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Australian Pesticides and Veterinary Medicines Authority

Paraquat final regulatory decision –

consideration of neurotoxicity submissions

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Executive summary

This report provides a review of the submissions received in response to the public consultation on the Proposed Regulatory Decision (PRD) on paraquat (PQ) and the Australian Pesticides and Veterinary Medicines Authority's (APVMA) responses to those submissions. The APVMA received 49 written submissions on health in response to the publication of the PRD, consisting of 26 submissions without supporting references calling on a ban of paraquat due to links to Parkinson's disease and 23 submissions that provided further information that has been considered by the APVMA. These submissions cited 156 references consisting of 134 peer reviewed papers, 22 additional documents (including reports, guidance documents, news articles), and one submission from industry which were screened at the title, abstract and/or paper level for relevance to the human health risk assessment (HHRA) of paraquat used as a herbicide. Of the 156 cited references those initially identified as potentially relevant to the HHRA were read and considered in their entirety. Of these papers, 17 were identified as published after completion of the 2016 APVMA review and were therefore considered in detail for this report. A further 4 relevant papers were identified in a literature search and were also considered in detail for this report.

Since the original APVMA review of paraquat (2016), the United States Environmental Protection Agency (US EPA) has published detailed and comprehensive reviews of the toxicology and epidemiology of paraquat used as a herbicide (2019). More recently, in December 2024 the Californian EPA Department of Pesticide Regulation (DPR) published a Preliminary Report of the Potential Human Health Outcomes Resulting from Paraquat Exposure, which relies substantively on the US EPA reviews. These reviews have been considered in the preparation of this report.

The 17 papers identified by the screening process as relevant to the HHRA report endpoints and markers related to Parkinson's disease (PD), end stage renal disease (ESRD) and reproductive toxicity in males and females. Where studies were designed, and capable, to be able to do so, none report a No Observed Adverse Effect Level (NOAEL) that is more sensitive than those already relied-on for health-based guidance values (HBGV) for paraquat. While the new studies build upon and extend the toxicological database, they do not introduce any endpoints (toxicological effects) that would alter the existing hazard and risk assessments for agricultural use of paraquat.

Animal studies that have become available subsequent to the completion of the 2016 APVMA paraquat review confirm and reinforce the previous conclusion that available animal data does not provide convincing evidence that paraquat presents a substantive risk of increasing Parkinson's like disease in humans from likely routes and magnitude of exposure arising from agricultural use. The previous conclusion of the APVMA paraquat technical report that paraquat in animal studies "does not induce neurotoxicity via the oral, dermal or intranasal exposure routes" remains appropriate. The 2019 US EPA review of the literature on the relationship between paraquat and Parkinson's disease concluded that "Overall, the limited, mixed findings in the animal literature were considered weak evidence of a Parkinson's disease-like response to paraquat exposure". No data has been identified from public submissions or the available literature that would alter the validity of these conclusions.

Epidemiology studies of the relationship between unequivocal and substantive paraquat exposure and the development of Parkinson's disease published since the 2016 APVMA review of paraquat have examined a UK workforce who manufactured paraquat between 1961 and 1995 and the very large US Agricultural Health Survey (AHS) study cohort.

These studies support and strengthen the overall conclusion that cause and effect for paraquat exposure and Parkinson's disease risk have not been convincingly demonstrated, a conclusion supported by the US EPA reviews, recent published reviews and to a large extent the Californian EPA DPR preliminary review (2024).

The overall conclusions of this report, the 2016 APVMA review of paraquat toxicology and epidemiology, and the US EPA (2019) reviews are that the evidence available to date does not convincingly demonstrate a direct causal association between exposure to paraquat occupationally and/or through residential exposure to paraquat used on nearby land, and an increased risk of developing Parkinson's disease. The existing HBGV remain appropriate and protective for consumers of treated produce, workers using paraquat in accordance with label directions and the general population.

Introduction

In 2016, the Australian Pesticides and Veterinary Medicines Authority (APVMA) published a detailed assessment of the mammalian toxicology and metabolism/toxicokinetics of paraquat (PQ) in 2 supplements: Supplement I: TOXICOLOGY, and Supplement II: NEUROTOXICOLOGY together with a summary report providing an overview of all relevant data available on paraquat relating to human health (APVMA, 2016 a,b,c).

In 2024 the Paraquat Review Technical Report (RTR) providing a summary of all the technical review components and the proposed regulatory decisions, was published on the APVMA website (APVMA, 2024) and public comments and submissions invited up to 29 October 2024. This final review report considers the material and comments submitted in response to the call for public comment and relevant new material in the public domain that has become available since 2016.

Review of public consultation submissions

The APVMA received 26 submissions without supporting references calling on a ban of paraquat due to links to Parkinson's Disease and 23 submissions that provided further information that has been considered by the APVMA.

International reviews considered

In 2019 the US EPA published detailed, thorough and comprehensive reviews of the toxicology and epidemiology of paraquat and worker exposure assessments, as part of its registration review program, which included material that became available only after completion of the Office of Chemical Safety (OCS)/APVMA toxicology reviews (US EPA, 2019 a,b,c,d).

In 2024 the Californian Department of Pesticide Registration (DPR) published its "Preliminary Report of the Potential Human Health Outcomes Resulting from Paraquat Exposure" which draws extensively upon the US EPA reviews (California EPA DPR, 2024).

The findings and observations of the above reviews of paraquat have been taken into consideration in the preparation of this report.

Screening of cited literature

The submissions received provided 156 references in total in support of the positions presented. Further details of the screening criteria are found in Appendix 1. Many of the submissions covered aspects of the chemical review of paraquat that were outside the scope of the human health risk assessment (HHRA) considerations.

Following identification and collation of all references cited by submissions to the public consultation, these were screened to identify references that might quantitatively or qualitatively inform the HHRA. During this screening, papers covering agronomics, economics, disease impact analysis, or environmental issues were excluded on the basis of the title and where appropriate a brief scan of the abstract. Editorial, opinion and clinical management papers were screened at the abstract level and excluded unless new data related to HHRA was provided. Papers that did not present new/original data were excluded at the abstract and/or paper level. Toxicology and epidemiology papers published prior to completion of the 2016 OCS/APVMA review were also excluded.

In vitro studies were generally excluded from detailed review. *In vitro* studies using cell cultures and similar techniques involve non-physiological exposure conditions and do not generally provide data that can directly guide or influence HHRA unless clear cross species toxicological effects have been demonstrated in *in vivo* animal or human studies. Where a toxicological effect has been demonstrated *in vivo* using a physiologically relevant route of exposure, in vitro studies may provide data relevant to a consideration of interspecies sensitivity to that effect and therefore papers describing such studies were scanned at the abstract and/or paper level for relevance.

In vivo studies demonstrating known, well characterised effects, at doses where existing Health Based Guidance Values (HBGV) remain protective, were scanned at the abstract and paper level to confirm their lack of relevance but not reviewed in detail. Where paraquat was not directly included in a published study, or exposure to paraquat was not detected, papers were generally excluded from detailed review.

Papers describing deliberate misuse or self-harm, currently managed through restricted access under Australia's Poisons Standard as a Schedule 7 Dangerous Poison, were excluded from detailed review except where long term follow up of survivors informs aspects of HHRA.

Papers cited in public submission that have previously been available for screening and consideration by the APVMA in published reviews have not been reviewed again in this report.

After screening, 17 papers were identified as published after completion of the 2016 APVMA reviews and of sufficient quality and of suitability of design to be of potential value for regulatory consideration. A further 4 recent relevant papers were identified in a literature search.

Consideration of cited studies

The 21 papers identified by the screening process report endpoints and markers related to Parkinson's disease (PD), end stage renal disease (ESRD) and reproductive toxicity in males and females. Where studies were designed, and capable, to be able to do so, none report a No Observed Adverse Effect Level (NOAEL) that is more sensitive than those already relied on for HBGV for paraquat. While the new studies build upon and extend the toxicological database, they do not introduce any endpoints (toxicological effects) that would alter the existing hazard and risk assessments for agricultural use of paraquat.

The most contentious aspect of paraquat toxicity is the postulated potential to increase the risk of contracting Parkinson's disease. The APVMA acknowledges that Parkinson's disease is unarguably a serious, debilitating and ultimately fatal disease that has a substantial personal, psychological and economic impact on affected individuals, their families and their communities. If a cause-and-effect relationship between paraquat and Parkinson's disease were established, these factors would be relevant for incorporation into risk management considerations, but they are not relevant factors in a determination of whether a cause-and-effect relationship actually exists between paraquat and Parkinson's disease.

Summary of new studies

For a detailed review of the studies refer to Appendix 2.

Animal studies

Fertility

Chen et al. (2017) examined the effects of paraquat on male fertility in rats at doses of 0.5 to 8 mg/kg bw/day administered by gavage for 8 weeks. Sperm abnormalities and testis tissue effects were observed in all dose groups, though the magnitude of change in these effects were small at 0.5 mg/kg bw/day and generally not statistically significant and therefore concluded to be non-adverse. A conclusion also drawn by the US EPA review: "Given the low magnitude of the change from controls, none of the reproductive effects observed in rats from the 0.5 mg/kg/day treatment group were indicative of an adverse response to treatment". Although the data presented in this study is new and is relevant to the hazard characterisation of paraquat the existing points of departure, and human health guidance values derived from them, are adequately protective and no changes to these are required.

In a poorly documented study, Sun et al. (2021) administered paraquat by gavage at a single dose of 10 mg/kg bw/day to female CD-1 mice for 21 days. Oocytes were collected and studied in culture. The authors report a range of effects on females and their maturing oocytes, including: reduced average ovary weight, decreased polar body extrusion (a measure of oocyte maturation), decreased metaphase II stage (MII) oocytes after 21 days paraquat administration, reduced rates of two-cell embryos and blastocysts after mating, decreased average number of pups per litter and decreased pup weight, increased incidence of aberrant spindle formation and misaligned chromosomes, increased clustering of mitochondria, an increased level of reactive oxygen species (ROS), increased early apoptosis, and a decreased level of histone methylation. This study appears to demonstrate that exposure of female mice to high oral doses of paraquat significantly affects a range of fertility parameters and leads to reduced numbers of pups per litter and reduced pup weights.

Previously assessed studies (Dial & Dial 1987, Hausburg et al. 2005) of paraquat administered to mice via intraperitoneal (ip) injection, at doses from mating through to delivery found a reduction in the number of dams pregnant at full term due to pre-implantation or early post implantation effects, but no effect on the pups per litter or pup weights. Collectively these 3 studies (Sun et al. 2021, Dial & Dial 1987, Hausburg et al. 2005) indicate paraquat effects on oocyte and/or early embryo development at comparatively high doses. Unfortunately, none of the studies individually or collectively provide a basis for quantitative assessment due to design deficiencies in the Sun et al. and Hausberg et al. studies (single doses) and variations in and between studies (dose frequency, duration, and route of administration). Hausberg et al. (2005) used a single ip dose of 30 mg/kg bw of paraquat on the day of ovulation.

Dial and Dial (1987) employed a more traditional 2 generation reproduction study design with paraquat administered in the diet at 0, 45, 90, or 125 mg paraquat cation/kg feed daily from pairing through to weaning of the pups. The dietary concentrations were equivalent to approximately 0, 7, 15, 21 mg/kg bw/day. The study employed a limited range of OECD Guideline parameters (no data on pre- and post-implantation losses was reported for example).

Multi-generation dietary reproduction studies in rats (Lindsey et al. 1982a,8b, Igarashi 1980) where administration of paraquat begins 10 weeks prior to mating and continued through to the end of pup weaning, found no effects on reproductive performance, including the percent of mated dams delivering a litter and the number of pups per litter, or the development of the reproductive organs of rats, when administered at dietary levels up to 15 mg/kg bw/d for 3 generations in one study and 20 mg/kg bw/day for 2 generations in a second study. A reduction in pup body weights at birth was observed in the 2-generation study at 14.4 mg/kg bw/day but not at the NOAEL of 7.2 mg/kg bw/day. In the 3 generation study the NOAEL for pups was set by effects in the lungs (Lindsay et al 1982a). Similarly, the US EPA report no reproductive effects of paraquat in rats of doses up to 7.5 mg/kg bw/day (US EPA 2019d).

Established HBGV for reproductive effects in males and females remain appropriate.

Studies on neurotoxicity and Parkinson's disease related effects

Demonstration of clinical signs of neurotoxicity in animal models similar to those associated with Parkinson's disease (motor impairment) in humans are largely limited to male mice and predominantly employ parenteral routes of administration (injections). The available data does not indicate that the data from male mice is predictive for female mice, and the relevance is questionable for rats or dogs. Parkinson's disease specific studies in rats are limited. Life-time studies in male and female mice (US EPA 2019d, Ishmael & Godley 1983, Ishmael 1987, Sotheran et al, 1981, Smith, 1986, 1990) of at least 2 strains (Swiss derived, JCL:ICR) and in 3 studies of male and female rats of both Wistar and Fischer 344 strains (Woolsgrove & Ashby 1985, Ashby & Finn 1983, Toyoshima et al 1982a, US EPA 2019d), all administered paraquat orally, daily, did not observe clinical signs consistent with motor impairment, or histological abnormalities in brain tissue, despite the highest doses administered being overtly toxic. Similarly, 90 day and 12-month studies in dogs, orally administered paraquat at overtly toxic doses were not associated with clinical signs of motor impairment or histological abnormalities in the brain (Sheppard 1981b, Kalinowski 1953 a,b, US EPA 2019d). The relevance of studies in male mice, as a model for humans for paraquat induced Parkinson's disease related effects, is uncertain.

Milanese et al. (2018) explored the DNA damage response (DDR) activation in 2 different synucleinopathy in vivo models and investigated possible causative mechanisms in vitro. Paraquat was not investigated directly. Alpha synuclein pre-formed fibrils (PFF) were implanted into the mouse substantia nigra and recombinant adeno-associated virus implantation was used to express human-α-synuclein (h-α-syn) in the mouse striatum. Immunohistochemistry, immunofluorescent and stereological techniques were used to identify Parkinson's disease related lesions and alterations. Four to 6 months after treatment lesions in the dopaminergic system were observed. Viral h-α-syn transduced mice had displayed significant striatal increase of h-α-syn levels in the ipsilateral hemisphere, accompanied by a reduction of striatal tyrosine hydroxylase (TH) immunoreactivity. Increased α-syn was also detected inside dopaminergic cell bodies in the substantia nigra pars compacta (SNpc). These alterations were associated with significant ipsilateral reduction in nigral dopaminergic cell bodies, as detected by stereological counts. In PFF treated mice reduced striatal TH immunoreactivity paralleled by α-synuclein stress (increased phospho-synuclein levels and reduced dopaminergic cell bodies in the SNpc) were reported. The authors concluded that these data demonstrate a causative link between α-syn proteotoxicity and DNA damage in dopaminergic neurons. This study provides further evidence for the potential role of α-synuclein in Parkinson's disease but adds no new data on paraquat specifically.

