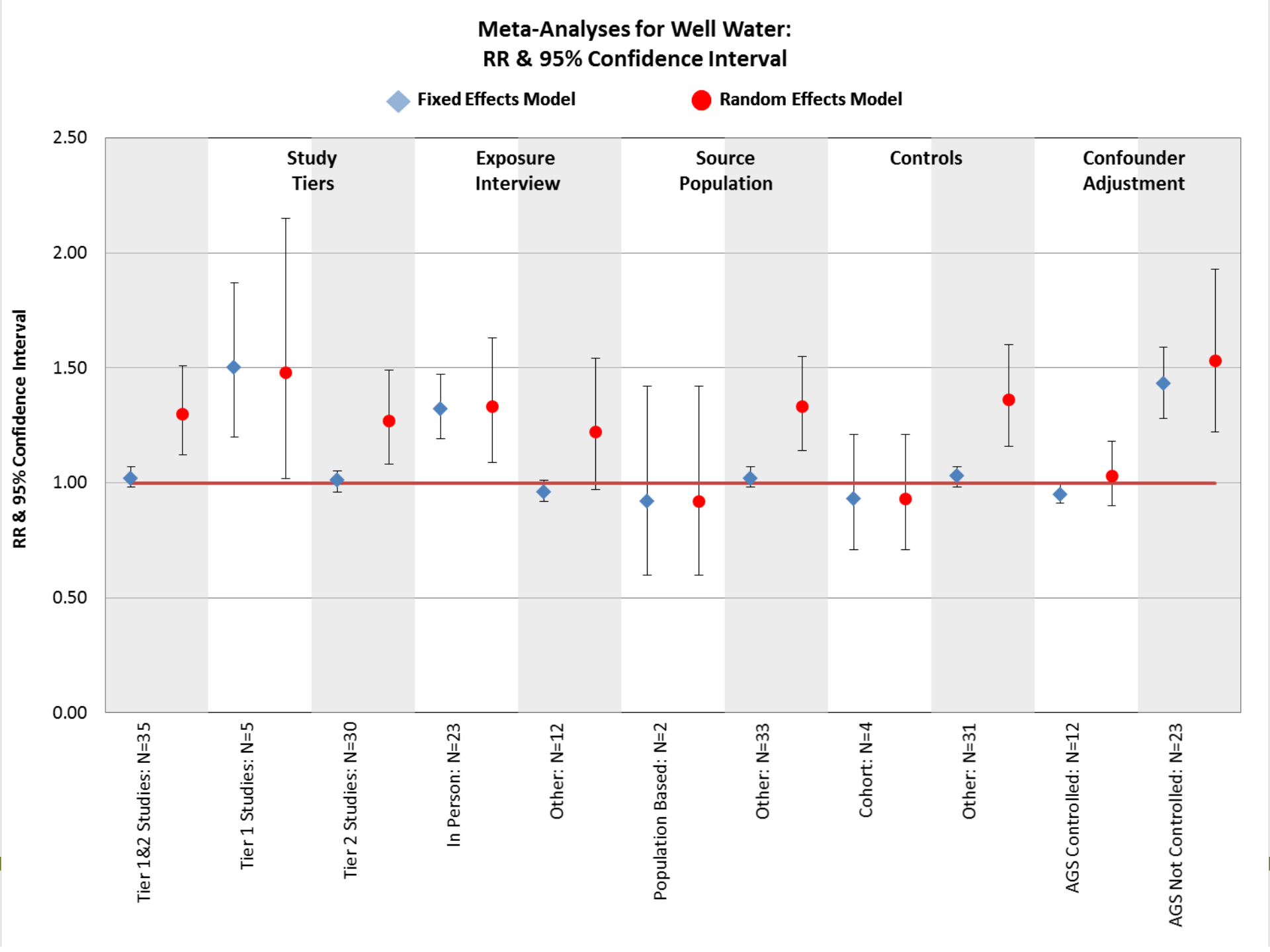
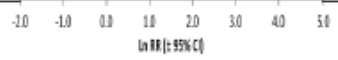

Meta-Analyses for Well Water:
RR & 95% Confidence Interval
Fixed Effects Model
Random Effects Model
RR & 95% Confidence Interval
2.50
2.00
1.50
1.00
0.50
0.00
Study Tiers
Exposure Interview
Source Population
Controls
Confounder Adjustment
Tier 1&2 Studies: N=35
Tier 1 Studies: N=5
Tier 2 Studies: N=30
In Person: N=23
Other: N=12
Population Based: N=2
Other: N=33
Cohort: N=4
Other: N=31
AGS Controlled: N=12
AGS Not Controlled: N=23

Association Between Farming and Parkinson's Disease

Year	Author	Tier	RR	LCL	UCL	Exposure
2003	Baldi [56]†	1	5.63	1.47	21.58	Occupational, men
2003	Baldi [56]†	1	1.02	0.22	4.82	Occupational, women
2005	Firestone [114]*	1	1.01	0.53	1.92	Occupational, men
2006	Ascherio [139]	1	1.8	1.3	2.5	Current or regular past use
2006	Frigerio [157]	1	1.5	0.8	2.9	Occupational or hobby, M&W
2008	Brighina [158]	1	1.11	0.89	1.38	Occupational or residential
2009	Costello [68]	1	1.52	1.08	2.14	Occupational, likely
2009	Gatto [69]*	1	1.66	1.04	2.66	≥ 12 of 26 pesticides in well water
2009	Ritz [70]*	1	1.44	1.01	2.06	Occupational, likely
2010	Firestone [57]†	1	0.6	0.30	1.29	Occupational, men
2010	Firestone [57]†	1	3.9	0.39	39.4	Occupational, women
2010	Skeie [140]	1	1.06	0.62	1.82	Occupational or private
2010	Vlajinac [115]	1	3.44	1.81	6.53	Occupational and residential
2011	Feldman [73]	1	0.9	0.5	1.3	Occupational
1989	Ho [116]	2	3.6	1.0	12.9	Previous use
1990	Hertzman [141]	2	1.34	0.71	2.52	Ever handled 11
1990	Koller [118]	2	1.05	0.67	1.65	Ever use
1992	Jiménez-J. [120]	2	1.34	0.85	2.13	Direct or indirect contact ≥ 1 yr
1992	Semchuk [143]	2	2.25	1.27	3.99	Occupational
1993	Hubble [54]	2	3.42	1.27	7.32	Factor: > 20 d/yr ever or > 5 yrs
1994	Hertzman [132]	2	2.32	1.10	4.88	Occupational – men, voter ctrls.
1994	Morano [100]	2	1.73	0.98	3.03	Direct or indirect exposure ≥ 1 yr
1995	Chaturvedi [19]	2	1.81	0.92	3.36	Occupational
1997	Liou [59]	2	2.89	2.28	3.66	Occupational or residential
1998	Chan [79]	2	0.75	0.26	2.22	During farming, M&W multivar.
1998	De Palma [123]*	2	2.92	1.38	6.14	Occupational or residential ≥ 10 yrs
1998	McCann [125]	2	1.2	0.8	1.5	Daily or weekly for > 6 mos
1998	Smargiassi [134]	2	1.15	0.56	2.36	Occupational for ≥ 10 consec. yrs
1999	Fall [80]	2	2.8	0.89	8.7	Handling for any occup., men
1999	Kuopio [81]	2	1.02	0.63	1.65	Regular and occasional use
1999	Taylor [102]	2	1.02	0.90	1.17	Years of use
1999	Werneck [126]	2	2.49	0.53	13.14	Inhaling/handling herb/pest ≥ 15 yrs
2000	Preux [127]	2	1.34	0.85	2.10	Work or leisure contact
2001	Engel [135]	2	0.8	0.5	1.2	Any
2002	Zorzon [128]	2	1.6	1.0	2.4	Exposure
2003	Baldereschi	2	3.68	1.57	8.64	Pesticide-use license
2003	Baldi [149]	2	2.20	1.11	4.34	Occupational
2003	Dong [84]	2	1.19	0.54	2.61	Use
2003	Duzcan [150]	2	2.96	1.31	6.69	Exp. > 20 days/yr for ≥ 10 yrs
2005	Galanaud [87]*	2	1.6	1.0	2.4	Gardening or professional
2005	Park RM [151]	2	1.14	1.09	1.20	Probable occupational
2005	Wright [129]	2	1.2	0.3	4.8	Occupational
2007	Dick [136]	2	1.25	0.97	1.61	Occupational or hobby, any
2007	Fong [89]	2	1.68	1.03	2.76	Use
2007	Kamel [18]	2	1.3	0.5	3.3	Ever use, incident PD
2007	Kamel [18]*	2	0.5	0.2	1.1	Ever use, prevalent PD
2008	Dhillon [92]	2	4.4	0.5	38.1	Agricultural pesticides in past yr.
2008	Hancock [137]	2	1.61	1.13	2.29	Ever applied
2008	Petersen [91]	2	6.00	0.62	57.68	Occupational, ever
2009	Elbaz [55]	2	1.7	1.0	2.9	Professional, both sexes
2009	Tanner [154]	2	1.90	1.12	3.21	Occupational
2010	Kiyohara [94]	2	0.79	0.57	1.10	Occupational or home
2010	Sanyal [130]	2	17.1	4.97	58.84	Exposure for ≥ 5 yrs
2011	Das [58]	2	6.18	3.71	10.29	≥ 3 hrs/day, > 3 mos/yr for 10 yrs
2011	Rugbjerg [156]	2	1.18	0.65	2.14	Hygiene-reviewed, any operation
2011	Tanaka [155]*	2	0.75	0.37	1.46	Occupational for ≥ 10 hrs/wk, > 1 yr
Meta (Tier 1, Random Effects)			1.40	1.06	1.85	
Meta (Tier 2, Random Effects)			1.61	1.39	1.87	
Meta (Tiers 1&2, Random Effects)			1.56	1.37	1.77	