Anderson et al. (2021) exposed male and female C57BL/6J mice whole body, to aerosolised paraquat at 130 µg/m3 for 4 hours per day, 5 days per week for 4 weeks. Exposed mice were then retained on test without further paraquat exposure for up to 303 days. Subsets of male mice were sacrificed on experimental days 10, 28, 56, and 303 and brain, lung, and kidney were collected for paraquat quantitation. Tissue levels of paraquat were largely confined to the lungs and olfactory bulb. Lactate dehydrogenase (LDH) enzyme activity and protein levels of bronchoalveolar lavage fluid was unaffected by treatment immediately following the end of exposure and there were no signs of lung fibrosis. In an olfactory discrimination test paraquat-exposed males made significantly more incorrect choices only at one concentration of scent. No effect was seen in females. The study does not alter the overall conclusion that exposure to paraquat via the nose leads to low, localised, levels of paraquat in the olfactory bulb that does not distribute to other areas of the brain. Under conditions of this study, paraquat levels achieved in brain regions were 2 to 3 orders of magnitude below those demonstrated to produce no damage to dopaminergic neurons of the substantia nigra pars compacta (SNpc) in mice.

Dwyer et al (2021) investigated the neurodegenerative, inflammatory, and stress effects of exposure to 7 (unstated) doses of paraquat administered over 2 weeks with animals sacrificed immediately after the last dose and at 1 and 6 months for investigations. The paper reports that paraquat induced a loss of dopaminergic SNpc neurons and activation of microglia that persisted over 6 months after the last injection although with reduced magnitude of SNpc neuron loss. The microglial proinflammatory actin-remodelling factor, WAVE2, and the inflammatory transcription factor, nuclear factor kappa B were also elevated within the brain. Corticosterone remained significantly elevated 1 month after paraquat. High-resolution MRI detected no striatal changes, but modest hemispheric differences in the SNpc and time-dependent volumetric enlargement of the ventricles in paraquat-treated mice were reported. The authors concluded that their data suggest that paraquat induces long-term nigrostriatal pathology and inflammatory changes and stress and trophic/apoptotic effects that appear to either increase with the passage of time or are evident for at least 1 month. This paper contains substantive deficiencies. The form, source and purity of the paraquat used were not stated. The dose administered and number of animals per group were not provided. Very small numbers of animals were examined in some key investigations (e.g. 4 per group for MRI). The dose administered appears to have produced general systemic toxicity as indicated by reduced body weights and increases in the organ weights of the heart and lungs. Paraquat was administered by ip injection, a non-physiologically relevant route of exposure for HHRA. Conversely a significant strength of the paper is that the immunohistochemistry was assessed by investigators blinded to treatment.

Studies in male mice also report inconsistent results. An exceptionally well-designed study by Smeyne et al. (2016) attempted to reproduce results from an earlier study in the same laboratory (Jiao 2012) that reported an ip paraquat induced decrease in SNpc dopamine neurons of approximately 50% in C57BL/6J male mice and approximately 10% in SWR/J male mice. Smeyne et al. (2016) investigate the potential basis for differences in the effects of paraquat on the SNpc reported in different laboratories [Jiao et al. 2012, Breckenridge et al. (2013), Minnema et al. (2014)] by conducting controlled, multisite experiments that systematically varied paraquat dose and dose frequency, the source and age of the C57BL/6 male mice, animal housing conditions and stereological and neuropathological methods. Unlike most of the published literature, this study used a relatively large number of replicates (n) per group and there was sufficient statistical power (0.994) to detect the average 26% reduction in the mean number of tyrosine hydroxylase positive (TH+) neurons reported in the statistically positive studies. Notably, stereology was performed by 2 independent investigators who were both blinded to treatment, and the neuropathological evaluations were conducted by another investigator, also blinded to treatment.

In contrast to the neurotoxin, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) used as a positive control, regardless of paraquat dose or dose frequency, the source of paraquat, age or husbandry of the C57BL/6J male mice evaluated, and the stereological or neuropathological methods employed, paraquat did not induce a loss of SNpc dopaminergic (DA) neurons, did not cause damage to DA axons/terminals or produce evidence of neuroinflammation. Importantly, the results were consistent among the 3 independent investigators.

In supplementary data to the main study the authors report a comprehensive review of 51 published studies (including their 2016 study) on the effects of paraquat on SNpc dopaminergic neurons. Twenty-eight of these published studies performed stereological assessments (73 group comparisons) of which 47 (of the 73) did not blind the investigators with respect to treatment. In 94% of these comparisons (44 of 47 not blinded), the mean number of TH+ neurons in the SNpc of paraquat treated mice were reduced (by an average of 26.5% compared to control). For the 26 comparisons (of 73) where investigators were blinded, 81% (i.e. 21 of 26) found no effect of paraquat. Aside from blinding, the most noticeable difference between studies showing a paraquat-induced cell loss and those that did not, was the observation that the average coefficient of variation (CV) among the “positive” studies (~ 6–7%) was statistically significantly less than the average CV observed in the negative studies (CV = 16.4%; $p < 0.0001$).

Sun et al. (2023) investigated the antioxidant properties of cryptotanshinone in paraquat- induced neurotoxicity in mice and in a human neuroblastoma cell line. Cryptotanshinone is a fat-soluble extract of Salvia miltiorrhiza with use in Chinese herbal medicine. Cryptotanshinone at a high concentration of 0.5 mM reportedly attenuated paraquat-induced oxidative stress in the cell line by upregulating antioxidant markers. In C57BL/6J mice administered by ip injection: paraquat 10 mg/kg bw once per week for 3 weeks and paraquat 10 mg/kg bw once a week for 3 weeks with 2 injections of cryptotanshinone 10 mg/kg bw each week, cryptotanshinone protected against behavioural deficits and protected dopaminergic neurons. The authors describe their study as demonstrating that cryptotanshinone can reduce reactive oxygen species (ROS) generation, thereby alleviating oxidative stress by elevating Nrf2 expression and promoting cell survival in differentiated SK-N-SH cells. Improved motor function and protection of Tyrosine hydroxylase-positive (TH+) neurons was also described. The paper provides no indication of blinding of histopathology investigators to treatment and overall, the study is of uncertain relevance.

Torres-Rojas et al. (2022) explored the relationship between genetic characteristics and paraquat reduction of tyrosine hydroxylase (TH) staining in the SN of various strains of male mice administered 5 mg/kg paraquat dichloride trihydrate by ip injection once weekly for 3 weeks. Processed brain sections were stereologically analysed for TH+ neurons, neurons, and nuclei in the SNpc by 2 investigators independently who were blinded to treatment. The authors concluded that there were significant effects of strain and treatment but not their interaction on TH+ neurons, with a TH+ loss ranging between 0 and 20% but there were no significant effects of strain treatment, or their interaction on neuron counts and query whether paraquat destroys TH+ neurons in the SNpc. This study did not address whether the loss of TH+ neuron staining is permanent or reversible and, if permanent, whether paraquat prevents the synthesis of TH but spares the neuron. The authors note a number of limitations of their study: a limited number of strains was investigated, males only were investigated, while the study identified strain differences in paraquat effects on TH staining, it did not observe a significant strain and treatment interaction. The study provides some support for genetic-based individual differences in susceptibility of male mice to paraquat neurotoxicity.

Wang et al (2016) investigated the link between defective DNA repair and age-associated neurodegenerative disorders such as Parkinson's disease through induction of DNA damage using X-ray irradiation and analysis of dopamine neuron degeneration in the A53T human α-synuclein over expressed mouse model and A53T-a-Syn Mouse Embryonic Fibroblast (MEF) cells. The authors describe their results as indicating that A53T-a-Syn MEFs show a prolonged DNA damage repair process and senescence phenotype. DNA damage preceded onset of motor phenotype in A53T-α-Syn transgenic mice and decreased the number of nigrostriatal dopaminergic neurons. Neurons of A53T-α-Syn transgenic mice were more fragile to DNA damage. The study did not investigate paraquat directly, and the sex of the mice used was not provided (but were likely male).

Overall, the newly available animal data reinforce the conclusion that the available animal studies do not provide convincing evidence that paraquat presents a substantive risk of increasing Parkinson's like disease in humans from likely routes of exposure arising from agricultural use. The previous conclusion of the APVMA paraquat technical report that paraquat in animal studies "does not induce neurotoxicity via the oral, dermal or intranasal exposure routes" remains appropriate. The US EPA review of the literature on the relationship between paraquat and Parkinson's disease (US EPA 2019a) concluded that "Overall, the limited, mixed findings in the animal literature were considered weak evidence of a Parkinson's disease-like response to paraquat exposure". No data has been identified from public submissions or the available literature that would alter the validity of these conclusions.

Key limitations of the body of available studies of paraquat in animal models is the use of non-physiologically relevant exposure routes, the concentration of studies on male mice and limited data on passage of paraquat across the blood brain barrier (BBB). A previously considered study in the rhesus monkey using radiolabelled paraquat and using PET scanning in live sedated animals (Bartlett et al 2009), found no penetration through the BBB, which raises questions as to the relevance of brain levels measured in brain tissue removed from mice postmortem. In a second study Bartlett et al (2011) used PET scanning to examine the uptake of paraquat administered to pregnant female rhesus monkeys, into the maternal and foetal brains. Minimal uptake of paraquat by both maternal and foetal brains was observed and the highest regional cerebral uptake in the maternal brain (0.0009% injected dose) was seen in the pineal gland, a structure known to lack a BBB. Bartlett however used only a single dose and studies in both rats and mice have shown accumulation in brain tissue after repeated doses. Clear differentiation between paraquat associated with blood vessels and that within brain tissue is limited. As concluded by the US EPA review (2019a), "it is plausible that environmentally relevant exposure to paraquat could lead to accumulation in brain tissue that increases with duration of exposure and persists after exposure ends" but the available data is not definitive particularly for environmentally relevant human routes of exposure and given the poor exposure quantification in epidemiological studies. Also as noted in the US EPA review (2019a) brain concentration in rats varies with age, and consequently extrapolation from rodent studies is complex. Improved data on brain paraquat levels in relevant animal species following administration by relevant exposure routes (dermal, oral, inhalational) would be advantageous.

Review of new epidemiology studies

End stage renal disease

McGwin & Griffin (2022) used an ecological study design to examine the association between end stage renal disease (ESRD) and inferred paraquat exposure based on the agricultural pesticide use estimates for counties across the USA maintained by the National Water Quality Assessment Program.

Cases were obtained from the US renal data system. The study concluded that: “The incidence of ESRD increased with increasing paraquat density. Based on a 20-year exposure lag, those in the highest paraquat density quartile had a 21% higher rate of ESRD compared to the lowest quartile whereas for a 15-year lag the increase was 26%. Adjusted associations were attenuated though still followed an increasing linear trend across quintiles”. The assignment of exposure based on application rates across counties of case residence at the time of diagnosis is tenuous at best. The study provides no basis for dose response considerations and the link between “exposure” as defined by paraquat use “density” and ESRD is weak. The study outcomes appear to be discordant with findings in the US Agricultural Health Study where mortality for private pesticide applicators associated with renal disease is generally comparable to controls (Shrestha et al. 2019, not stratified for paraquat specifically however).

Parkinson’s disease

The origins and causes of Parkinson’s disease are multifactorial. A combination of genetic, environmental, ageing and lifestyle conditions appear to contribute to an individual’s susceptibility to develop Parkinson’s disease, however the specific causal factor(s) remain unknown. These issues are discussed at length in the US EPA and the recent Californian DPR reports and are not addressed in further detail here. Regardless of any link between paraquat and Parkinson’s disease, the overwhelming majority of Parkinson’s disease cases cannot be reliably linked to pesticide exposure in general or to any specific chemical.

Ayton et al. (2019) used dispensing data for Parkinson’s disease medications from Pharmaceutical Benefits Scheme (PBS) records as an indication of Parkinson’s disease prevalence (unique patients) across 79 Local Government Areas (LGAs) in Victoria, Australia as a percentage of the LGA population. Data from the Australian Bureau of Statistics (ABS) was used to identify agricultural commodity production characteristics in each of these LGAs. The study made no attempt to quantify the identity or usage rates of specific pesticides in the various LGAs. A notable cluster of increased Parkinson’s disease prevalence was identified in the central western area of Victoria. Although the prevalence of Parkinson’s disease was higher in rural (1.02%) compared to urban localities (0.80%; P=0.001) when Parkinson’s disease prevalence was adjusted to account for median age, proportion male, and the Socio-Economic Indexes for Areas, SEIFA socioeconomic indicator, rurality in itself was no longer predictive of prevalence rates. The authors used Monte Carlo modelling to estimate the probability of the cluster of the 4 highest prevalent LGAs forming randomly, through 20,000 reconstructions of the map of Victoria with randomized allocation of prevalence rates in each of the 79 LGAs and identified only 19 occasions where this might have occurred giving a probability of P=0.00095, indicating chance is an unlikely source for the observations. In the absence of availability of data on the use of agricultural chemicals across Victoria, the study examined the types of commodities produced in each of the LGAs and identified 5 commodities which were produced in higher intensity in the high prevalence areas: barley, chickpeas, faba beans, lentils, and vetches. With the exception of barley, the identified commodities were all from the pulse family of crops. The primary significance of this paper is that it illustrates the challenges of identifying causative relationships between such clusters, and any specific agent, without careful exposure analysis involving direct measurement of worker and bystander exposure to suspected causative agents, particularly where studies arbitrarily restrict the range of potential causative parameters examined.

Krzyanowski et al. (2025) used a case control design study involving patients with Parkinson’s disease and matched controls from the Rochester Epidemiology Project (REP) from 1991 – 2015, to investigate the association between living near to golf courses and development of Parkinson’s disease. Patients were identified retrospectively from medical records.

Cases were only required to live in the area of interest (Olmsted County) at the time of symptom onset/diagnosis. Controls were recruited from across 27 counties as covered by the REP. No attempt was made to identify or quantify exposure to any environmental factor other than proximity to a golf course. The study provides no new data relevant to a consideration of paraquat human health risk.