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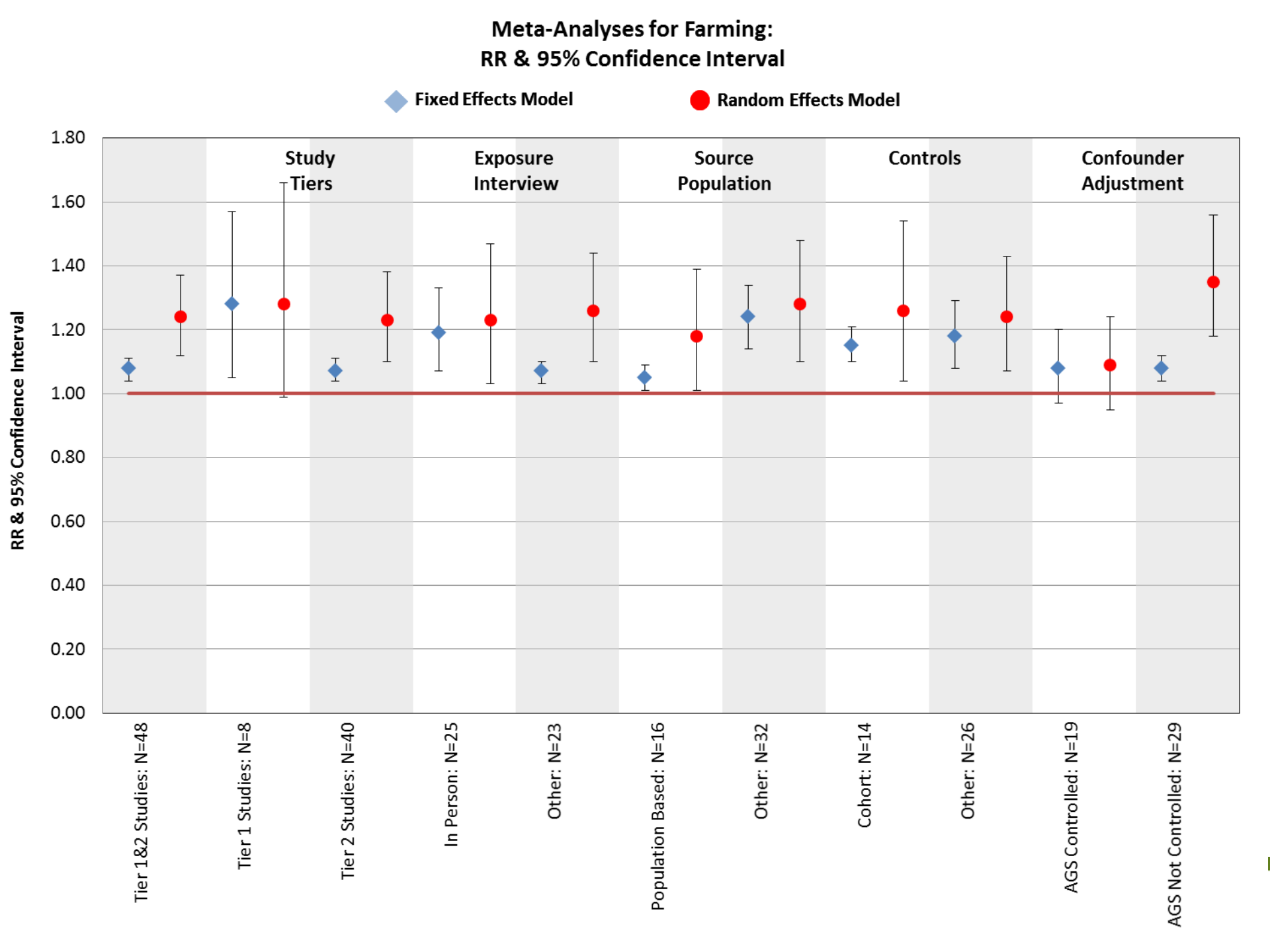
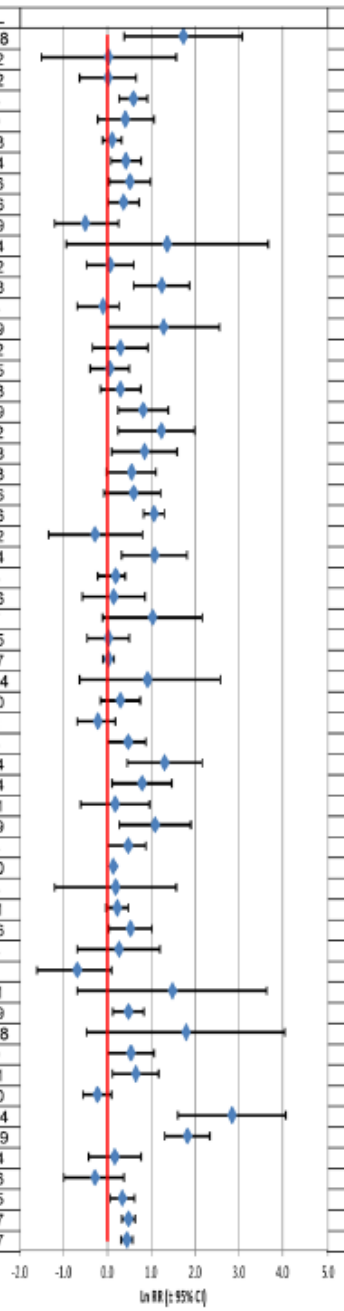

Meta-Analyses for Farming:
RR & 95% Confidence Interval
Fixed Effects Model
Random Effects Model
RR & 95% Confidence Interval
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Study Tiers
Exposure Interview
Source Population
Controls
Confounder Adjustment
Tier 1&2 Studies: N=48
Tier 1 Studies: N=8
Tier 2 Studies: N=40
In Person: N=25
Other: N=23
Population Based: N=16
Other: N=32
Cohort: N=14
Other: N=26
AGS Controlled: N=19
AGS Not Controlled: N=29

Association Between Pesticide Use and Parkinson's Disease

Year	Author	Tier	RR	LCL	UCL	Exposure
2003	Baldi [56]†	1	5.63	1.47	21.58	Occupational, men
2003	Baldi [56]†	1	1.02	0.22	4.82	Occupational, women
2005	Firestone [114]*	1	1.01	0.53	1.92	Occupational, men
2006	Ascherio [139]	1	1.8	1.3	2.5	Current or regular past use
2006	Frigerio [157]	1	1.5	0.8	2.9	Occupational or hobby, M&W
2008	Brighina [158]	1	1.11	0.89	1.38	Occupational or residential
2009	Costello [68]	1	1.52	1.08	2.14	Occupational, likely
2009	Gatto [69]*	1	1.66	1.04	2.66	≥ 12 of 26 pesticides in well water
2009	Ritz [70]*	1	1.44	1.01	2.06	Occupational, likely
2010	Firestone [57]†	1	0.6	0.30	1.29	Occupational, men
2010	Firestone [57]†	1	3.9	0.39	39.4	Occupational, women
2010	Skeie [140]	1	1.06	0.62	1.82	Occupational or private
2010	Vlajinac [115]	1	3.44	1.81	6.53	Occupational and residential
2011	Feldman [73]	1	0.9	0.5	1.3	Occupational
1989	Ho [116]	2	3.6	1.0	12.9	Previous use
1990	Hertzman [141]	2	1.34	0.71	2.52	Ever handled 11
1990	Koller [118]	2	1.05	0.67	1.65	Ever use
1992	Jiménez-J. [120]	2	1.34	0.85	2.13	Direct or indirect contact ≥ 1 yr
1992	Semchuk [143]	2	2.25	1.27	3.99	Occupational
1993	Hubble [54]	2	3.42	1.27	7.32	Factor: > 20 d/yr ever or > 5 yrs
1994	Hertzman [132]	2	2.32	1.10	4.88	Occupational – men, voter ctrls.
1994	Morano [100]	2	1.73	0.98	3.03	Direct or indirect exposure ≥ 1 yr
1995	Chaturvedi [19]	2	1.81	0.92	3.36	Occupational
1997	Liou [59]	2	2.89	2.28	3.66	Occupational or residential
1998	Chan [79]	2	0.75	0.26	2.22	During farming, M&W multivar.
1998	De Palma [123]*	2	2.92	1.38	6.14	Occupational or residential ≥ 10 yrs
1998	McCann [125]	2	1.2	0.8	1.5	Daily or weekly for > 6 mos
1998	Smargiassi [134]	2	1.15	0.56	2.36	Occupational for ≥ 10 consec. yrs
1999	Fall [80]	2	2.8	0.89	8.7	Handling for any occup., men
1999	Kuopio [81]	2	1.02	0.63	1.65	Regular and occasional use
1999	Taylor [102]	2	1.02	0.90	1.17	Years of use
1999	Werneck [126]	2	2.49	0.53	13.14	Inhaling/handling herb/pest ≥ 15 yrs
2000	Preux [127]	2	1.34	0.85	2.10	Work or leisure contact
2001	Engel [135]	2	0.8	0.5	1.2	Any
2002	Zorzon [128]	2	1.6	1.0	2.4	Exposure
2003	Baldereschi	2	3.68	1.57	8.64	Pesticide-use license
2003	Baldi [149]	2	2.20	1.11	4.34	Occupational
2003	Dong [84]	2	1.19	0.54	2.61	Use
2003	Duzcan [150]	2	2.96	1.31	6.69	Exp. > 20 days/yr for ≥ 10 yrs
2005	Galanaud [87]*	2	1.6	1.0	2.4	Gardening or professional
2005	Park RM [151]	2	1.14	1.09	1.20	Probable occupational
2005	Wright [129]	2	1.2	0.3	4.8	Occupational
2007	Dick [136]	2	1.25	0.97	1.61	Occupational or hobby, any
2007	Fong [89]	2	1.68	1.03	2.76	Use
2007	Kamel [18]	2	1.3	0.5	3.3	Ever use, incident PD
2007	Kamel [18]*	2	0.5	0.2	1.1	Ever use, prevalent PD
2008	Dhillon [92]	2	4.4	0.5	38.1	Agricultural pesticides in past yr.
2008	Hancock [137]	2	1.61	1.13	2.29	Ever applied
2008	Petersen [91]	2	6.00	0.62	57.68	Occupational, ever
2009	Elbaz [55]	2	1.7	1.0	2.9	Professional, both sexes
2009	Tanner [154]	2	1.90	1.12	3.21	Occupational
2010	Kiyohara [94]	2	0.79	0.57	1.10	Occupational or home
2010	Sanyal [130]	2	17.1	4.97	58.84	Exposure for ≥ 5 yrs
2011	Das [58]	2	6.18	3.71	10.29	≥ 3 hrs/day, > 3 mos/yr for 10 yrs
2011	Rugbjerg [156]	2	1.18	0.65	2.14	Hygiene-reviewed, any operation
2011	Tanaka [155]*	2	0.75	0.37	1.46	Occupational for ≥ 10 hrs/wk, > 1 yr
Meta (Tier 1, Random Effects)			1.40	1.06	1.85	
Meta (Tier 2, Random Effects)			1.61	1.39	1.87	
Meta (Tiers 1&2, Random Effects)			1.56	1.37	1.77	



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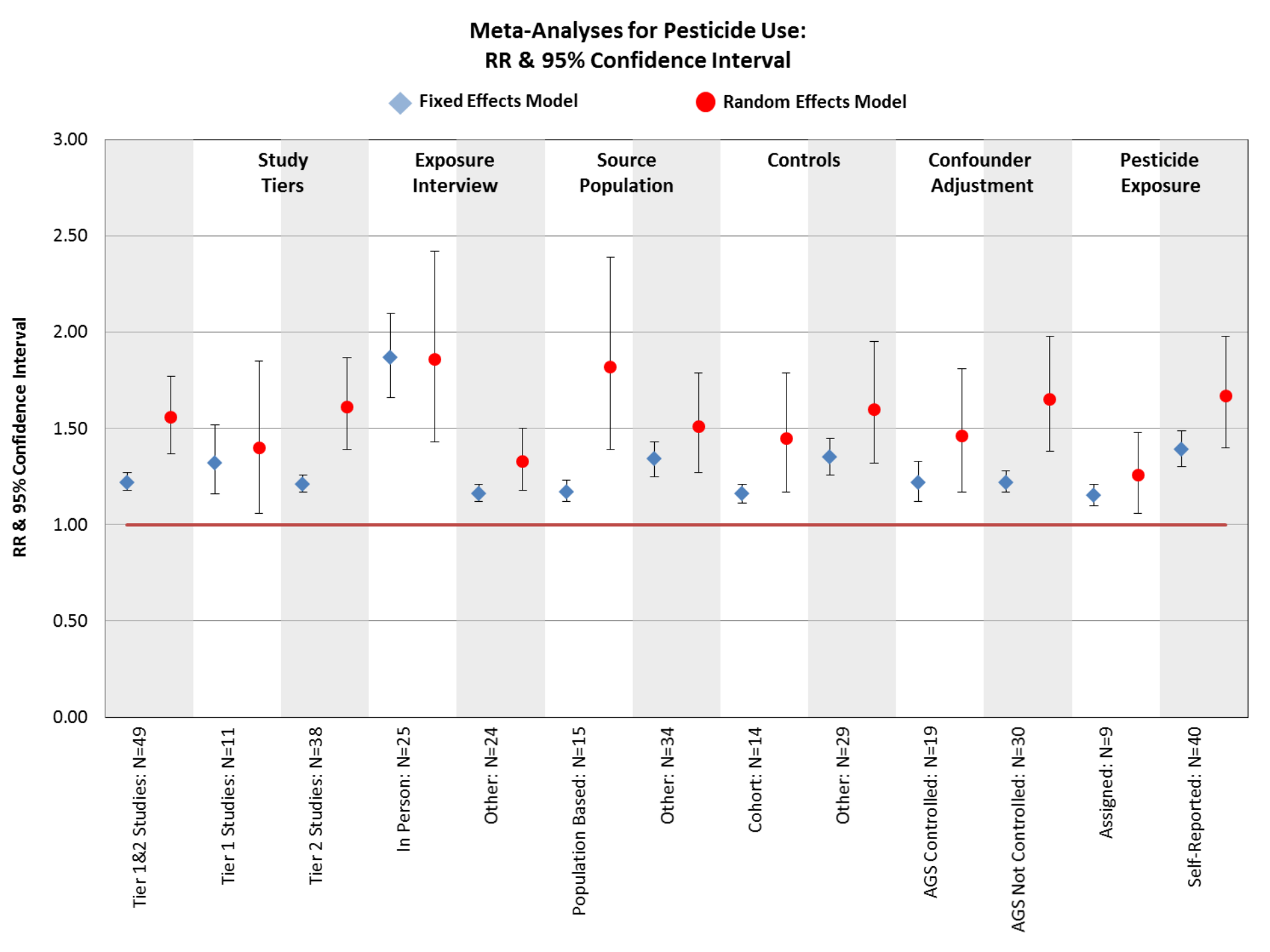
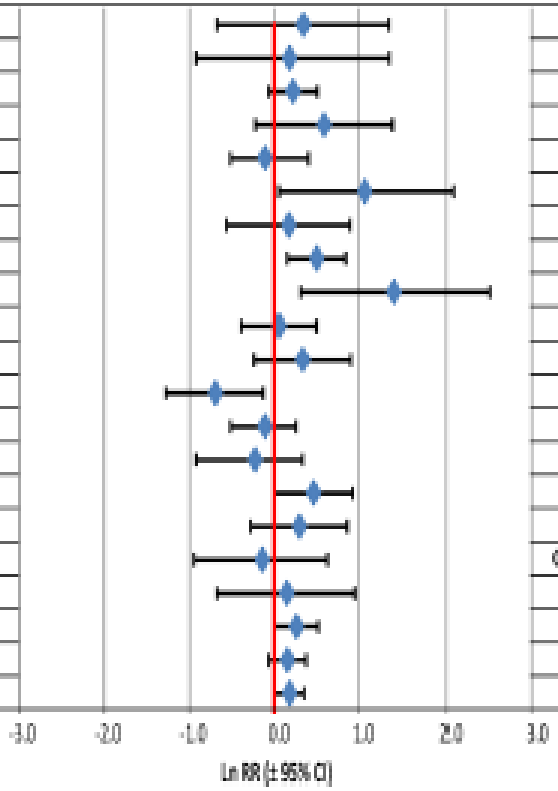

Meta-Analyses for Pesticide Use:
RR & 95% Confidence Interval
Fixed Effects Model
Random Effects Model
RR & 95% Confidence Interval
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Study Tiers
Exposure Interview
Source Population
Controls
Confounder Adjustment
Pesticide Exposure
Tier 1&2 Studies: N=49
Tier 1 Studies: N=11
Tier 2 Studies: N=38
In Person: N=25
Other: N=24
Population Based: N=15
Other: N=34
Cohort: N=14
Other: N=29
AGS Controlled: N=19
AGS Not Controlled: N=30
Assigned: N=9
Self-Reported: N=40