Paul et al (2024) used a case control design drawing data from the Parkinsons Environment and Genes (PEG) study which comprised 829 Parkinson's disease patients and 824 community controls across 3 Californian counties. This data was then used for an ecological study where the authors estimated residential and workplace proximity to commercial agricultural applications in the 3 counties since 1974 using the California pesticide use reporting (PUR) data and land use maps. "Exposure" was expressed in terms of use of paraquat and the duration and average intensity of use, in pounds/active constituent/year of paraquat across 4 time periods at the agricultural locations. The authors conclude that their study demonstrated Parkinson's disease was associated with paraquat "exposure" at both residence and workplace. For workplace and residential proximity to commercial paraquat applications odds of contracting Parkinson's disease were elevated [Workplace odds ratio (OR) = 2.15, 95% confidence interval (CI) = 1.46, 3.19], (residential: OR=1.91, 95% CI=1.30, 2.83). Risk estimates were comparable for men and women, and the strongest odds were observed for those diagnosed at or before 60 years of age. A major weakness of this study is that while the ecological study design using GIS pesticide use data for exposure inference removes recall bias, it does not provide quantitative or qualitative data on exposure for any individual study participant (the "ecological fallacy"), Chang et al., 2014. At best the design provides evidence of potential exposure at unknown levels. The distance between a subject's residence and workplace and the location of paraquat application and amount applied were the only evidence of potential exposure. Important differences between subjects and controls in this study included controls being younger, more likely to be female and non-white. While these factors were controlled statistically using the logistic regression models, there is a potential for error with this approach. Notably while the authors documented around 190 odds ratios (and their 95% CI estimates) they focussed their abstract and discussion on only 4 of these with ORs greater than 1.0 and 95% confidence intervals that did not include 1.0. The ORs for 77 (41%) of the total number of comparisons had 95% confidence intervals that did include 1.0 and the paper does not correct for multiple comparisons for reasons not explained in the paper.

Sanders et al. (2017) report analysis of 619 patients and 854 population based controls drawn from the Californian PEG study, to investigate the relationship between paraquat exposure and single nucleotide polymorphisms (SNPs) in base excision repair (BER) genes. Exposure inference was derived from GIS-based paraquat usage data and considered both residential and occupation address. Study subjects provided blood and saliva samples in order to determine the genetic data. The study reports evidence of a positive association between paraquat residential/workplace exposure and Parkinson's disease (OR = 1.54, 95% CI: 1.23-1.93, n = 245 exposed cases). For the interaction between paraquat exposure and genetic susceptibility, the study reports no evidence of a significant positive association between paraquat exposure in subjects with no more than 1 risk allele (OR = 1.13, 95% CI: 0.75-1.70, n = 48 exposed cases) compared to those with 1 or fewer risk alleles and a strong positive association in subjects with 2 or more risk alleles (OR = 2.38, 95% CI: 1.44-3.95, n = 22 exposed cases). The primary strength of this and other PEG studies is the recruitment of cases with clinically confirmed Parkinson's disease diagnosis and the use of GIS data to infer exposure not subject to recall bias. GIS based data, however, does not provide reliable quantitative exposure estimates and cannot isolate paraquat exposure specifically as opposed to general residential/workplace proximity to agricultural land. No published information on the measurement of paraquat residue levels in residential or workplace environments is available for the counties of residence or workplace for study participants.

In the absence of exposure validation/quantification, the relationship between being present at addresses within 500 m of agricultural land and actual paraquat exposure is indeterminate. Controls were recruited using a population-based approach which may have introduced selection bias if cases and controls represent populations with different characteristics.

Shrestha et al. (2019) compared the observed mortality rates in private pesticide applicators from the US Agricultural Health Study (AHS) with the mortality rates of the general population and found largely comparable rates of Parkinson's disease between the 2 and a significantly lower all-cause mortality in pesticide applicators (SMR overall=0.69, 95% CI: 0.67–0.70). There are numerous epidemiology studies which have investigated the potential link between the use of paraquat, other specific pesticides, or pesticides in general, and the development of Parkinson's disease. A number of studies have concluded that exposure to pesticides in general (i.e. collectively or generically) increases the risk of developing Parkinson's disease. Exposure estimation in these studies is generally very weak however, and do not allow an assessment of dose response relationships, if any exist. Additionally, a causal association between pesticides in general and Parkinson's disease, or any other specific disease, is biologically implausible. Pesticides cover a broad range of chemical structures, and pesticidal modes of action are specific to the pest being controlled. The modes of action of pesticides for the control of bacteria, fungi, nematodes, molluscs, weeds, insects and mammalian pests are specific to the biochemistry of those organisms. The likelihood of such a wide range of substances and pesticidal mechanisms producing the same or closely related disease outcomes in humans, particularly where exposure is very low and infrequent, such as for persons living in rural communities but not handling pesticides for example, is at most negligible. The outcomes reported are therefore likely to reflect various sources of experimental bias and confounding inherent to the study design, and particularly the inference of exposure from indirect data. This observation does not however preclude specific chemical or mechanistic classes of pesticide being associated with human disease outcomes, including Parkinson's disease, or for specific pesticides with different but interacting mechanisms producing adverse health effects when exposure is combined. The nature of such specific associations, if any, are however not discernible from the available studies.

Shrestha et al. (2020) investigated the relationship between incident Parkinson's disease cases and 50 specific pesticides, including paraquat in participants in the Agricultural Health Study. Detailed information on both duration and frequency of use was collected at enrolment or in take home questionnaires with an additional questionnaire asking about pesticide use in the most recent year prior to the study. Exposure estimations utilised a range of information to provide more robust estimates than are possible from more common approaches such as GIS based use rates for example. Exposure intensity weights were derived using a previously described algorithm that incorporates information on; mixing practices, application methods, equipment repair status, and personal protective equipment use (as described in Coble et al., 2011). The study then used intensity-weighted lifetime days (IWLD) of pesticide use (i.e., the product of years of use and days used per year weighted by exposure intensity) as a measure of cumulative exposure for applicators, creating a four-category exposure variable (never use and 3 categories among users with cut-points at tertiles of IWLD). There was no association for ever use of paraquat and Parkinson's disease (HR = 1.09, 95% CI: 0.84-1.19). When analysed for lifetime days of use, there was again no evidence of an association and no evidence of a trend across lifetime use days. The hazard ratios through enrolment were 1.03 (0.58 – 1.81), 1.42 (0.86 – 2.33), and 0.74 (0.37 – 1.49) for the first, second, and third tertile of use respectively indicating no dose-response based on increasing exposure. The same pattern was observed through phase two: 0.92 (0.51 – 1.63), 1.49, (0.92 – 2.41), 0.69 (0.34 – 1.38). The study found a significant interaction between occupational paraquat exposure and head injury with the development of Parkinson's disease, with a hazard ratio for those with head injuries being 3.2 (95% CI,1.38–7.45).

There were only 23 participants with both Parkinson's disease and a history of prior head injury across the entire study population and co-exposure of Parkinson's disease and head injury cases with multiple pesticides confounds attribution of Parkinson's disease to any specific combination of head injury and a specific (or class of) pesticide exposure with any confidence.

A recent updated study of Parkinson's disease and mortality from major causes of death among a UK workforce who manufactured paraquat in any of 4 plants in the UK (between 1961 and 1995 (Tomenson & Campbell. 2021) found no association between Parkinson's disease and paraquat exposure despite unequivocal signs and symptoms of paraquat toxicity in workers prior to improved occupational health and safety (OHS) practices in the early 1980s. The study followed 926 male and 42 female workers through to 2017 and compared the rate of mortalities and cause of death for males with national and local rates. Exposure data was available from 1330 static monitoring results at one site between 1979 and 1993 and 100 personal monitoring results were collected between 1983 and 1993. There was insufficient sampling information available to perform a quantitative exposure assessment, however. A limited qualitative exposure assessment of male workers based on their highest level of exposure to 11 substances including paraquat and its manufacturing precursor was also available. Approximately 300 of the 729 male workers included in the initial mortality investigation were assessed in the mid-1980s to have had high or medium exposure to paraquat. Of 394 males who had died by the end of follow up, 4 death certificates mention Parkinson's disease (as cause of death or present prior to death combined) compared to the expected number of 6 giving a SMR for Parkinson's disease of 0.67 (95% CI 0.18 - 1.72). Unlike many case-control studies where the exposure estimates/inference for cases is somewhat tenuous, a strength of this study is the likely higher exposure of workers engaged in paraquat production, particularly before the 1980s, as evidenced by overt symptoms of significant exposure recorded by workers. Many of the workers at 2 plants reported skin lesions and nose bleeds (common signs of paraquat dermal and nasal exposure) following exposure to dust and the prevalence of nose bleeds was considerably higher than that seen in cross-sectional surveys of paraquat sprayers. These symptoms reflect substantial exposure of workers, at markedly higher levels, on a daily basis, than is plausible for residents living in rural areas near to agricultural land or for applicators of diluted sprays applied on a seasonal (infrequent) basis. Improved OHS requirements after the 1970s substantially lowered exposure of workers and personal monitoring after 1983 yielded estimated daily intakes of 25.8 mg of paraquat ion. The authors estimate that the daily exposure of workers in the plants from the mid-1980s onwards was comparable to that of an agricultural sprayer, although factory worker exposure was more frequent and prolonged. A key strength of this study is that participants were followed over a protracted period of time from exposure to outcome, which, when compared to other observational studies, provides a clearer temporal sequence and strengthens causal inferences about the relationship between exposure and outcome, or lack thereof. The study established temporality and used biomarker and environmental monitoring measurements of exposure, avoiding self-reporting of paraquat exposure and recall bias. The authors conclude that "The study provided no evidence of an increased risk of Parkinson's disease, or increased mortalities from other causes among paraquat production workers whose exposure to paraquat on a daily basis was at least comparable to that of a paraquat sprayer or mixer/loader". There was also no evidence of increased mortality from other causes including cancer. As the authors note, although other studies have determined that approximately 30 to 50% of death certificates for a deceased person with Parkinson's disease do not mention Parkinson's disease, this does not bias the SMR as the same degree of underreporting applies to national and local mortality rates.

Yuan et al. (2022) identified individuals reporting unusually high pesticide exposures from 1983 to 1997 from the AHS study and examined self-reported "dream enacting behaviours" (DEB) from 2013 to 2015 as a surrogate for rapid eye movement sleep behaviour (RBD). DEB is a characteristic feature of RBD, the most specific prodromal marker of synucleinopathies.

Occurrence of DEB was self-identified by participants in response to specific questions regarding typical symptoms. Of a total of 11,248 eligible participants 939 (8.3%) reported DEB. Compared with farmers without DEB, those who reported such behaviours were more likely to be current smokers, married or living as married, had histories of head injury and depression, and reported more nonspecific symptoms and major chronic conditions at baseline. A history of high pesticide exposure events was reported by 1847 farmers. Farmers reporting high pesticide exposures were significantly more likely to also report DEB, OR = 1.74 (95% CI 1.49-2.05). For paraquat specifically, farmers reporting a high exposure event were significantly more likely to also report DEB, OR = 3.48 (95% CI 1.37 – 8.81) but the absolute number of high exposure subjects was small at 26 with only 6 of these reporting DEB.

Brent and Schaeffer (2011) examined the entire body of published reports of clinical outcomes of survivors of paraquat poisoning and found no connection between high dose paraquat exposure in humans and the development of Parkinson's disease. If paraquat exposure is causally related to Parkinson's disease development, the absence of reports of Parkinson's disease related symptoms in patients requiring treatment for paraquat poisoning, and the absence of increased Parkinson's disease risk in industrial workers with clear clinical signs and symptoms of significant paraquat exposure is notable, and discordant. More recent studies utilising the very large AHS study cohort (Shrestha et al. 2019, Shrestha et al. 2020) support and strengthen the overall conclusion that cause and effect for paraquat exposure and Parkinson's disease risk have not been convincingly demonstrated, a conclusion supported by the US EPA reviews, recent published reviews (Weed, 2021, Weed, 2024) and to a large extent the Californian DRP preliminary review (2024). The potential for a combination effect of head injury and paraquat exposure has been suggested (Shrestha 2020, Lee et al. 2012) but case numbers in those studies were small, co-exposure to multiple pesticides was a major confounder and the significance of the observation is, as yet, uncertain.

Much of the epidemiological information about a possible association between Parkinson's disease and paraquat exposure is provided by case-control studies many of which had potential for exposure recall bias, small numbers of subjects exposed to paraquat and/or limited and/or inaccurate exposure information, and few were able to control for confounding. The most robust epidemiological studies of unequivocally exposed subjects, with the highest level, most frequent and prolonged exposures do not provide convincing evidence of a causal relationship between Parkinson's disease and occupational paraquat exposure.

Exposure uncertainties

The most substantive data gap across all of the available epidemiology studies relates to exposure quantification and characterisation, most especially exposure of persons not involved in paraquat application but living in agricultural regions where paraquat is applied. Some level of non-negligible exposure of workers manufacturing or mixing, loading, and/or spraying paraquat over periods of decades can reasonably be assumed but reliable quantification studies are few. One available exposure study relevant for safety assessment for workers applying paraquat in an agricultural setting is comparatively strong, however. The US EPA Draft HHRA Report for (US EPA 2019d, page 53) summarises a now quite old worker exposure study submitted by Zeneca[1] where systemic exposure of 17 workers applying paraquat using open cab tractors and ground boom spraying was assessed from urinary excretion of paraquat over 3 days post application. Cumulative urinary paraquat provides a direct measure

[1] MRID 43644202: Paraquat: Worker Exposure During Mixing, Loading, and Application of GRAMOXONE® EXTRA to Pecans Using Vehicle-Mounted Ground Boom Equipment.

of actual achieved systemic exposure from all sources of contact, removing considerable uncertainties associated with less direct measures of exposure (sample patches on worker clothing for example). Workers individually selected what personal protective equipment (PPE) to wear based on their normal work practices (as opposed to specific label directions), resulting in a variety of protection being used ranging from baseline clothing only (n=9), the addition of chemical resistant gloves only when mixing (4) and the remainder (4) adding various combinations of protective clothing, goggles and face shield. The study found that only 6 of the 17 urine samples collected contained detectable paraquat. All 6 of these samples were taken from Day 0 (day of product application) samples. Of the 6 workers with detectable paraquat exposure, none wore protective equipment while handling the formulation. There was no discernible trend between the amount of pesticide handled and the resultant exposure. Despite the negligible PPE used, use of an open cab tractor and close proximity to paraquat spray, actual daily systemic exposure was < 1.1 µg/kg bw/day. The low systemic exposure predominantly reflects the poor dermal absorption of paraquat.

Paraquat is predominately applied by ground boom spraying which is less likely to give rise to aerosolised particulates than spray operations with other pesticides, misting application on vines or trees for example. Paraquat has low mobility in soil, due to binding to soil particles, further reducing potential quantitative distribution beyond agricultural sites. There is therefore considerable uncertainty regarding actual exposure of rural residents at distance beyond a few tens of metres from farm boundaries but, given the exceedingly low systemic exposures found in poorly protected workers applying paraquat from open cab tractors, such exposures would be expected to be negligible at most.

Studies monitoring paraquat distribution (amount and form) over a radius of 10 to 2000 m from agricultural sites for some days following paraquat spray operations would considerably improve interpretation of the available epidemiology studies.

As highlighted by the Kamel et al. (2007) study, recall bias in prevalent cases self-reporting paraquat exposure is another substantive confounder in many epidemiological studies. This paper identified substantial differences between the odds ratio for the association between paraquat and Parkinson’s disease between incident and prevalent cases. The paper reports a non-significant but positive association of paraquat exposure with prevalent Parkinson’s disease (OR = 1.8; 95% CI, 1.0-3.4, n = 14 paraquat exposed cases) but no evidence of an association with incident Parkinson’s disease (OR = 1.0; 95% CI, 0.5-1.9, n = 11 paraquat exposed cases).

Overall conclusions

The overall conclusions of this report, the 2016 OCS/APVMA review of paraquat neurotoxicology and the extensive US EPA (2019) reviews is that the evidence available to date does not convincingly demonstrate a direct causal association between exposure to paraquat occupationally and/or through residential exposure to pesticides used on nearby land, and an increased risk of developing Parkinson’s disease. As noted by the US EPA (2019a) although Parkinson’s disease-like effects are seen in some animals administered paraquat via injection, given the substantial differences in toxicokinetic behaviour from anticipated routes of exposure and parenteral dosing in animals, toxicity data reported for injection studies is of limited use to assessing human risk from pesticidal uses of paraquat.

Appendix 1

Appendix 1: Screening criteria of cited references

The APVMA received 23 written submissions for health with supporting references in response to the publication of the Proposed Regulatory Decision. These submissions cited 156 references consisting of 134 peer reviewed papers, 22 additional documents (including reports, guidance documents, news articles) and one industry submission which were screened at the title, abstract and/or paper level for relevance to the Human Health Risk Assessment (HHRA) of paraquat used as an herbicide. An additional 4 published papers were identified from literature search and included for review. Many cited references although not relevant for HHRA, and therefore excluded from review, may have relevance for other aspects of the APVMA chemical review of paraquat such as environmental risk assessment.

Inclusion criteria

Cited references were included for detailed review where they:

1. contain new, original, data directly relevant to HHRA
2. include paraquat in the study protocol
3. have not previously been reviewed or screened out of the review by the APVMA (i.e. published or made available after 2015)
4. are of adequate quality of design and level of reporting detail to be reliable for risk assessment purposes
5. were conducted using human subjects (e.g. epidemiology, worker exposure studies) or common mammalian experimental animals (e.g. rats, mice, dogs, rabbits, guinea pigs, primates).

Cited references meeting some but not all of the above criteria were considered on a case-by-case basis for inclusion in the detailed review where they present data informative for the review of public comments.

Cited references, and new papers from a literature search, identified for detailed review are listed in Table A1.

Table A1: Cited and new references identified for detailed review

Author (Date)	Title (Comment)
Anderson et al. (2021)	Paraquat Inhalation, a Translationally Relevant Route of Exposure: Disposition to the Brain and Male-Specific Olfactory Impairment in Mice.
Ayton, D. et al. (2019).	Parkinson's disease prevalence and the association with rurality and agricultural determinants. Parkinsonism and related disorders.
Chen et al. (2017)	Oxidative damage of the male reproductive system induced by paraquat.
Dwyer et al. (2021)	Characterizing the protracted neurobiological and neuroanatomical effects of paraquat in a murine model of Parkinson's disease.
Jiao, Y. et al. (2012)	Genetic Dissection of Strain Dependent Paraquat-induced Neurodegeneration in the Substantia Nigra Pars Compacta (identified from a literature search, read in conjunction with the Smeyne 2016 paper).

Author (Date)	Title (Comment)
Kamel, F. et al. (2007)	Pesticide Exposure and Self-reported Parkinson's Disease in the Agricultural Health Study. American Journal of Epidemiology, 2006. 165(4): p. 364-374.
Krzyzanowski, B. et al. (2025)	Proximity to Golf Courses and Risk of Parkinson Disease (identified from a literature search).
Lee, P.C. et al. (2012)	Traumatic brain injury, paraquat exposure, and their relationship to Parkinson disease. Neurology, 2012. 79(20): p. 2061-6.
McGwin, G. and Griffin, R.L. (2022)	An ecological study regarding the association between paraquat exposure and end stage renal disease. Environmental Health21, 127
Milanese et al. (2018)	Activation of the DNA damage response in vivo in synucleinopathy models of Parkinson's disease.
Paul et al. (2024)	Agricultural paraquat dichloride use and Parkinson’s disease in California Central Valley. Int J Epidemiol. 2024 Feb 1;53(1):dyae004.
Sanders et al. (2017)	Base Excision Repair Variants and Pesticide Exposure Increase Parkinson’s Disease Risk. Toxicological Sciences, 158(1), 188–198
Shrestha, S. et al. (2020)	Pesticide use and incident Parkinson's disease in a cohort of farmers and their spouses. Environ Res 191: 110186.
Shrestha et al. (2019)	Overall and cause-specific mortality in a cohort of farmers and their spouses (identified from a literature search).
Smeyne et al. (2016)	Assessment of the effects of MPTP and paraquat on dopaminergic neurons and microglia in the substantia nigra pars compacta of C57BL/6 mice (identified from a literature search).
Sun et al. (2023)	Cryptotanshinone protects against oxidative stress in the paraquat-induced Parkinson's disease model.
Sun et al. (2021)	Paraquat Reduces the Female Fertility by Impairing the Oocyte Maturation in Mice.
Tomenson. J.A. and Campbell, C. (2021)	Mortality from Parkinson's disease and other causes among a workforce manufacturing paraquat: an updated retrospective cohort study. J Occup Med Toxicol 16, 20 (2021).
Torres-Rojas et al. (2021)	Paraquat Toxicogenetics: Strain-Related Reduction of Tyrosine Hydroxylase Staining in Substantia Nigra in Mice.
Wang et al. (2016)	DNA damage preceding dopamine neuron degeneration in A53T human alpha synuclein transgenic mice.
Yuan et al. (2022)	High Pesticide Exposure Events and Dream-Enacting Behaviors Among US Farmers. Mov Disord. 2022;37(5):962-71.

Exclusion criteria

Cited references were excluded from detailed review for the HHRA section of the paraquat chemical review where they:

1. reported studies on non-mammalian species, studies of environmental effects of paraquat, or economic, agronomic, or general agricultural analysis or investigation, Table A2
2. consist of reviews, opinions, editorial comment or clinical management considerations, that present no new data relevant to HHRA, Table A3
3. were published or became publicly available before 2015 and therefore available for the 2016 APVMA review of paraquat, Table A4
4. do not include paraquat in the study protocol, or present results demonstrating known, well characterised effects (e.g. deliberate self-harm, known effects on lungs or other target tissues) at doses that do not affect existing points of departure for derivation of human health guidance values (e.g. Acceptable Daily Intake (ADI), Acute Reference Dose (ARfD)), or were conducted using non-physiological conditions (e.g. in vitro studies), or one or more authors have been flagged as unreliable, Table A5.

Although individual cited references may have been excluded on the basis of multiple criteria each reference is included only once in the following tables. Some older studies previously available for screening or reviewed by the APVMA in the 2016 review reports and therefore excluded from detailed review in the current evaluation of public comments, have been reconsidered where updated or additional data has been subsequently published, or similar findings have been more recently published, and the older studies inform interpretation of the newer papers. These papers are referenced in the main review of public comments wherever discussed.

Table A2: Cited references presenting data not relevant to HHRA (i.e. data on non-mammalian species, studies of environmental effects of paraquat, or economic, agronomic, or general agricultural analysis or investigation)

Author (Date)	Title (Comment)
Adegaye, A. et al. (2023)	Effects of 2 commonly used herbicides on soil microbial activity under conservation tillage. Environmental Advances 13, 100424
Aribisala, O.A., Sogbanmu, T.O. and Kemabonta, K.A. (2022)	Genotoxic, biochemical and histological biomarkers of subacute concentrations of paraquat and glyphosate in Nile Tilapia. Environ Anal Health Toxicol, 37, e2022012-2022010
Augustyniak, M. et al. (2015)	DNA damage in grasshopper Chorthippus brunneus (Orthoptera) hatchlings following paraquat exposure. Chemosphere,125, 212-219.
Ayanda, O.I., Tolulope, A. and Oniye, S.J. (2021)	Mutagenicity and genotoxicity in juvenile African catfish, Clarias gariepinus exposed to formulations of glyphosate and paraquat. Sci Prog, 104, 368504211021751.
Bohingamu Mudiyanselage, S. et al. (2017)	Cost of Living with Parkinson's Disease over 12 Months in Australia: A Prospective Cohort Study. Parkinson's disease, 2017, 5932675. https://doi.org/10.1155/2017/5932675

Author (Date)	Title (Comment)
Bora, S. et al. (2021)	Paraquat exposure over generation affects lifespan and reproduction through mitochondrial disruption in C. elegans. Toxicology, 447, 152632.
da Silva et al. (2024)	Exploring Caenorhabditis elegans as Parkinson's Disease Model: Neurotoxins and Genetic Implications. Neurotox Res, 42(1): p. 11.
Donaher, S. E. and P. Van den Hurk (2023)	Ecotoxicology of the herbicide paraquat: effects on wildlife and knowledge gaps. Ecotoxicology 32(9): 1187-1199.
Doyle, J.M. and Croll, R.P. (2022)	A Critical Review of Zebrafish Models of Parkinson's Disease. Front Pharmacol, 13: p. 835827.
el-Abidin Salam et al. (1993)	The mutagenicity of Gramoxone (paraquat) on different eukaryotic systems. Mutat Res, 319, 89-101.
Gupta, S., Garg, N.K. and Shekhawat, K. (2022)	Regulation of Paraquat for wheat crop contamination. Environ Sci Pollut Res Int. 2022 Oct;29(47):70909-70920. doi: 10.1007/s11356-022-20816-8. Epub 2022 May 20. PMID: 35595893.
Huang, Y. et al. (2019)	Paraquat Degradation From Contaminated Environments: Current Achievements and Perspectives." Front Microbiol 10: 1754.
Muangphra, P., Kwankua, W. and Gooneratne, R. (2014)	Genotoxic effects of glyphosate or paraquat on earthworm coelomocytes. Environ Toxicol, 29, 612-620
Qian, H. et al. (2009)	Inhibitory effects of paraquat on photosynthesis and the response to oxidative stress in Chlorella vulgaris. Ecotoxicology 18(5):537–543.
Rathee, V. et al. (2024)	Effective attenuation of Paraquat induced oxidative stress and Genotoxicity in testicular germ cells by vitamin E in Caprines. Toxicol Res (Camb), 13, tfae153
Silva, A.M. et al. (2019)	Ginkgo biloba L. Leaf Extract Protects HepG2 Cells Against Paraquat-Induced Oxidative DNA Damage. Plants (Basel), 8.
Snapshot of Australian Agriculture	https://www.agriculture.gov.au/abares/products/insights/snapshot-of-australian-agriculture
Stuart, A.M. et al, (2023)	Agriculture without paraquat is feasible without loss of productivity—lessons learned from phasing out a highly hazardous herbicide. Environ Sci Pollut Res Int. Jan 9;30(7):16984–17008.
Tagun, R. and Boxall, A. (2018)	The Response of Lemna minor to Mixtures of Pesticides That Are Commonly Used in Thailand. Bull Environ Contam Toxicol 100(4):516–523.
Walsh, A. and Kingwell, R. (2021)	Economic implications of the loss of glyphosate and paraquat on Australian mixed enterprise farms. Agricultural Systems. Online from 25 June 2021.

Author (Date)	Title (Comment)
Yang, W. et al. (2020)	Current and projected future economic burden of Parkinson's disease in the U.S. NPJ Parkinsons Dis, . 6: p. 15.

Table A3: Cited references presenting, reviews, opinions, editorials, commentary or clinical opinions or advice

Author (Date)	Title (Comment)
Ben-Shlomo, Y. et al. (2024)	The epidemiology of Parkinson's disease. Lancet. 403 (10423): 283-92.
Berg, D. et al. (2022)	Path to Parkinson Disease Prevention: Conclusion and Outlook. Neurology, 99 (7 Suppl 1): p. 76-83. doi: 10.1212/WNL.0000000000200793. PMID: 35970586.
Berry et al. (2010)	Paraquat and Parkinson's disease. Cell Death & Differentiation 17, pages 1115–1125.
Blanco-Ayala, T., Andérica-Romero, A.C. and Pedraza-Chaverri, J. (2014)	New insights into antioxidant strategies against paraquat toxicity. Free Radical Research 48(6):623–640.
Breckenridge, C.B. et al. (2016)	Association between Parkinson's Disease and Cigarette Smoking, Rural Living, Well-Water Consumption, Farming and Pesticide Use: Systematic Review and Meta-Analysis
Calne, D.B. and Langston, J.W. (1983)	Aetiology of Parkinson's disease. Lancet 2, 1457-1459.
Tanner. C.M. and Ostrem, J.L. (2024)	Parkinson's Disease. New England Journal of Medicine Aug 1;391(5):442-452. doi: 10.1056/NEJMra2401857
Clark et al. (1966)	The toxicity of paraquat.
Darweesh, S.K.L. et al. (2022)	Exposure to Pesticides Predicts Prodromal Feature of Parkinson's Disease: Public Health Implications. Mov Disord. 2022; 37(5): 883-5.
Darweesh, S.K., Vermeulen, R., and Bloem, B. (2024)	Paraquat and Parkinson's Disease: has the burden of proof shifted? International Journal of Epidemiology. Volume 53, issue 5, October 2024.
Day, J.O. and Mullin, S. (2021)	The Genetics of Parkinson's Disease and Implications for Clinical Practice" Genes 12, no. 7: 1006.https://doi.org/10.3390/genes12071006
Dorsey, E.R. and Bloem, B.R. (2024)	Parkinson's Disease Is Predominantly an Environmental Disease. J Parkinsons Dis 14, 451-465.
Dorsey, E.R. and Bloem, B.R. (2018)	The Parkinson Pandemic- A Call to Action. JAMA Neurol 75, 9-10.
Dorsey, E.R. et al. (2024)	The Body, the Brain, the Environment, and Parkinson's Disease. J Parkinsons Dis. 14(3):363-81.