Association Between Herbicide Use and Parkinson's Disease

Year	Author	Tier	RR	LCL	UCL	Exposure
Panel a: Herbicide						
2005	Firestone [114]	1	1.41	0.51	3.88	Occupational, men
2006	Frigerio [157]	1	1.20	0.40	3.90	Occupational or hobby, men
2008	Brighina [158]	1	1.25	0.94	1.66	Occupational or residential
2010	Vlajinac [115]	1	1.80	0.80	4.04	Occupational and residential
1991	Stern [119]	2	0.90	0.60	1.50	Any exposure vs. none
1992	Semchuk [143]	2	2.91	1.06	8.01	Herb. / insect. / fung. - agricultural
1994	Hertzman [132]	2	1.19	0.57	2.45	Occupational - men, voter ctrls
1996	Seidler [121]	2	1.65	1.17	2.33	Neighbors, stratified sample
1998	Gorell [122]	2	4.10	1.37	12.24	All occupations
1999	Taylor [102]	2	1.06	0.68	1.65	Yrs of use
1999	Kuopio [81]	2	1.40	0.79	2.48	Regular and occasional use
2001	Behari [104]	2	0.50	0.28	0.88	Exposure, matched analysis
2001	Engel [135]	2	0.90	0.60	1.30	Any
2008	Dhillon [92]	2	0.80	0.40	1.40	Home/agricultural in past yr
2008	Hancock [137]	2	1.59	1.00	2.54	Ever applied
2009	Elbaz [55]	2	1.35	0.76	2.37	Professional, men
2011	Tanaka [155]	2	0.87	0.39	1.88	Occupational for ≥ 10 hrs/wk, > 1 yr
2011	Rugbjerg [156]	2	1.16	0.51	2.60	Hygiene-reviewed, any operation
Meta (Tier 1, Random Effects)			1.30	1.01	1.68	
Meta (Tier 2, Random Effects)			1.17	0.94	1.46	
Meta (Tiers 1&2, Random Effects)			1.20	1.00	1.43	



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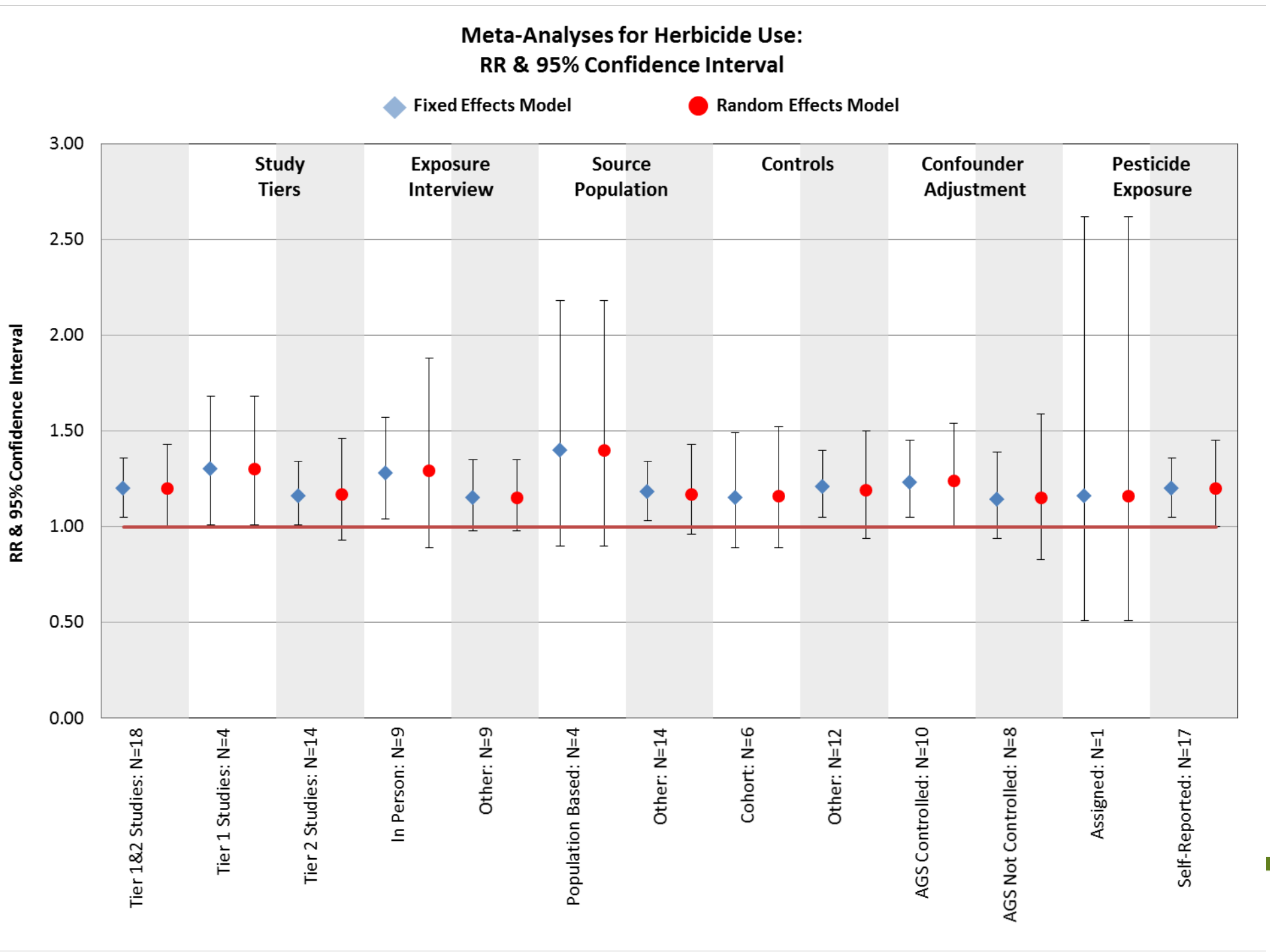
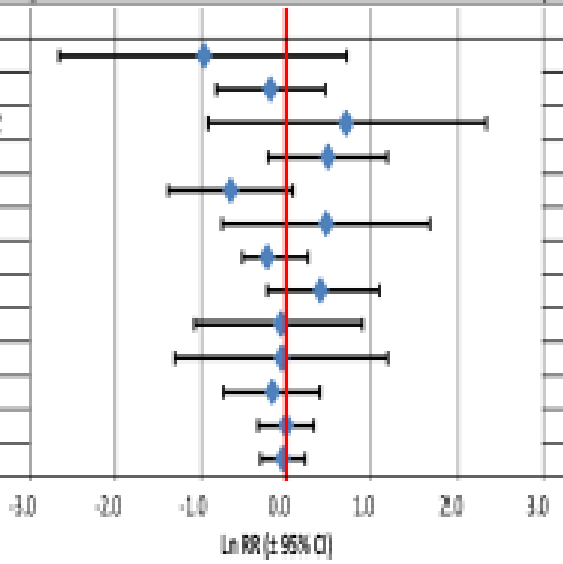

Meta-Analyses for Herbicide Use:
RR & 95% Confidence Interval
Fixed Effects Model
Random Effects Model
RR & 95% Confidence Interval
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Study Tiers
Exposure Interview
Source Population
Controls
Confounder Adjustment
Pesticide Exposure
Tier 1&2 Studies: N=18
Tier 1 Studies: N=4
Tier 2 Studies: N=14
In Person: N=9
Other: N=9
Population Based: N=4
Other: N=14
Cohort: N=6
Other: N=12
AGS Controlled: N=10
AGS Not Controlled: N=8
Assigned: N=1
Self-Reported: N=17

Association Between Fungicide Use and Parkinson's Disease

Year	Author	Tier	RR	LCL	UCL	Exposure
Panel b: Fungicide						
2005	Firestone [114]	1	0.38	0.07	2.05	Men - occupational
2008	Brighina [158]	1	0.83	0.44	1.59	Occupational or residential
2010	Vlajinac [115]	1	2.03	0.40	10.22	Occupational and residential
1992	Semchuk [143]	2	1.63	0.81	3.29	Occupational
1994	Hertzman [132]	2	0.52	0.25	1.08	Occupational - men, voter ctrls
1998	Gorell [122]	2	1.60	0.47	5.45	All occupations
2001	Engel [135]	2	0.80	0.60	1.30	Any
2009	Elbaz [55]	2	1.50	0.80	3.00	Professional, men
2011	Tanaka [155]	2	0.94	0.34	2.47	Occupational for ≥ 10 hrs/wk, > 1 yr
2011	Rugbjerg [156]	2	0.95	0.27	3.31	Hygiene-reviewed, any operation
Meta (Tier 1, Random Effects)			0.85	0.48	1.49	
Meta (Tier 2, Random Effects)			1.00	0.72	1.39	
Meta (Tiers 1&2, Random Effects)			0.96	0.74	1.25	