Author (Date)	Title (Comment)
Dorsey, E.R. and Ray, A. (2023)	Paraquat, Parkinson's Disease, and Agnotology. Mov Disord. 38(6):949-52.
Dorsey, E.R. et al. (2018)	The Emerging Evidence of the Parkinson Pandemic. Journal of Parkinson's disease, 8(s1), S3–S8. https://doi.org/10.3233/JPD-181474
Flafel, H.M. et al. (2024)	Unveiling the hazards: comprehensive assessment of paraquat herbicide's toxicity and health effects. Euro-Mediterranean Journal for Environmental Integration.
Gawarammana, I.B. and Buckley, N.A. (2011)	Medical management of paraquat ingestion. Br J Clin Pharmacol 2011;72: 745-57.
Gonzalez-Hunt, C.P. and Sanders, L.H. (2021)	DNA damage and repair in Parkinson's disease: recent advances and new opportunities. J Neurosci Res, 99, 180-189.
Gunnarsson, L.G. and Bodin, L. (2017)	Parkinson's disease and occupational exposures: a systematic literature review and meta-analyses. Scand J Work Environ Health 43, 197-209 (2017).
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Jeppesen, D.K., Bohr, V.A. and Stevnsner, T. (2011)	DNA repair deficiency in neurodegeneration. Prog Neurobiol, 94, 166-200.
Kamel, F. (2013)	Paths from pesticides to Parkinsons." Science Vol 341, Issue 6147 pp 722-723. https://www.science.org/doi/10.1126/science.1243619
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Kulcsarova, K. et al. (2023)	Pesticides and the Microbiome-Gut-Brain Axis: Convergent Pathways in the Pathogenesis of Parkinson's Disease. J Parkinsons Dis. 13(7):1079-106.
Li, Y.L. et al. (2022)	Decoding the Role of Familial Parkinson's Disease-Related Genes in DNA Damage and Repair. Aging Dis, 13, 1405-1412.
Lin, X. et al. (2020)	Contributions of DNA Damage to Alzheimer's Disease. Int J Mol Sci, 21.
Madabhushi, R., Pan, L. and Tsai, L.H. (2014)	DNA damage and its links to neurodegeneration. Neuron, 83, 266-282.

Author (Date)	Title (Comment)
Navneet, A., Wadhera, S and Dhibar, P.D. (2021)	"Paraquat Poisoning: 'What we do and do not know.'." J Clin Toxicol. S 19 (2021).
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Sharma et al. (2024)	Paraquat (herbicide) as a cause of Parkinson's Disease. Parkinsonism Relat Disord. Feb:119:105932.
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Sun, Y. et al. (2020)	The role of DNA damage response in amyotrophic lateral sclerosis. Essays Biochem, 64, 847-861.
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Tangamornsuksan, W. et al. (2019)	Paraquat exposure and Parkinson's disease: A systematic review and metaanalysis. Arch Environ Occup Health 74, 225-238.
Utyasheva, L., Prabath Amarasinghe, Michael Eddleston et al. (2024)	Paraquat at 63
Vaccari, C. et al. (2019)	Paraquat and Parkinson's disease: a systematic review and meta-analysis of observational studies. J Toxicol Environ Health B Crit Rev. 22(5-6):172-202.
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Wang, H. et al. (2021)	DNA Damage-Induced Neurodegeneration in Accelerated Ageing and Alzheimer's Disease. Int J Mol Sci, 22.

Author (Date)	Title (Comment)
Wang, Z.X. et al. (2023)	DNA Damage-Mediated Neurotoxicity in Parkinson's Disease. Int J Mol Sci, 24.
Welch, G. and Tsai, L.H. (2022)	Mechanisms of DNA damage-mediated neurotoxicity in neurodegenerative disease. EMBO Rep, 23, e54217.

Table A4: Cited references previously available for screening or reviewed by the APVMA for the 2016 chemical review of paraquat

Author (Date)	Title (Comment)
Ali, S. et al. (1996)	Paraquat induced DNA damage by reactive oxygen species. Biochem Mol Biol Int, 39, 63-67.
Alizadeh et al. (2002)	Paraquat induced oxidative stress, DNA damage, and cytotoxicity in lymphocytes. Heliyon, 8, e09895.
Costello, S. et al. (2009)	Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the Central Valley of California.
Dawson, A.H. et al. (2010)	Acute human lethal toxicity of agricultural pesticides: a prospective cohort study.
Dhillon, A.S. et al. (2008)	Pesticide/environmental exposures and Parkinson's disease in East Texas.
Dial, C.A. and Dial, N.A. (1987)	Effects of paraquat on reproduction and mortality in 2 generations of mice. Arch Environ Contam Toxicol, 16, 759-764.
D'Souza et al. (2006)	Dermal exposure to the herbicide-paraquat results in genotoxic and cytotoxic damage to germ cells in the male rat.
Goldman, S.M. et al. (2012)	Genetic modification of the association of paraquat and Parkinson's disease. Mov Disord, 2012. 27(13): p. 1652-8.
Hausburg et al. (2005)	Effects of paraquat on development of preimplantation embryos in vivo and in vitro.
Kamel, F. et al. (2014)	Dietary fat intake, pesticide use, and Parkinson's disease. Parkinsonism Relat Disord, 2014. 20(1): p. 82-7.
Liou, H. et al. (1997)	Environmental risk factors and Parkinson's disease: a case-control study in Taiwan. Neurology 48(6): 1583-1588.
McCormack et al. (2005)	Role of oxidative stress in paraquat-induced dopaminergic cell degeneration. J Neurochem 93, 1030-1037
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Mamane, A. et al. (2015)	Occupational exposure to pesticides and respiratory health. Eur Respir Rev 24: 306-19
Manning-Bog et al. (2002)	The herbicide paraquat causes up-regulation and aggregation of alpha-synuclein in mice: paraquat and alpha synuclein.

Author (Date)	Title (Comment)
Mehdi, S.H. and Qamar, A. (2002)	Paraquat-induced ultrastructural changes and DNA damage in the nervous system is mediated via oxidative-stress-induced cytotoxicity in Drosophila melanogaster. Toxicol Sci, 134, 355-365.
Menegon, A. et al. (1998)	Parkinson's disease, pesticides, and glutathione transferase polymorphisms
Minnema et al. (2014)	Dietary administration of paraquat for 13 weeks does not result in a loss of dopaminergic neurons in the substantia nigra of C57BL/6J mice.
Ortiz et al. (2000)	Genotoxicity of paraquat: micronuclei induced in bone marrow and peripheral blood are inhibited by melatonin
Pasi, A. et al. (1974)	Assessment of the mutagenic properties of diquat and paraquat in the murine dominant lethal test. Mutat Res, 26, 171-175.
Peng et al. (2004)	The Herbicide Paraquat Induces Dopaminergic Nigral Apoptosis through Sustained Activation of the JNK Pathway,
Peter, B. et al. (1992)	Role of lipidperoxidation and DNA damage in paraquat toxicity and the interaction of paraquatwith ionizing radiation. Biochem Pharmacol, 43, 705-715.
Peters, C.M. et al (2006).	Prevalence of Parkinson's disease in metropolitan and rural Queensland: a general practice survey. J Clin Neurosci, 13(3), 343-348.http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16540321
Petrovska, H. and Dusinska, M. (1999)	Oxidative DNA damage in human cells induced by paraquat. Altern Lab Anim, 27, 387-395.
Rojo et al. (2007)	Chronic inhalation of rotenone or paraquat does not induce Parkinson's disease symptoms in mice or rats.
Ross, W.E., Block, E.R. and Chang, R.Y. (1979)	Paraquat-induced DNA damage in mammalian cells. Biochem Biophys Res Commun, 91, 1302-1308.17.
Senator, A. et al. (2004)	Prion protein protects against DNA damage induced by paraquat in cultured cells. FreeRadic Biol Med, 37, 1224-1230.
Shimizu et al. (2001)	Carrier-mediated processes in blood--brain barrier penetration and neural uptake of paraquat. Brain Res 906, 135-142 (2001).
Tanner, C.M. et al. (2011)	Rotenone, paraquat, and Parkinson's disease. Environmental health perspectives, 119(6), 866–872.
Tanner, C.M. et al. (2009)	Occupation and risk of parkinsonism: a multicenter case-control study. Arch Neurol, 2009. 66(9): p. 1106-13.
Todorovic, M. et al. (2015)	Comprehensive Assessment of Genetic Sequence Variants in the Antioxidant 'Master Regulator' Nrf2 in Idiopathic Parkinson's Disease. Plos One, 10(5).
Tokunaga et al. (1997)	Determination of 8-hydroxy-deoxyguanosine formation in rat organs: assessment of paraquat evoked oxidative DNA damage

Author (Date)	Title (Comment)
Tomenson, J.A. and Campbell, C. (2011)	Mortality from Parkinson's disease and other causes among a workforce manufacturing paraquat: a retrospective cohort study. BMJ Open 1, e000283 (2011).
Wang et al. (2011)	Parkinson's disease risk from ambient exposure to pesticides
Wills et al. (2012)	Paraquat, but not maneb, induces synucleinopathy and tauopathy in striata of mice through inhibition of proteasomal and autophagic pathways

Table A5: Paraquat not included in the study protocol, reference presents data on deliberate self-harm (known effects), in vitro studies, author flagged as unreliable

Author (Date)	Title (Comment)
Barron, C.J. et al. (2022)	In vitro cytotoxicity and genotoxicity of single and combined pesticides used by Bolivian farmers. Environ Mol Mutagen, 63, 4-17.
Braun et al. (2024)	Neurotoxic mixture effects of chemicals extracted from blood of pregnant women. Science 386,301-309.DOI:10.1126/science.adq0336
Dusinska, M. et al (2021)	Responses of alveolar macrophages and epithelial type II cells to oxidative DNA damage caused by paraquat. Carcinogenesis, 19, 809-812.
Gu, Q. et al. (2024)	Unravelling paraquat-induced toxicity on mouse neural stem cells: Dose-response metabolomics insights and identification of sensitive biomarkers for risk assessment. Environmental pollution (Barking, Essex : 1987), 355, 124211. https://doi.org/10.1016/j.envpol.2024.124211
Eddleston, M. and Phillips, M.R. (2004)	"Self poisoning with pesticides." BMJ 328(7430): 42-44
Eun Shil Cha et al. (2016)	Impact of paraquat regulation on suicide in South Korea, International Journal of Epidemiology, Volume 45, Issue 2, April 2016, Pages 470–479, https://doi.org/10.1093/ije/dyv304
Figueiredo, D. et al. (2022)	Pesticides in doormat and floor dust from homes close to treated fields: Spatio-temporal variance and determinants of occurrence and concentrations. Environ Pollut. 301:119024.
Goldman, S.M. et al. (2023)	Risk of Parkinson disease among service members at Marine Corps Base Camp Lejune. JAMA Neurology 2023, 80, 673-681.
Paul, K.C. et al. (2023)	A pesticide and iPSC dopaminergic neuron screen identifies and classifies Parkinson-relevant pesticides. Nat Commun. 2023;14(1):2803.
Straw, E.A. et al. (2023)	Self-reported assessment of compliance with pesticide rules, Ecotoxicology and Environmental Safety, Volume 254, 2023, https://doi.org/10.1016/j.ecoenv.2023.114692.
Gunnell, D. et al. (2007)	"The global distribution of fatal pesticide self-poisoning: systematic review."
Stephenson, L. et al. (2023)	Features of fatal pesticide ingestion in South Australia. Med Sci Law. Sep 4:258024231197914. doi: 10.1177/00258024231197914. Epub ahead of print. PMID: 37661826.

Author (Date)	Title (Comment)
Thiruchelvam, M. et al. (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 (2003).
Yamamoto, H.A. and Mohanan, P.V. (2001)	Effects of melatonin on paraquat or ultraviolet light exposure-induced DNA damage. J Pineal Res, 31, 308-313.

* Dr Mona Thiruchelvam was found by the US Office of Research Integrity (ORI) to have engaged in research misconduct, by falsifying and fabricating data in a paper investigating an aspect of paraquat and Parkinson's disease. Papers including Dr Thiruchelvam as a co-author are therefore of uncertain scientific reliability.

Other documents and references cited in public submissions

A number of documents were submitted by registrants and industry bodies that did not contain paraquat specific, new or original data, or information impacting HHRA and were not therefore reviewed in detail, Table A6. Where these documents commented on proposed outcomes of the paraquat review these have been considered and addressed in the responses to the public submissions.

Table A6: Documents provided by registrants and industry bodies

Submitted by	Citation
Syngenta	Duncan, R (Study Author) The acute oral toxicity of Diquat weed Killer (SX1085). SOCAL 1396, 13 Dec 1985. Chevron Environmental Health Centre. Richmond, California.
	EFSA, 2015, CONCLUSION ON PESTICIDE PEER REVIEW, Conclusion on the peer review of the pesticide risk assessment of the active substance diquat. EFSA Journal 2015;13(11):4308
	FERA, 2010, Collection and evaluation of relevant information on crop interception for the revision of the Guidance Document on Persistence in Soil. Prepared by Wendy van Beinum and Sabine Beulke, The Food and Environment Research Agency, Sand Hutton YO41 1LZ York UK.
	Gutierrez-Expositi, C., Russ, A., Sainz-Elipe, R., Wolf, C., and Krageten, S. (2023). Energy Content, Moisture Content, and Energy Assimilation Efficiency by Birds and Mammals of Oil-Containing Seeds and Implications for Seed Treatment Risk Assessments for Birds and Mammals. Environmental Toxicology and Chemistry, Volume 43, Number 9, pp. 2080–2085
	Johnson I, 2003. Diquat 200 g/l SL formulation (A1412A): Acute Oral Toxicity Study In The Rat –Up-And-Down-Procedure. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No. CTL/AR7412/REGULATORY/REPORT, 6 August 2003. Unpublished. (Syngenta File No. PP901/1383)
	McCall, J. (Study Director) ICI Central Toxicology Laboratory. 1990, Diquat Dibromide: Acute Oral Toxicity to the rat. CTL Study number AR5024, Document reference; 61050164
	Pooles, A. (Study Director). Diquat SL (A1412H)- Acute Oral Toxicity in the Rat – Up-and-Down-Procedure. Safepharm Laboratories Limited, Shardlow Business Park, Shardlow, Derbyshire, DE72 2GD, UK. Report Number: 2364/0323. Task Number: T005408-07

Submitted by	Citation
	Syngenta, Submission to APVMA regarding paraquat/diquat review, specifically addressing phase in timelines of new labels.
	US Fish and Wildlife Service, 1975, Lethal dietary toxicities of environmental pollutants to birds. Prepared by Hill, E., Heath, R., Spann, J., and Williams, J. Special Scientific Report – Wildlife No. 191.
	Washington State Department of Ecology, Nov 2002, Final Risk Assessment For Diquat Bromide, The Water Quality Program
Cotton Australia	AgEcon. Oct 2024. Economic analysis of paraquat and diquat loss in cotton production.
	Australian Environmental Agency Pty Ltd. Oct 2024. Consideration of APVMA Technical Review Reports for Paraquat and Diquat with specific consideration of modelling outcomes for birds and mammals

Background, guidance and reference documents

The following documents cited in public submissions provided background or regulatory guidance, or expressed the view of a specific organisation, but did not contain new, original data directly impacting the HHRA and were therefore screened but not reviewed in detail for this aspect of the chemical review (but may be relevant for other review areas). Documents expressing the view of a specific organisation were addressed as appropriate in responses to public submissions.

Table A7: Background, position, guidance and reference documents

Submitted by	Citation
South Australia State government guidance	Understanding product labels #1, Responsible chemical use, SA Government, Adelaide and Mount Lofty Ranges Natural Resources Management Board, undated. https://cdn.environment.sa.gov.au/landscape/docs/hf/responsible-chemical-use-product-labels1-fact.pdf
APVMA guidance	APVMA Agricultural Labelling Code Review (2021) https://www.apvma.gov.au/news-and-publications/news/agricultural-labelling-code-review
APVMA guidance	APVMA Compliance Plan (2022-2023). https://www.apvma.gov.au/sites/default/files/publication/49591-apvma_2022-23_compliance_plan.pdf
APVMA guidance	APVMA Understanding Pesticide Chemical Labels (2020). https://www.apvma.gov.au/sites/default/files/publication/67431-understanding_labels_booklet_2020.pdf
APVMA guidance	Correction of ABC Reporting (2024) https://www.apvma.gov.au/news-and-publications/media-releases/correction-abc-reporting-apvmas-proposed-regulatory-decision-paraquat

Submitted by	Citation
APVMA review reports	Australian Pesticides and Veterinary Medicines Authority | Strategic Review Report | Clayton Utz, July 2023. https://www.agriculture.gov.au/sites/default/files/documents/APVMA%20-%20Strategic%20Review%20Report.PDF
APVMA review reports	Future structure and governance arrangements for the APVMA, Ken Matthews, AO, 20 October 2023. https://www.agriculture.gov.au/sites/default/files/documents/apvma-rapid-evaluation-final-report.pdf
CDC factsheet on paraquat exposure	CDC Emergency Preparedness and Response to Paraquat accessed on the web on 16/9/2024 at https://emergency.cdc.gov/agent/paraquat/basics/facts.asp
Deloitte report	Deloitte Access Economics Living with Parkinson's Disease: An Updated Economic Analysis 2014. 2015. https://www.parkinsonstasmania.org.au/deloitte-access-economics-report
FAO standard	FAO specifications and evaluations for agricultural pesticides paraquat dichloride. 1 1,1'-dimethyl-4,4'-bipyridinium dichloride. https://openknowledge.fao.org/server/api/core/bitstreams/05621e49-cae8-4985-865d-9be6f1c6be40/content
Farm practices survey ABARES	Coelli, R 2021, Natural Resource Management and Drought Resilience – survey of farm practices, ABARES research report 21.12, Canberra, https://doi.org/10.25814/99n0-7q92 https://daff.ent.sirsidynix.net.au/client/en_AU/search/asset/1032761/0
News item	Australia's top neurologists call for chemical regulator to ban paraquat herbicide over links with Parkinson's disease. ABC regional investigations, Monday 28/10/24. https://www.abc.net.au/news/2024-10-28/neurologistsdoctors-call-for-paraquat-ban-over-parkinsons-link/104502044
News item	Rosic, N, et.al. 'One sip can kill': why a highly toxic herbicide should be banned in Australia, The Conversation, May 19, 2021, https://theconversation.com/one-sip-can-kill-why-a-highly-toxic-herbicide-should-be-banned-inaustralia-159333
OS Regulatory decision	Environmental Protection Agency, Paraquat Dichloride: Interim Registration Review Decision Case Number 0262" (Washington, D.C.: Environmental Protection Agency, July 13, 2021), https://www.regulations.gov/document/EPA-HQ-OPP-205-0307.
OS Regulatory decision	Reregistration Eligibility Decision (RED) Paraquat Dichloride, United States Prevention, Pesticides EPA 738-F-96-018 Environmental Protection And Toxic Substances, August 1997, Agency (7508W) https://archive.epa.gov/pesticides/reregistration/web/pdf/0262red.pdf
PAN list	Highly Hazardous Pesticides (2009 – link to the slightly updated 2021 version) https://pan-international.org/wp-content/uploads/PAN_HHP_List.pdf
PAN article	PAN International's Dirty Dozen (1985) https://panap.net/timeline/1985/
Product MSDS	AIRR Apparent Pty Ltd, 'Safety Data Sheet - Apparent Paraquat 250 Herbicide,' 1 June 2021, retrieved from: https://apparentag.com.au/documents/msds/66103_APPARENT_PARAQUAT_250_HERBICIDE_MSDS_1.pdf
Report commissioned by Parkinsons Australia	Mellick, G. Ecosystem of Parkinson's in Australia Project Report. 2024. https://www.parkinsons.org.au/reports-and-submissions/

Submitted by	Citation
Parkinson's Australia	Ban paraquat in Australia. https://www.parkinsons.org.au/wpcontent/uploads/2024/10/APVMA_submission_ParkinsonsAustralia_2024.pdf
Fight Parkinson's	Parkinson's Prevalence (2022) retrieved from: https://www.fightparkinsons.org.au/news-resources/parkinsons-prevalence/
Australian Animal Poison Hotline webpage	accessed on 16/9/2024 https://animalpoisons.com.au/news/paraquat#:~:text=In%20Australia%20and%20New%20Zealand,is%20recommended%20in%20all%20cases.

Appendix 2

Appendix 2: Review of new studies

General toxicology studies

Chen, Q., Zhang, X., Zhao, J.Y., Lu, X.N., Zheng, P.S. and Xue, X. (2017): Oxidative damage of the male reproductive system induced by paraquat. J Biochem MolToxicol, 31.

Study objectives and design. In order to investigate the effects of paraquat (PQ) on male fertility, male rats were administered paraquat at 0, 0.5, 2 and 8 mg/kg bw/day by gavage for 8 weeks. The animals were examined for clinical signs daily, and body weights and food consumption were recorded every 3 days. At the end of the treatment period the testes and epididymis were dissected, removed, and weighed. The cauda epididymides were excised, sperm removed, and the sperm count, viability, morphology and motility were analysed. The tissues of the right testis were used to examine the levels of apoptosis, cytochrome c expression, and oxidative stress.

Results. Body weights were unaffected at all doses. Decreased absolute testes weights and increased absolute epididymis weights were observed in treated groups compared to controls but there was no clear dose response relationship. After correcting for body weight, the testis weight was significantly decreased only at 2 and 8 mg/kg bw/day and epididymis weights were increased only at 8 mg/kg bw/day. Paraquat decreased sperm number (statistically significant) in all treated groups and sperm viability was significantly decreased at 2 and 8 mg/kg bw/day.

Sperm motility was only significantly affected at 8 mg/kg bw/day. The percentage of head, tail, and multiple sperm abnormalities were significantly increased at 2 and 8 mg/kg bw/day. When these parameters were combined, total sperm abnormalities count was significantly elevated above controls in all treatment groups.

Statistically significantly dose-dependent decreases in superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) levels were observed at 2 and 8 mg/kg bw/day accompanied by a significant, dose-dependent increase in lipid peroxidation in these groups.

An increase in testis germ cell apoptosis was observed at 2 and 8 mg/kg bw/day accompanied by a significant dose-dependent increase in caspase-3 and caspase-9 activity and cytochrome c expression. Caspase-3 and cytochrome c expression were also significantly elevated at 0.5 mg/kg bw/day, but the magnitude was lower compared to the other treatment groups and was not accompanied by significant germ cell apoptosis.

Conclusions. Sperm abnormalities and testis tissue effects were observed in all dose groups, though the magnitude of change in these effects were small at 0.5 mg/kg bw/day, generally not statistically significant, and the dose response at 0.5 mg/kg bw/day was very weak.

The US EPA review of this study (US EPA 2019d) concluded:

> **"Absolute testis and epididymis weight in the 0.5 mg/kg/day group were <13% different from controls and the changes in weight were not significant after normalizing for body weight. Sperm number in the 0.5 mg/kg/day treatment group decreased by <10% relative to controls, the increase in total percentage of abnormal sperm was marginal, and no significant impact on sperm motility or viability was observed. Testis tissue from rats in this treatment group also did not exhibit evidence of oxidative stress or apoptosis. Given the low magnitude of the change from controls,**

> **none of the reproductive effects observed in rats from the 0.5 mg/kg/day treatment group were indicative of an adverse response to treatment. Rats from the 2 and 8 mg/kg/day treatment groups exhibited a wider array of changes in the male reproductive tissues that were significantly different from the controls and generally of higher magnitude relative to the 0.5 mg/kg/day group".**

The EPA conclusions are reasonable and appropriate. The data presented in this study is new and is relevant to the hazard characterisation of paraquat. The existing Points of Departure (POD), and human health reference values derived from them, are adequately protective and no changes to these are required.

Sun, Y.L., Wang, X.L., Yang, L.L., Ge, Z.J., Zhao, Y., Luo, S.M., Shen, W., Sun, Q.Y. and Yin, S. (2021): Paraquat Reduces the Female Fertility by Impairing the Oocyte Maturation in Mice. Front Cell Dev Biol, 8, 631104.

Study objectives and design. The authors report a study of the effects of paraquat on female fertility. Female CD-1 mice (source not stated) in groups of 18-20 were administered 10 mg/kg bw/day paraquat (source and purity not stated) by gavage for 21 days. The control groups were administered normal saline. Oocytes were collected from control and paraquat treated mice and cultured to the MI or MII stage for examination of α-tubulin, centromere formation, distribution of mitochondria, production of intracellular reactive oxygen species (ROS), and early apoptosis. Female mice were mated and the next morning oviductal ampullae were broken by syringe to release the cumulus oocyte complexes (COCs). Cumulus cells of COCs were processed to get cumulus-free oocytes and cultured until blastocyst stage. Other mated female groups (number of females not specified) were allowed to deliver and rear pups for examination of effects on the pups.

The study methodology is poorly documented particularly the purity of the paraquat and the disposition of experimental animals (numbers of treated and control animals used for each investigation group, total animals used, whether a single control/treatment cohort was used for each investigation or some cohorts were used for more than one investigation), and only one relatively high dose was administered. The study provides qualitative data only.

Paraquat treatment produced a range of statistically significant effects on maturing oocytes including:

- Reduced average ovary weight,
- Decreased polar body extrusion (a measure of oocyte maturation)
- Decreased MII oocytes after 21 days paraquat administration
- Reduced rates of two-cell embryos and blastocysts after mating
- Decreased average number of pups per litter and decreased pup weight
- Increased incidence of aberrant spindle formation and misaligned chromosomes
- Increased clustering of mitochondria
- An increased level of ROS
- Increased early apoptosis
- Decreased level of histone methylation.

Paraquat treatment of the females however had no effects on the rates of IVF (59.33%, n = 84, control vs. 55.8 %, n = 114, PQ treated group; P > 0.05).

Conclusions. Overall, this study appears to demonstrate that exposure of female mice to high oral doses of paraquat significantly affected a range of fertility parameters and leads to reduced numbers of pups per litter and reduced pup weights. Previously assessed studies (Dial & Dial 1987, Hausburg et al. 2005) of paraquat administered to mice (in the diet continuously and a single intraperitoneal (ip) dose at ovulation respectively) found a reduction in the number of dams pregnant likely due to pre-implantation or early post implantation effects, but no effect on the pups per litter or pup weights. In the Dial & Dial study effects on number of dams pregnant was only seen at the top dietary dose equivalent to approximately 21 mg/kg bw/day with no effects at approximately 15 mg/kg bw/day. The broad range of reproductive effects observed in the Sun et al. study at the lower dose of 10 mg/kg bw/day by gavage compared to the absence of any effects at 15 mg/kg bw/day dose administered in the diet in the Dial and Dial is notable. The use of a single dose in the Sun et al. study precludes investigation of dose effect relationships. Toxicokinetic differences arising from gavage versus dietary administration may have a bearing on the discordant observations but is speculative given the absence of toxicokinetic data for these studies.

Collectively these 3 studies indicate paraquat effects on oocyte and/or early embryo development in mice at comparatively high doses is likely. Unfortunately, none of the studies individually or collectively provide a robust basis for quantitative assessment due to design deficiencies in the Sun et al. and Hausberg studies (single doses) and variations in and between studies (dose frequency, duration, and route of administration). Hausberg et al. (2005) used a single ip dose of 30 mg/kg bw of paraquat on the day of ovulation. Dial and Dial (1987) employed a more traditional 2 generation reproduction study design with paraquat administered in the diet at 0, 45, 90, or 125 mg paraquat cation/kg feed daily from pairing through to weaning of the pups. The dietary concentrations were equivalent to approximately 0, 7, 15, 21 mg/kg bw/day. The study employed a limited range of OECD Guideline parameters (no data on pre and post implantation losses was reported for example).

Multi-generation dietary studies in rats (Igarashi 1980, Lindsay et al. 1982a,b,) where administration of paraquat begins 10 weeks prior to mating, found no effects on reproductive performance, including the percent of mated dams delivering a litter and the number of pups per litter, or the development of the reproductive organs of rats, when administered at dietary levels up to 15 mg/kg bw/d for 3 generations in one study and 20 mg/kg bw/day for 2 generations in a second study. A reduction in pup body weights at birth was observed in the 2-generation study (Lindsay et al 1982a,b) at 14.4 mg/kg bw/day but not at the NOAEL of 7.2 mg/kg bw/day. In the 3 generation study the NOAEL for pups was set by effects in the lungs (Igarashi 1980).