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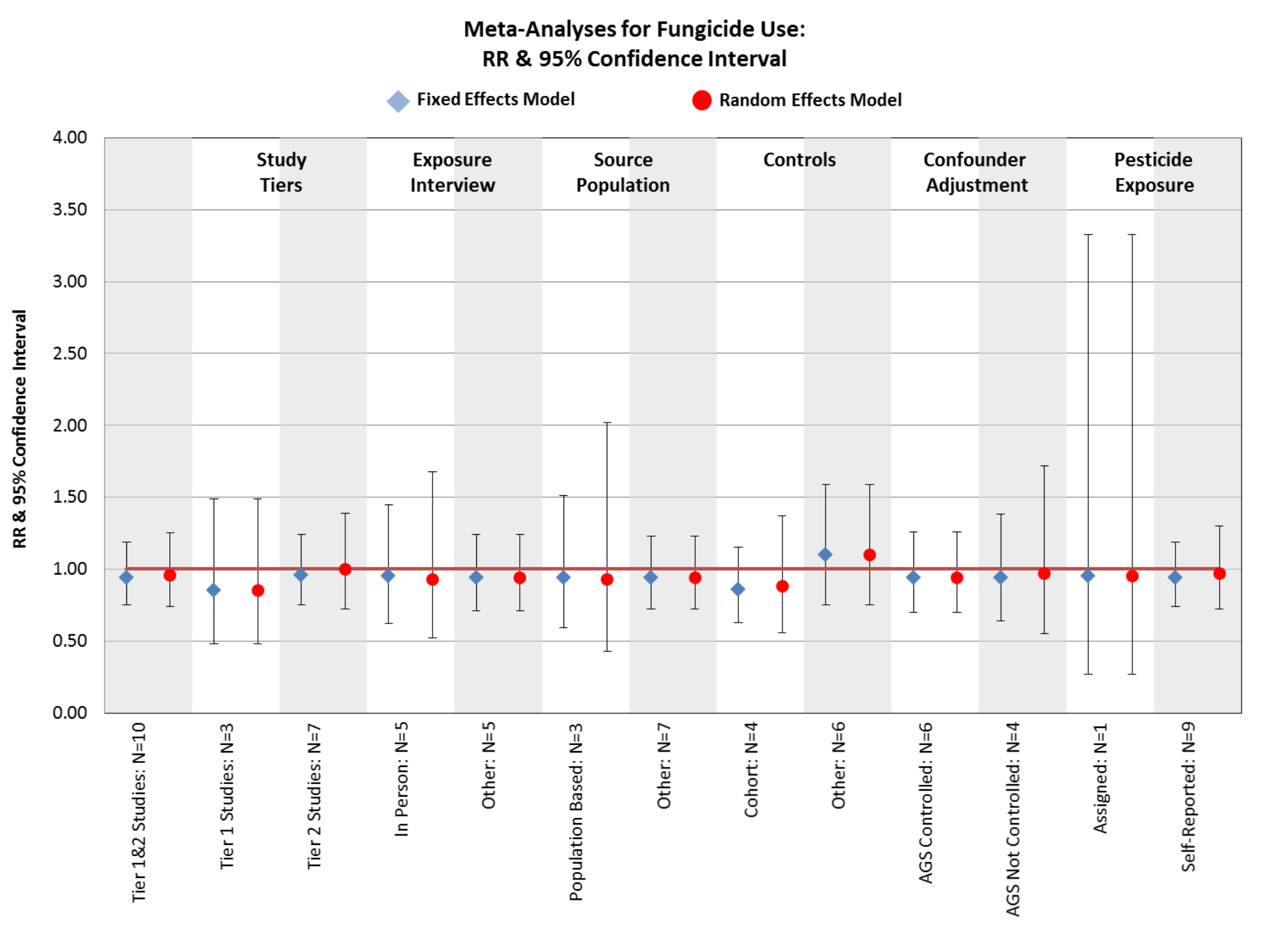
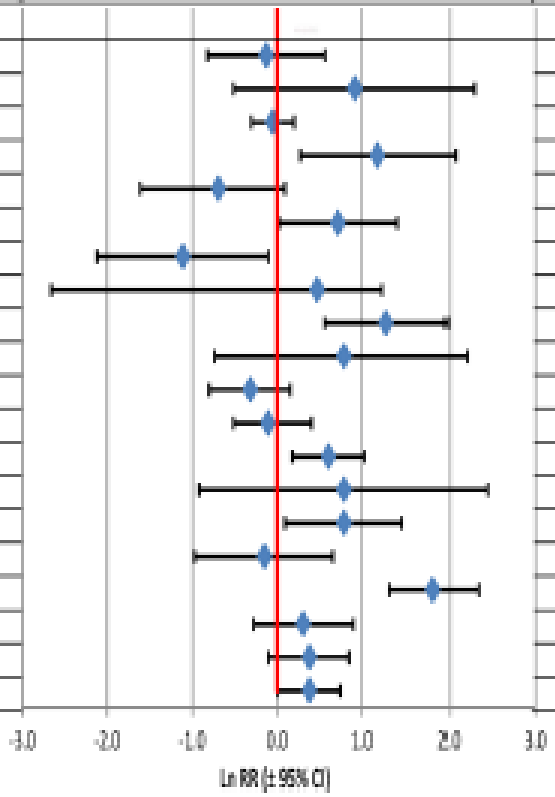

Meta-Analyses for Fungicide Use:
RR & 95% Confidence Interval
Fixed Effects Model
Random Effects Model
RR & 95% Confidence Interval
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Study Tiers
Exposure Interview
Source Population
Controls
Confounder Adjustment
Pesticide Exposure
Tier 1&2 Studies: N=10
Tier 1 Studies: N=3
Tier 2 Studies: N=7
In Person: N=5
Other: N=5
Population Based: N=3
Other: N=7
Cohort: N=4
Other: N=6
AGS Controlled: N=6
AGS Not Controlled: N=4
Assigned: N=1
Self-Reported: N=9

Association Between Insecticide Use and Parkinson's Disease

Year	Author	Tier	RR	LCL	UCL	Exposure
Panel c: Insecticide						
2005	Firestone [114]	1	0.88	0.44	1.76	Men - occupational
2006	Frigerio [157]	1	2.50	0.60	9.80	Occupational or hobby, men
2008	Brighina [158]	1	0.95	0.74	1.22	Occupational or residential
2010	Vlajinac [115]	1	3.22	1.32	7.87	Occupational and residential
1991	Stern [119]	2	0.50	0.20	1.10	Use; any vs. none
1992	Semchuk [143]	2	2.05	1.03	4.07	Occupational
1994	Hertzman [132]	2	0.33	0.12	0.90	Occupational - men, voter ctrls
1996	Seidler [121]	2	1.60	0.07	3.40	Neighbors, age > 80 years
1998	Gorell [122]	2	3.55	1.75	7.18	All occupations
1999	Fall [80]	2	2.20	0.48	9.00	Handling for agriculture, men
2001	Behari [104]	2	0.73	0.45	1.17	Exposure, matched analysis
2001	Engel [135]	2	0.90	0.60	1.50	Any
2008	Hancock [137]	2	1.83	1.20	2.81	Ever applied
2008	Dhillon [92]	2	2.20	0.40	11.40	Appl. to/for farm crops in past yr
2009	Elbaz [55]	2	2.20	1.10	4.30	Professional, both sexes
2011	Rugbjerg [156]	2	0.86	0.38	1.93	Hygiene-reviewed, any operation
2011	Das [58]	2	6.18	3.71	10.29	≥ 3 hrs/day, > 3 mos/yr for 10 yrs
Meta (Tier 1, Random Effects)			1.36	0.76	2.46	
Meta (Tier 2, Random Effects)			1.46	0.90	2.34	
Meta (Tiers 1&2, Random Effects)			1.46	1.01	2.11	



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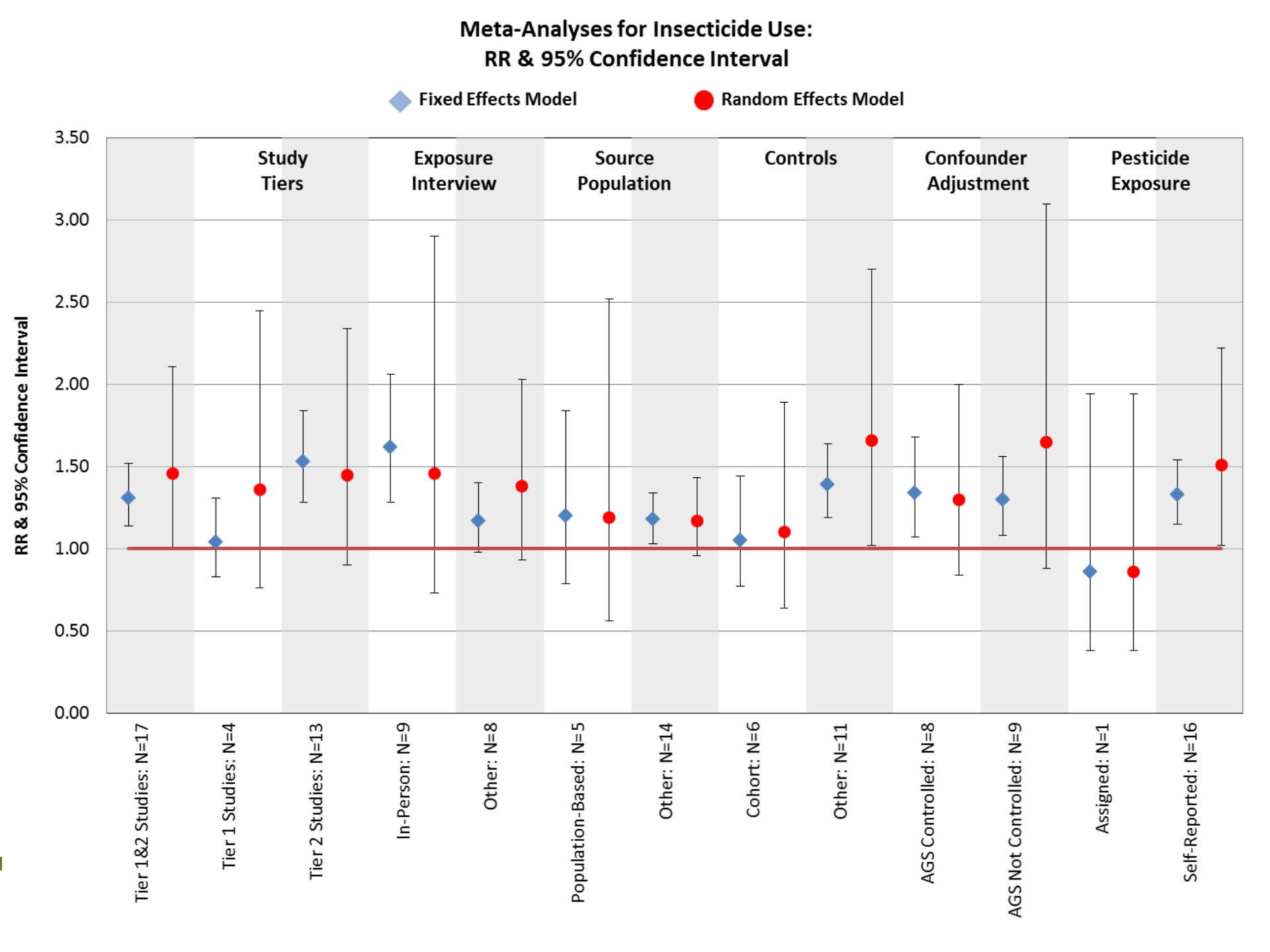
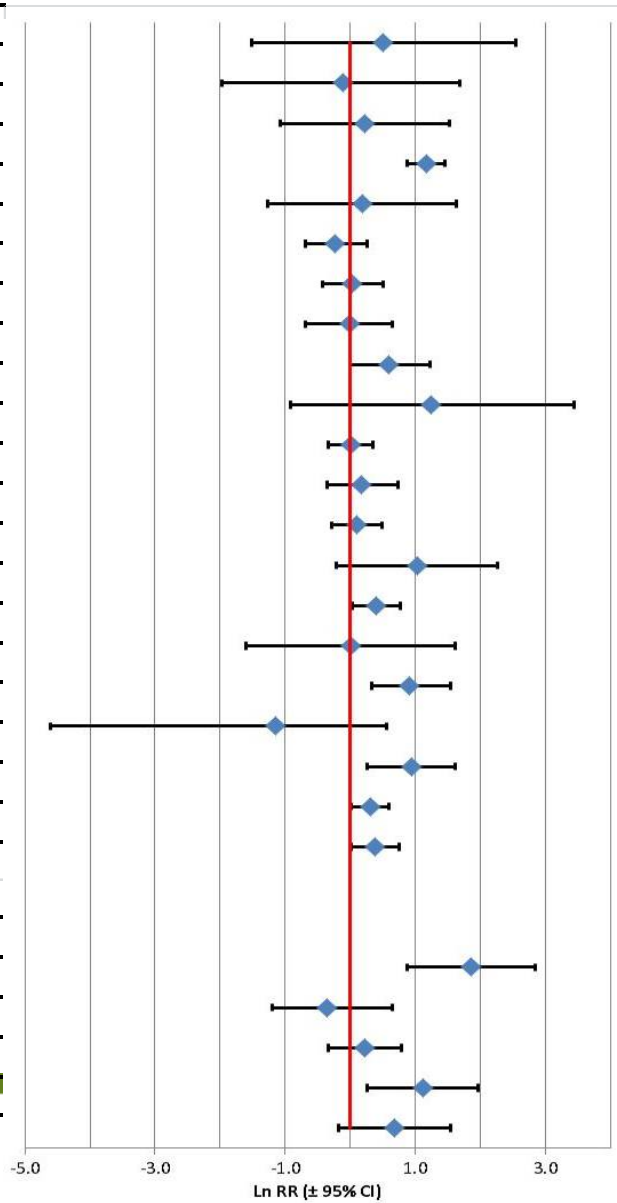

Meta-Analyses for Insecticide Use:
RR & 95% Confidence Interval
Fixed Effects Model
Random Effects Model
RR & 95% Confidence Interval
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Study Tiers
Exposure Interview
Source Population
Controls
Confounder Adjustment
Pesticide Exposure
Tier 1&2 Studies: N=17
Tier 1 Studies: N=4
Tier 2 Studies: N=13
In-Person: N=9
Other: N=8
Population-Based: N=5
Other: N=14
Cohort: N=6
Other: N=11
AGS Controlled: N=8
AGS Not Controlled: N=9
Assigned: N=1
Self-Reported: N=16

Association Between Paraquat Use and Parkinson's Disease

Year	Author	Tier	RR	LCL	UCL	Exposure
Panel a: Ever used Paraquat						
2005	Firestone (72)*	1	1.67	0.22	12.8	Occupational, men
2010	Firestone (56)	1	0.90	0.14	5.43	Men
1994	Hertzman (180)	2	1.25	0.34	4.63	Gen pop
1997	Liou (58)	2	3.22	2.41	4.31	Use
1999	Kuopio (135)	2	1.21	0.28	5.13	Use; compr. meta.
2001	Engel (183)	2	0.80	0.50	1.30	Ever
2004	Elbaz (73)*	2	1.04	0.65	1.66	Use, men
2007	Kamel (18)	2	1.00	0.50	1.90	Incident PD cases
2007	Kamel (18)*	2	1.80	1.00	3.40	Prevalent PD cases
2008	Dhillon (146)	2	3.50	0.40	31.60	Ever
2009	Costello (68)*	2	1.01	0.71	1.43	Only 1974-1999
2009	Elbaz (54)	2	1.20	0.70	2.10	Use, all men, mult. Imput.
2009	Gatto (69)*	2	1.10	0.75	1.63	Use
2009	Tanner (201)	2	2.80	0.81	9.72	Use
2011	Wang (70)*	2	1.50	1.03	2.18	Residential and occup.
2011	Rugbjerg (203)	2	1.01	0.20	5.01	Exposure
2011	Tanner (66)	2	2.50	1.40	4.70	Ever
2011	Tomenson (205)	2	0.32	0.01	1.76	Male production workers
2012	Goldman (71)*	2	2.60	1.30	5.00	Ever, men
2012	Lee (67)	2	1.36	1.02	1.81	Residential and workplace
Meta (Tiers 1&2, Random Effects)			1.47	1.01	2.13	
Panel b: High use of Paraquat						
1997	Liou (58)	2	6.44	2.41	17.20	≥20 yrs of using
2001	Engel (183)	2	0.70	0.30	1.90	683-9,950 acre-yrs
2009	Gatto (69)	2	1.26	0.72	2.20	High exposure level
2012	Goldman (71)	2	3.10	1.30	7.20	Lifetime use > med.= 4 yrs
Meta (Tiers 1&2, Random Effects)			1.99	0.84	4.71	