Animal studies investigating paraquat and Parkinson's disease

Anderson, T., Merrill, A., Eckard, M., Marvin. E., Conrad, K., Welle, K., Oberdörster, G., Sobolewski, M., and Cory-Slechta, D. (2021): Paraquat Inhalation, a Translationally Relevant Route of Exposure: Disposition to the Brain and Male-Specific Olfactory Impairment in Mice. Toxicol Sci 180, 175-185 (2021).

Study objectives and design. The study was conducted to investigate the disposition and toxicity of paraquat in mice following inhalational exposure. C57BL/6J mice (Jackson Labs) were assigned to groups of 8–9/treatment/behaviour group for males and 5/treatment/behaviour group for females. Paraquat from Sigma-Aldrich was > 98.9% pure.

Male and female C57BL/6J mice were exposed, whole body, to either HEPA-filtered air (99.9% effective) or aerosolised/vaporised paraquat (130 µg/m3) for 4 hrs/day, 5 days per week for 28 days and then retained without further paraquat exposure for up to 303 days. Subsets of male mice were sacrificed on experimental days 10, 28, 56, and 303 and brain, lung, and kidney were collected for paraquat quantitation. Only males were used for tissue level analysis as a previous study they cited had established that there is no difference in toxicokinetic profile of paraquat between sexes.

Bronchoalveolar lavage fluid was collected from a subset of males immediately following the end of exposure and at 303 days to assess the acute and long-term effects of paraquat inhalation on the lung. Olfactory discrimination training/testing began on experimental day 163, 135 days after the end of exposure. All mice were weighed weekly.

Results. No significant treatment-related changes in body weight were observed in either sex. There was no statistically significant difference in lactate dehydrogenase (LDH) activity of the bronchoalveolar lavage fluid between air control and paraquat-exposed males immediately following the end of exposure nor was there any difference in the lavage fluid protein content. Histological analysis of the lungs at the end of the experiment did not identify any signs of fibrosis. Paraquat-exposed males made significantly more incorrect choices in the olfactory discrimination test only at one concentration of scent. No effect was seen in females. Tissue levels of paraquat were highest in the lungs (20.8 ng/g) and olfactory bulb (5.4 ng/g) with very low levels in other areas of the brain (all <1.1 ng/g) including the striatum.

Conclusions. This study exposed mice to paraquat in a form and in a manner that is not representative of occupational use patterns, bystander or consumer exposures. The paraquat was delivered to the mice as dried aerosolised particulates. Application in agricultural settings is as a comparatively coarse wet spray applied from a tractor mounted, calibrated spray with workers wearing personal protective equipment. These differences substantially alter likely achieved air concentrations and achievable exposures. As the authors themselves note:

> **"It is difficult to compare exposure parameters from this study directly to what workers may be exposed to in the field. Few studies have examined the concentration of airborne paraquat in the field during spraying. Seiber and Woodrow (1981) reported that paraquat was present at a range of 4.31–10.7 µg/m3, 1m downwind of the field, during spraying. Although the highest values detected on the perimeter of the field are about 10-fold lower than the concentration used in this study, workers within the field may experience higher levels".**

Animals were not restrained from grooming, and a substantial proportion of the systemic exposure is likely to have been oral. Notably paraquat levels were highest in the lungs and a substantial proportion of brain levels measured may have resulted through absorption across the lungs. Nonetheless the achieved brain levels are substantially below those achieved in the Smeyne et al. (2016) study, reviewed later in this report. Following twice weekly ip injection of 10 mg/kg bw of paraquat for 3 weeks, the brain concentration in the Smeyne study was 540 ng/g of tissue, 2-3 orders of magnitude higher than observed here with no evidence of damage to dopaminergic neurons in the substantia nigra pars compact (SNpc).

The study does not alter the overall conclusion that exposure to paraquat via the nose leads to low, localised, levels of paraquat in the olfactory bulb that does not distribute to other areas of the brain. Under conditions of this study paraquat levels achieved in brain regions were 2 to 3 orders of magnitude below those demonstrated to produce no damage to dopaminergic neurons of the SNpc in mice.

Jiao Y, Lu L, Williams RW, Smeyne RJ (2012): Genetic Dissection of Strain Dependent Paraquat-induced Neurodegeneration in the Substantia Nigra Pars Compacta. PLoS ONE 7(1): e29447. https://doi.org/10.1371/journal.pone.0029447

Study objectives and design. This study was primarily conducted to investigate the genetic basis for strain specific sensitivity to MPTP induced effects on tissues of the SNpc of C57BL/6J and SWR/J mice (Jackson Laboratories). Male and female C57BL/6J and SWR/J mice were crossed by mating male C57BL/6J with female SWR/J and female C57BL/6J with male SWR/J stock. F1 hybrids were backcrossed to SWR/J to generate a set of 61 backcross (N2) progeny that were used to map chromosomal regions that harbor crucial gene variants that modulate risk, called quantitative trait loci (QTLs). The primary investigations of interest in the context of paraquat (Sigma analytical standard, assumed 98%+), however, were stereological investigations of the dopaminergic neurons in the SNpc to identify Parkinson's Disease (PD) like effects of paraquat.

Mice were given a total of 60 mg/kg of paraquat, using a dosage regimen of 10 mg/kg bw per week for 3 weeks administered by injection (presumably ip but not explicitly stated). All mice that survived the injection protocol were sacrificed one week after the final paraquat administration.

Brain tissue was perfused with saline and fixed in situ and prepared for histology. Immuno-histochemical methods were used to visualise tyrosine hydroxylase (TH) to identify dopamine neurons in the SNpc. Dopaminergic neurons in the SNpc were quantified using stereological techniques. Researchers performing histological and stereological analysis were not blinded to treatment.

A subsequent, greatly expanded, study (reviewed below) repeated this protocol (using mice with the same genetic characteristics) but with researchers from 2 independent labs performing histological and stereological assessment blinded to the treatment group of the brain sections being assessed, was unable to reproduce the findings reported in this study. Because the reported effects relevant to Parkinson's Disease (C57BL/6J mice reportedly lost, 50% of their SNpc DA neurons, whereas inbred Swiss-Webster (SWR/J) mice showed no loss), were not reproducible in the same laboratory using mice of the same genetic provenance (C57BL/6J), in a considerably better designed and conducted study protocol, the genetic analysis of sensitivity to the reported effects in the current study of paraquat are therefore not relevant and are not reported here.

Results. The authors report a decrease in SNpc dopamine neurons of approximately 50% in male C57BL/6J mice and approximately 10% in SWR/J mice and present histological photomicrographs to illustrate the difference between control and paraquat treated mice.

Comment. As discussed in the following consideration of the paper by Smeyne et al. (2016) failure to blind researchers assessing the histology and stereology of the brain sections is a substantive source of experimental bias. The findings as reported here were not reproducible by Smeyne et al. (2016), in the same laboratory, where investigators were blinded to treatment. The lead author of this paper (Jiao, Y) was also an author of the subsequent Smeyne paper.

Smeyne RJ, Breckenridge CB, Beck M, Jiao Y, Butt MT, Wolf JC, Zadory D, Minnema DJ, Sturgess NC, Travis KZ, Cook AR, Smith LL, and Botham PA. (2016): Assessment of the effects of MPTP and paraquat on dopaminergic neurons and microglia in the substantia nigra pars compacta of C57BL/6 mice. PLoS One. 11(10): e0164094

Study objectives and design. This study utilises 2 independent laboratories (WIL research laboratories & SJCRH[2]) for the bulk of the study and a number of specialised analytical laboratories for specific analyses. The lead author for this paper (Smeyne, R) was a co-author for the Jiao et al. (2012) paper discussed above and Jiao is a co-author for the current paper. The study was financially supported by a registrant.

As stated in the paper's introduction:

> **"The purpose of this study was to investigate the potential basis for differences in the effects of paraquat on the SNpc reported in different laboratories [Jiao et al. 2012, Breckenridge et al. (2013), Minnema et al. (2014)] by conducting controlled, multisite experiments that systematically varied paraquat dose and dose frequency, the source and age of the C57BL/6 male mice, animal housing conditions and stereological and neuropathological methods. The in-life phases of these studies were conducted in 2 laboratories. Stereology was performed by 2 investigators who were blinded to treatment, and the neuropathological evaluations were conducted by another investigator, also blinded to treatment."**

The study was designed to assess the effect of paraquat (Syngenta, 99.7% pure, Batch ID 550055) on the total number of TH+ neurons in the SNpc in male C57BL/6 mice (Jacksons Laboratories) of 2 ages at the time of dosing (9- or 16-weeks) with different dose levels and dose frequency (10 mg/kg bw x 6 doses, 20 mg/kg bw x 3 doses administered ip); via 2 different stereological assessment methods (design or model-based stereology[3]); and employing semi-quantitative neuro-histopathological assessments (TH+ staining reduction, neurodegeneration, apoptosis or gliosis). MPTP (Sigma, 100%, Lot #128K1549) was used as a positive control.

The stereology was performed at SJCRH and Experimental Pathology Laboratories, Inc (EPL, Sterling VA), while the qualitative neuropathology was performed at Tox Path Specialists LLC (TPS, Frederick MD).

Due to the large number of groups of mice evaluated, age effects were assessed by comparing 9-week-old paraquat and MPTP-treated animals (Experiment 1a) to 16-week-old mice (Experiment 1b). Sixteen-week-old mice in the WIL study (Experiment 1b) were also statistically compared to 16-week-old control mice in the study conducted at SJCRH (Experiment 2) and 16-week old paraquat and MPTP groups at WIL (Experiment 1b) were compared to the corresponding 16-week-old paraquat and MPTP groups at SJCRH (Experiment 2, TH+ stereology only).

Paraquat was administered by ip injection twice weekly at a dose of 10 mg paraquat (salt)/kg bw for 3 weeks (60 mg/kg bw total across 6 injections), or once weekly at 20 mg paraquat (salt)/kg bw for 3 weeks (60 mg/kg across 3 injections). Mice administered MPTP-HCl (19.4 mg/kg bw at WIL; 20 mg/kg bw at SJCRH) were injected ip at 2-

[2] St. Jude Children's Research Hospital, Dept. of Developmental Neurobiology, Memphis, TN (SJCRH)

[3] Dumelle et al. (2022). The design-based and model-based approaches rest on fundamentally different foundations. In the design-based approach, inference relies on random sampling. In the model-based approach, inference relies on distributional assumptions.

hour intervals for a total of 4 doses (total dose of 80 mg/kg). Control mice were administered 0.9% saline at the same volume as the paraquat mice.

Brain paraquat concentration was determined in 5 C57BL/6J mice at SJCRH 24 hours after the final (sixth) paraquat dose (10 mg/kg/dose). Brains, excluding olfactory bulbs, were removed homogenized and analysed for paraquat concentration using LC-MS/MS.

The total number of TH+ (DA) neurons in the SNpc were estimated using both Model-Based (2-D) and Design-Based (3-D) stereology on brain sections stained for TH+ dopaminergic neurons. All of the stereological evaluations were performed blinded to treatment group.

A block of the caudate/putamen section of the brain (all of the structures defined as basal ganglia including the striatum, globus pallidus, substantia nigra pars compact (SNpc), and subthalamic nuclei) was serial sectioned and stained for:

- Tyrosine hydroxylase (TH), an enzyme involved in the synthesis of DA, to identify dopaminergic neurons and their neuronal processes (axons, dendrites and synaptic terminals).
- Glial fibrillary acidic protein (GFAP) to selectively identify protein filaments unique to activated astrocytes.
- Ionised Calcium Binding Adaptor Molecule 1 (Iba-1), a protein expressed by activated microglia, to detect microglial cell activation.
- Amino Cupric Silver (AmCuAg) selectively stains the disintegrating elements of dead neuronal cell bodies and the neuronal processes (axons, dendrites and synaptic terminals).
- Caspase 3 is an enzyme that is expressed by cells during apoptotic cell death. The caspase 3 cleavage product was labelled to detect neurons that are in the processes of dying via an apoptotic mechanism. Only SNpc sections were evaluated for caspase 3.
- Thionine a general morphological stain to detect Nissl substance, revealing nuclear details within a variety of cell types.
- Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) to detect DNA fragmentation. When used in combination with cleaved caspase 3 immunoreactivity, it provides a sensitive indicator of apoptosis. Only sections of the SNpc were processed for TUNEL.

Results. A statistically significant reduction in body weight and food consumption was observed in mice treated with paraquat at 20 mg/kg/week for 3 weeks after the first dose, and less so after subsequent doses. There were minimal clinical signs and no effects on survival in either laboratory. MPTP-treated mice displayed hunched posture and hypoactivity after dosing and body weight was transiently reduced on the day of treatment. There were no effects of MPTP-treatment on survival in either laboratory.

In contrast to the neurotoxin MPTP, regardless of paraquat dose or dose frequency, the source of paraquat, the source, age or husbandry of the C57BL/6J male mice evaluated, and the stereological or neuropathological methods employed paraquat did not induce a loss of SNpc dopaminergic (DA) neurons, did not cause damage to DA axons/terminals or produce evidence of neuroinflammation. The results were robust and consistent among the 3 independent, blinded, investigators.

After 6 ip injections of 10 mg/kg bw paraquat (i.e. twice weekly for 3 weeks) the mean brain concentration of paraquat was 0.54 ± 0.03 µg/g tissue, at 24 hours after the final dose representing less than 0.03% of the total dose injected.

The brain tissue concentration reported for mice exposed to 6 doses of 10 mg ion/kg over 3 weeks (0.54 ng /mg tissue) exceeded the whole brain concentration predicted for 13 weeks of daily dietary exposure to 10.2 mg ion/kg/day in the same strain of mouse (0.42 ng/mg tissue; Minnema et al. 2014) using a physiologically based pharmacokinetic (PBPK) model. Although the comparison is imperfect it does demonstrate that ip exposure to a similar dose level at lower frequency and across a shorter exposure period results in greater paraquat accumulation in brain tissues compared to prolonged daily oral exposure. Levels of paraquat in brain were determined on homogenised tissue which incorporates paraquat associated with the lumen of blood vessels (ie outside the blood brain barrier) and is therefore likely to overestimate actual tissue levels to some, unknown, extent.