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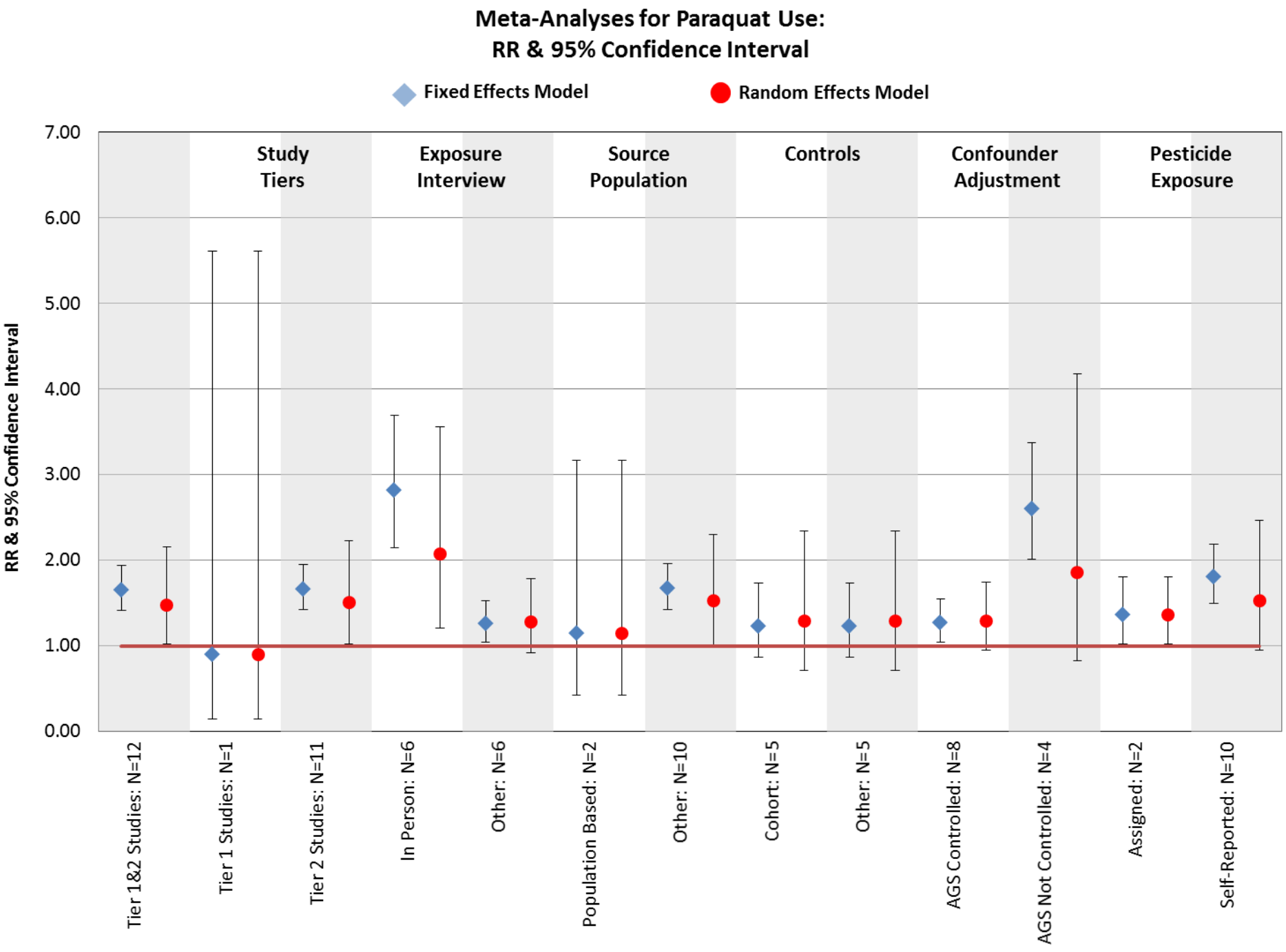
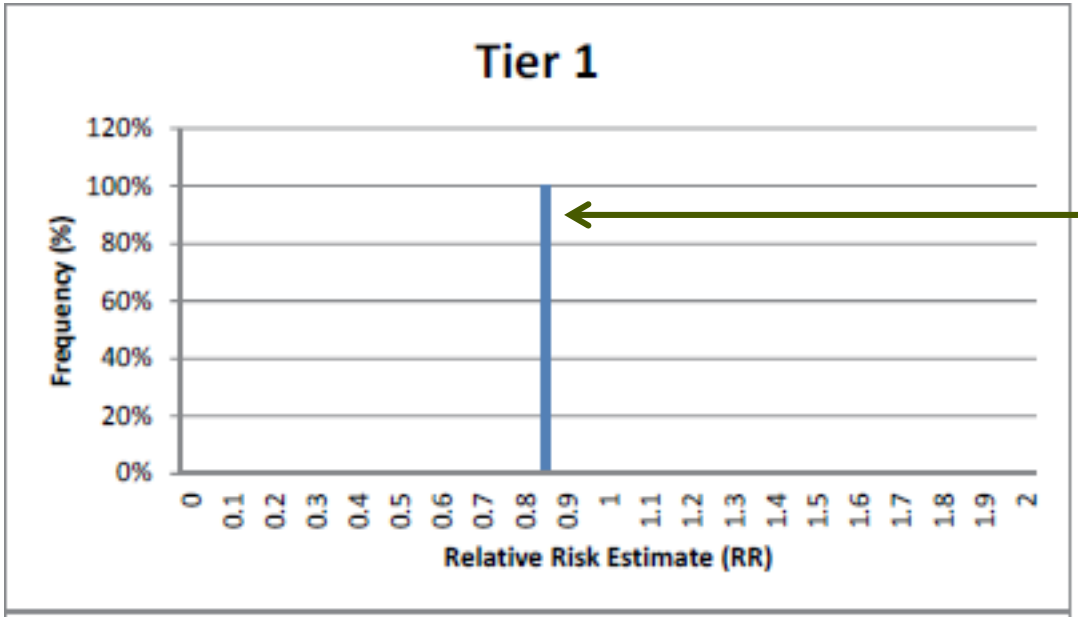
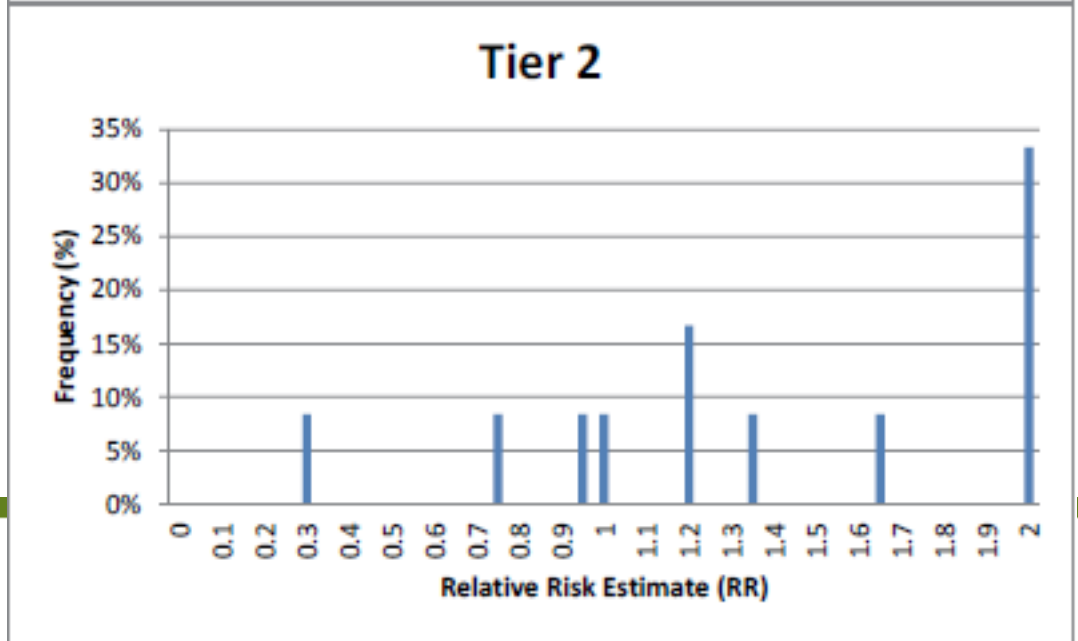

Meta-Analyses for Paraquat Use:
RR & 95% Confidence Interval
Fixed Effects Model
Random Effects Model
RR & 95% Confidence Interval
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
Study Tiers
Exposure Interview
Source Population
Controls
Confounder Adjustment
Pesticide Exposure
Tier 1&2 Studies: N=12
Tier 1 Studies: N=1
Tier 2 Studies: N=11
In Person: N=6
Other: N=6
Population Based: N=2
Other: N=10
Cohort: N=5
Other: N=5
AGS Controlled: N=8
AGS Not Controlled: N=4
Assigned: N=2
Self-Reported: N=10

Association Between Paraquat Use and Parkinson's Disease: Distribution of Tier 1 (1 study) vs. Tier 2 Study (12 studies) Results



Firestone et al. (2010)



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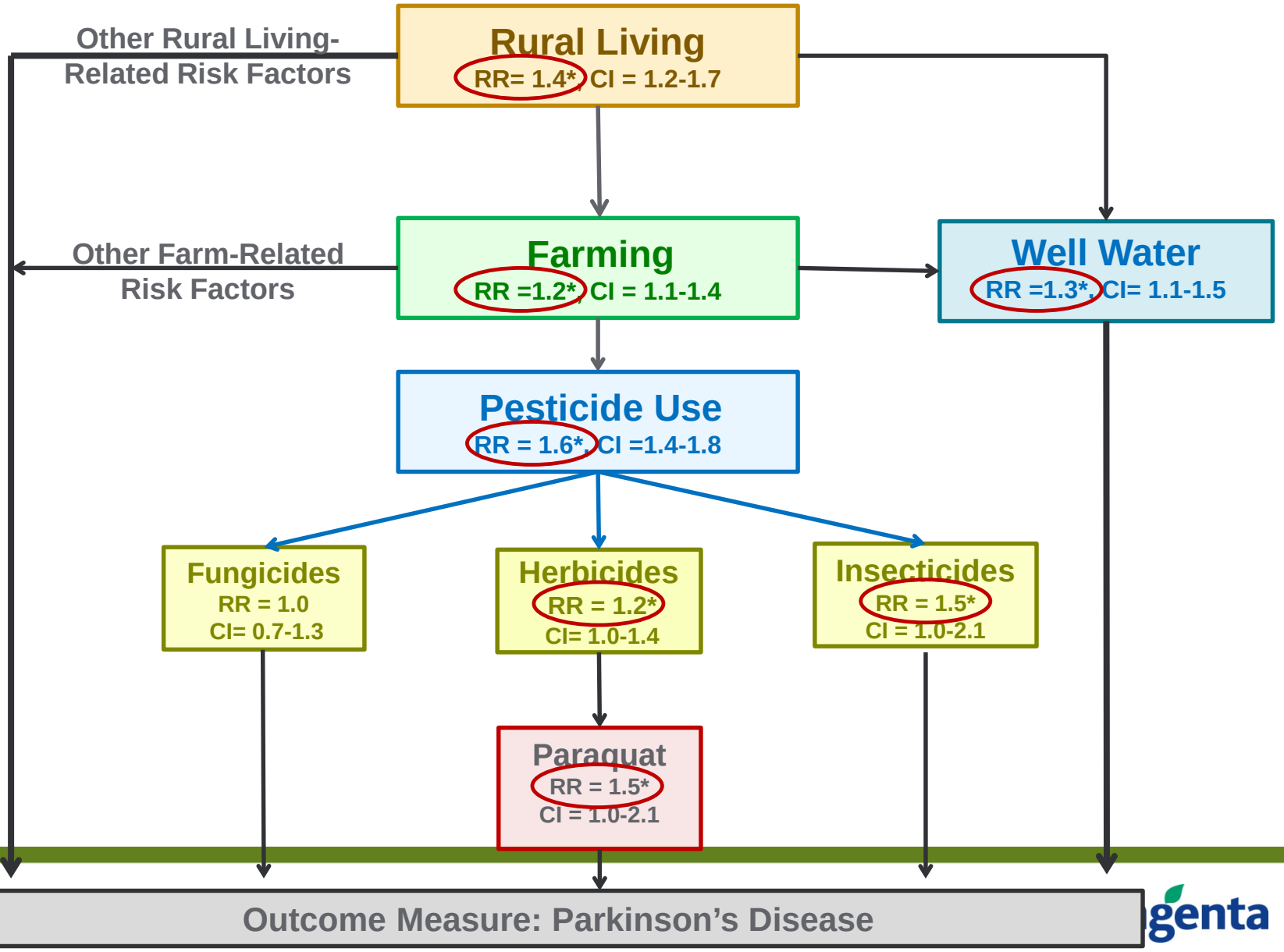
Potential Risk Factors for PD are Intercorrelated
Meta-Analysis Random Effects Model: RR (95% CI)

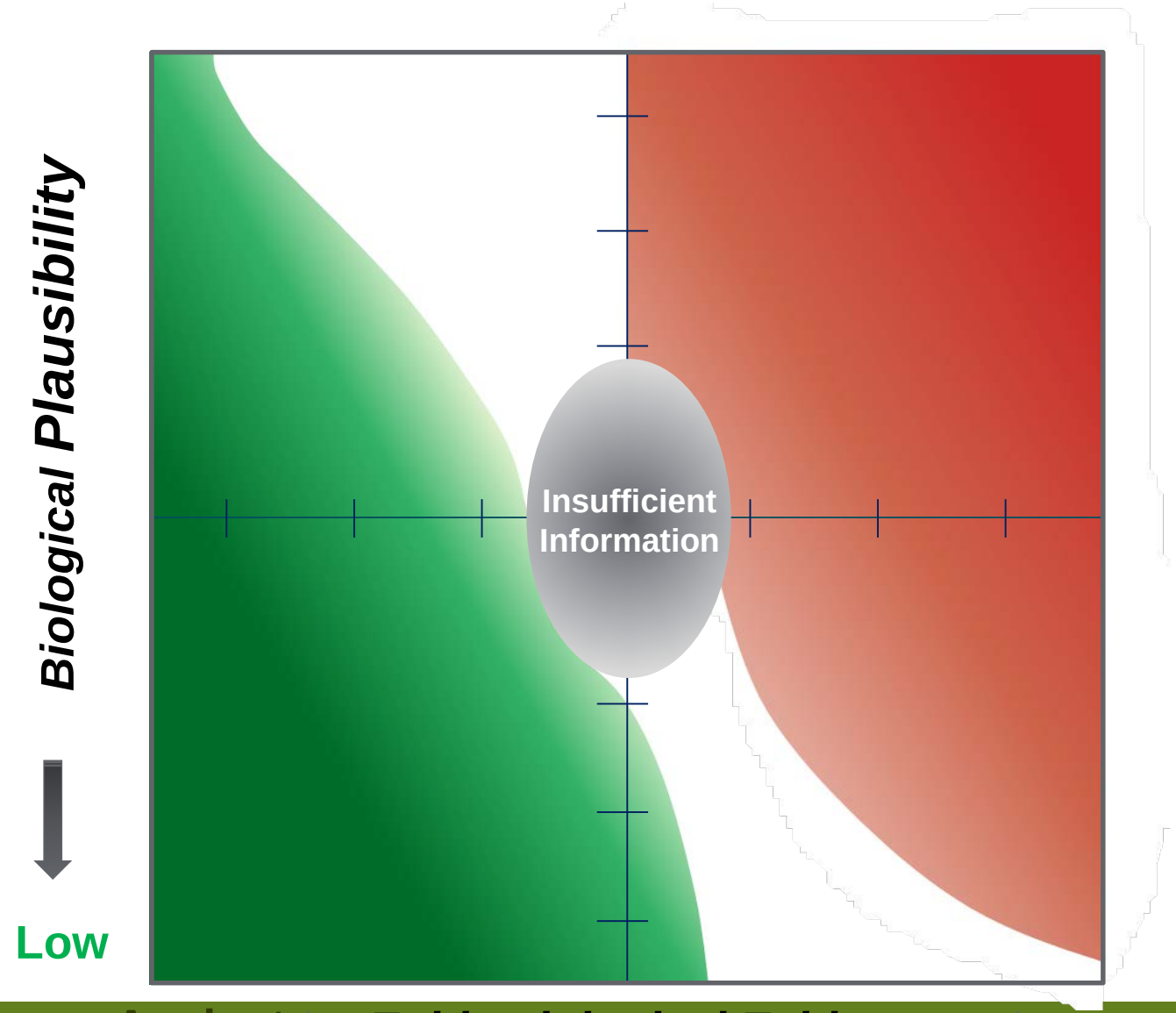


Weight of Evidence Assessment of Causality Based Upon Bradford Hill's Viewpoints

Bradford Hill Viewpoint	Cigarette Smoking	Rural Living	Well-Water Consumption	Farming	Any Pesticide Use	Herbicide Use	Fungicide Use	Insecticide Use	Paraquat Use
Strength of Association (RR) in Tier 1 Studies	0.54*	1.28*	1.50*	1.28*	1.14*	1.26†	0.85†	1.36†	0.90‡
Biological Gradient	Pack-Years Assessed	Not Evaluated	Not Evaluated	Not Evaluated	Not Evaluated	No High-Use Tier 1 Studies	Limited High-Use Tier 1 Studies	Limited High-Use Tier 1 Studies	No High-Use Tier 1 Studies
Temporality	Not Established	Not Established	Not Established	Not Established	Not Established	Not Established	Not Established	Not Established	Not Established
Consistency	Consistent	Inconsistent	Inconsistent	Inconsistent	Consistent	Inconsistent	Consistently Null	Inconsistent	Inconsistent
Specificity	Not Specific	Not Specific	Not Specific	Not Specific	Not Specific	Not Specific	Not Specific	Not Specific	Highly Specific
Plausibility	Uncertain§	No	No	No	No	No	No	No	Uncertain‖
Coherence	Moderate	No	No	No	No	No	No	No	No
Experimental Evidence	No Studies	No Studies	No Studies	No Studies	No Studies	No Studies	No Studies	No Studies	No Studies
Analogy	Analogies Exist	No Analogies	No Analogies	No Analogies	No Analogies	No Analogies	No Analogies	No Analogies	Analogies Exist

* Stronger statistically significant RR (from fixed or random effects model) after correction for reporting bias.
† Stronger statistically non-significant RR (from fixed or random effects model) after correction for reporting bias.
‡ Statistically non-significant RR from one study.
§ Although the epidemiological data show a consistent, approximately two-fold reduction in PD risk in individuals who smoke cigarettes, no constituent of cigarette smoke has been identified as being neuroprotective [112] and a mechanism of action has not been elucidated.
‖ Because paraquat is capable of redox recycling, it is plausible that paraquat could damage dopaminergic neurons in the substantia nigra. However, controversy exists in the published literature as to whether there are effects of paraquat in animal models or whether paraquat, under conditions of human exposure, reaches critical regions of the brain at concentrations sufficient to trigger adverse effects [113].

Has a Causal Relationship Between Paraquat Use and Parkinson's Disease been Established Based on Epidemiology and Animal Studies? No



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Conclusions

1. Paraquat has no effect on neurochemical, stereological or neuropathological endpoints in the SNpc or striatum of a sensitive strain of mouse given maximum tolerated doses.

2. Production workers known to have been exposed to paraquat at a manufacturing site did not display increased risk of PD-related death (Tomenson & Campbell, 2011).

3. Humans exposed to high, near lethal doses of paraquat did not display any parkinson-like symptoms during follow-up (Brent & Schaeffer, 2011)

4. A causal relationship between paraquat use and Parkinson's disease is not supported in a weight-of-the evidence assessment (Breckenridge, et al., 2016) because the epidemiological studies:

   - Display weak (inverse, null or positive) and inconsistent associations.
   - Inadequately characterize exposure and have not assessed biological gradient.
   - Have not assessed the temporal relationship between exposure and disease onset.
   - Have not sufficiently controlled for other factors that might contribute to PD.

Discussion