In supplementary data published with the main study the authors report a comprehensive review of 51 published studies (including the current study) on the effects of paraquat on SNpc dopaminergic neurons. Twenty-eight of these published studies performed stereological assessments (73 group comparisons) of which 47 (of the 73) did not blind the investigators with respect to treatment. In 94% of these comparisons (44 of 47), the mean number of TH+ neurons in the SNpc of paraquat treated mice were reduced (average 26.5% compared to control). For the 26 comparisons (of 73) where investigators were blinded 81% (i.e. 21 of 26) found no effect of paraquat. Aside from blinding, the most noticeable difference between studies showing a paraquat -induced cell loss and those that did not, was the observation that the average coefficient of variation (CV) among the “positive” studies (~ 6–7%) was statistically significantly less than the average CV observed in the negative studies (CV = 16.4%; $p < 0.0001$).

The authors observe that unlike most of the published literature, the current study used a relatively large number of replicates (n) per group and there was sufficient statistical power (0.994) to detect the average 26% reduction in the mean number of TH+ neurons reported in the statistically positive studies. No effect of paraquat treatment was observed.

Comments. This study in conjunction with those of Breckenridge et al. (2013, industry supported) which used ip dosing, and Minnema et al. (2014) [published version of the unpublished Beck 2012 regulatory study] which used oral dosing, raises substantial doubt regarding studies reporting damage to dopaminergic neurons following administration of paraquat. This study used 3 methods of assessment performed by 3 separate groups, each blinded to treatment for a series of neuropathological indices to evaluate 2 age groups and 2 sub-strains of male C57BL/6 mice, from 2 animal supply houses, housed under different conditions in 2 laboratories and administered paraquat at maximum tolerated doses. Paraquat did not induce any neuropathogenic effects.

Dwyer, Z., Rudyk, C., Farmer, K., Beauchamp, S., Shail, P., Derksen, A., Fortin, T., Ventura, KI., Torres, C., Ayoub, K. and Hayley, S. (2021): Characterizing the protracted neurobiological and neuroanatomical effects of paraquat in a murine model of Parkinson's disease. Neurobiol Ageing, 2021. 100: p. 11-21

Study objectives and design. The study investigated the neurodegenerative, inflammatory and stress effects of exposure to 7 (unstated) doses of paraquat administered over 2 weeks to male C57Bl6 mice (from Charles River Laboratories) with animals sacrificed immediately after the last dose and at 1 and 6 months for investigations.

Results. The paper reports that paraquat induced a loss of dopaminergic SNpc neurons and activation of microglia that persisted over 6 months after the last injection although with reduced magnitude for SNc neuron loss. The microglial proinflammatory actin-remodelling factor, WAVE2, and the inflammatory transcription factor, nuclear factor kappa B were also elevated within the brain. Corticosterone remained significantly elevated 1 month after paraquat. High-resolution MRI detected no striatal changes, but modest hemispheric differences in the SNpc and time-dependent volumetric enlargement of the ventricles in paraquat-treated mice were reported. The authors concluded that their data suggest that paraquat induces long-term nigrostriatal pathology and inflammatory changes and stress and trophic/apoptotic effects that appear to either increase with the passage of time or are evident for at least 1 month.

Comment. As this study was published after the APVMA and EPA paraquat review reports it was reviewed at the paper level but was found to contain substantive deficiencies. The form, source and purity of the paraquat used were not stated. The dose administered at each time point and in total, and the number of animals per group were not provided. Very small numbers of animals were examined in some key investigations (eg. 4 per group for MRI). The dose administered appears to have produced general systemic toxicity as indicated by reduced body weights and increases in the organ weights of the heart and lungs. Paraquat was administered by ip injection, a non-physiologically relevant route of exposure for HHRA. In the absence of information of the dose administered, the significance of the findings is uncertain.

Milanese, C., Cerri, S., Ulusoy, A., Gornati, S.V., Plat, A., Gabriels, S., Blandini, F.,Di Monte, D.A., Hoeijmakers, J.H. and Mastroberardino, P.G. (2018): Activation of the DNA damage response in vivo in synucleinopathy models of Parkinson's disease. Cell Death Dis, 9, 818

Study objectives and design. This study explores the DNA Damage Response (DDR) activation in 2 different synucleinopathy in vivo models and investigated possible causative mechanisms in vitro. Paraquat was not investigated directly.

Alpha synuclein pre-formed fibrils (PFF) were synthesised in vitro and surgically implanted into the mouse substantia nigra. Recombinant adeno-associated virus was used to express human-α-synuclein (h-α-syn) in the mouse striatum through surgical implantation/injection. Immunohistochemistry, immunofluorescent and stereological techniques were used to identify Parkinson’s disease related lesions and alterations. Additionally, dopaminergic SH-SY5Y neuroblastoma cells were maintained in culture to produce differentiated neurons which were then incubated with PFF and processed for immunohistochemistry.

Six months after injection, viral h-α-syn transduced mice displayed significant striatal increase of human α-syn protein levels in the ipsilateral hemisphere, which was paralleled by a remarkable reduction of striatal TH immunoreactivity. Increased α-syn was also detected inside dopaminergic cell bodies in the SNpc. These alterations were associated with significant ipsilateral reduction in nigral dopaminergic cell bodies, as detected by unbiased stereological counts.

In these mice a significant ipsilateral increase in the DDR markers γH2AX and 53BP1 foci, and phospho-ATM immunoreactivity was detected. To eliminate the possibility that the DDR activation was due to non-specific viral toxicity and/ or non-specific protein overexpression, a separate group of animals was injected intrastriatally with the same viral vector carrying only green fluorescent protein. The absence of significant changes within GFP-transduced neurons, supported the conclusion that DDR activation is a specific α-synuclein effect.

Lesions in the dopaminergic system were detected also in PFF treated mice, 4 months after injection, which showed reduced striatal TH immunoreactivity paralleled by α-synuclein stress as evidenced by increased phospho-synuclein levels and reduced dopaminergic cell bodies in the SNpc when compared to saline injected animals. DDR markers were also identified in these mice but not saline treated mice.

The authors concluded that these data demonstrate a causative link between α-syn proteotoxicity and DNA damage in dopaminergic neurons.

The effects of the thiol-based reactive species scavenger and antioxidant N-acetylcysteine (NAC) on DDR activation in differentiated SH-SY5Y were explored in cells exposed to PFF. NAC reduced levels in both γ-H2AX and 53BP1 foci. Cells were also treated with the nitric oxide (NO) donor nitrite, which triggers expression of endogenous antioxidant genes via S-nitrosation of Keap1 and consequent activation of the Nrf2 pathway. Nitrite significantly reduced PFF-induced formation of γ-H2AX foci. The authors interpreted these results to indicate that pro-oxidants mechanistically participate in DDR activation induced by proteotoxic α-syn.

Mitochondrial bioenergetics were explored in SH-SY5Y cells exposed to PFF. Proteotoxic stress reduced both basal respiration, reserve capacity, and rotenone sensitive respiration, i.e., attributable to complex I. Potentiation of exogenous or endogenous antioxidant defenses by NAC or nitrite supplementation, which attenuated the DDR, also reversed these defects. The authors interpret this data as substantiation of the nexus between proteotoxicity, oxidative stress, DNA damage, and mitochondria.

Comments. This study does not directly investigate paraquat but provides further evidence for the potential role of α-synuclein in Parkinson's disease.

Sun, J., Agarwal, S.,Desai , T., Ju, D., Chang, Y., Liao, S., Ho, T., Yeh, Y., Kuo, W., Lin. Y., and Huang, C. (2023): Cryptotanshinone protects against oxidative stress in the paraquat-induced Parkinson's disease model. Environmental toxicology, 38(1), 39–48

Study objectives and design. The study investigated the antioxidant properties of cryptotanshinone in paraquat-induced neurotoxicity in mice and in a neuroblastoma cell line. Cryptotanshinone is a fat-soluble extract of Salvia miltiorrhiza, with use in Chinese herbal medicine.

Apoptosis and oxidative stress were assessed in a human neuroblastoma cell line exposed to paraquat at 0.5 mM with or without the herbal medicine derived cryptotanshinone at various concentrations. Cryptotanshinone attenuated paraquat-induced oxidative stress by upregulating antioxidant markers.

C57BL/6J mice (2 months old) in groups of 6 were administered by ip injection; paraquat 10 mg/kg bw once per week for 3 weeks, paraquat 10 mg/kg bw once a week for 3 weeks with 2 injections of cryptotanshinone 10 mg/kg bw each week, or control (material administered not detailed). Cryptotanshinone is reported to have protected against behavioural deficits and protected dopaminergic neurons.

Results. The authors describe their study as demonstrating that cryptotanshinone can reduce ROS generation, thereby alleviating oxidative stress by elevating Nrf2 expression and promoting cell survival in differentiated SK-N-SH cells. Improved motor function and protection of Tyrosine hydroxylase-positive neurons was also described.

Comment. The paper provides no indication of blinding of histopathology investigators to treatment.

Torres-Rojas, C., Zhao, W., Zhuang, D., P O'Callaghan, J., Lu, L., Mulligan, M., Williams, R., and Jones, B. (2022): Paraquat Toxicogenetics: Strain-Related Reduction of Tyrosine Hydroxylase Staining in Substantia Nigra in Mice. Front Toxicol, 2021. 3: p. 722518

Study objectives and design. This study aimed to explore the relationship between genetic characteristics and paraquat reduction of TH staining in the SN of various strains of mice.

Male mice from 6 BXD strains (4 ± 2 mice per strain, from the University of Tennessee Health Science Centre) were administered 5 mg/kg paraquat dichloride trihydrate (paraquat, Sigma product # 36541) in saline by ip injection once weekly for 3 weeks and euthanised the day following the third injection. Control mice were administered saline under the same protocol.

Brains were perfused and fixed in situ then postfixed after removal for 24 hrs then processed for cryostatic serial sectioning of coronal sections containing the entire SN and processed for immunohistochemistry analysis of TH (post mitotic neurons (NeuN) and nuclei (DAPI-a blue-fluorescent dye that binds to AT-rich regions in double-stranded DNA).

Processed brain sections were stereologically analysed for TH+ neurons, neurons, and nuclei in the SNpc by 2 investigators independently who were blinded to treatment.

Results. The authors concluded that there were significant main effects of strain and treatment but not their interaction on TH+ neurons, with a TH+ loss ranging between 0 and 20% but there were no significant effects of strain, treatment, or their interaction on neuron counts and query whether paraquat actually destroys TH+ neurons in the SNpc.

Comment. This study does not address whether the loss of TH+ neuron staining is permanent or reversible and, if permanent, whether paraquat prevents the synthesis of TH but spares the neuron.

The authors note a number of limitations of their study:

- A limited number of strains was investigated (30 strains are minimal for identification of candidate genes that underlie individual differences in sensitivity).
- Males only were investigated (inclusion of both sexes is necessary because in some toxicological studies similarities and important sex differences are observed).
- While the study identified strain differences in paraquat effects on TH staining, it did not observe a significant strain and treatment interaction.

The study provides some support for genetic-based individual differences in susceptibility to paraquat neurotoxicity.

Wang, D., Yu, T., Liu, Y., Yan, J., Guo, Y., Jing, Y., Yang, X., Song, Y. and Tian, Y. (2016): DNA damage preceding dopamine neuron degeneration in A53T human alpha-synuclein transgenic mice. Biochem Biophys Res Commun, 481, 104-110.13.

Study objectives and design. This study investigated the link between defective DNA repair and age-associated neurodegenerative disorders such as Parkinson's disease through induction of DNA damage using X-ray

irradiation and analysis of dopamine neuron degeneration in the A53T human α-Synuclein over expressed mouse model and A53T-a-Syn Mouse Embryonic Fibroblast (MEF) cells.

Results. The authors describe their results as indicating that A53T-a-Syn MEFs show a prolonged DNA damage repair process and senescence phenotype. DNA damage preceded onset of motor phenotype in A53T-α-Syn transgenic mice and decreased the number of nigrostriatal dopaminergic neurons. Neurons of A53T-α-Syn transgenic mice were more fragile to DNA damage.

Comments. The study did not investigate paraquat directly. The sex of the mice used was not provided.

Epidemiology studies

Ayton, D. Ayton, S., Barker, A., Bush, A. and Warren, N. (2019): Parkinson's disease prevalence and the association with rurality and agricultural determinants. Parkinsonism & related disorders, 61, 198–202. https://doi.org/10.1016/j.parkreldis.2018.10.026

Study objectives and design. This study used dispensing data for Parkinson's disease medications from Pharmaceutical Benefits Scheme (PBS) records as an indication of Parkinson's disease prevalence across 79 Local Government Areas (LGAs) in Victoria, Australia. The data indicated the number of individual patient prescriptions, by dispensing pharmacy, of all Parkinsonian drugs for the year 2011. The number of Parkinson's disease patients in each LGA was expressed as a percentage of the LGA population and used to estimate Parkinson's disease prevalence rates in each LGA. Data from the Australian Bureau of statistics (ABS) was used to identify agricultural commodity production characteristics in each of these LGAs. The study made no attempt to quantify the identity or usage rates of pesticides in the various LGAs.

Results. A notable cluster of increased Parkinson's disease prevalence was identified in the central western area of Victoria. Although the prevalence of Parkinson's disease was higher in rural (1.02%) compared to urban localities (0.80%; P=0.001) when Parkinson's disease prevalence was adjusted to account for median age, proportion male, and the SEIFA socioeconomic indicator, rurality in itself was no longer predictive of prevalence rates.

An elevated prevalence rate in Ballarat was attributed by the authors to it being the only rural area in Victoria with a specialised movement disorder clinic and the data collection method being based on the dispensing pharmacy as an indication of patient locality. Patients travelling to this clinic from other areas of Victoria to see a Parkinson's disease specialist would likely collect new scripts for their medication in Ballarat. This assumption is reasonable. The authors do not however entirely discount other potential causes for the apparent clustering.

The study found no difference in the median age or in the percentage of people over the age of 65 in the high prevalent LGA cluster compared to the other rural LGAs.

The study used Monte Carlo modelling to estimate the probability of the cluster of the 4 highest prevalent LGAs forming randomly, through 20,000 reconstructions of the map of Victoria with randomised allocation of prevalence rates in each of the 79 LGAs. The analysis identified 19 occasions where this might have occurred giving a probability of P=0.00095 indicating chance is an unlikely source for the observations. In the absence of availability of data on the use of agricultural chemicals across Victoria, the study examined the types of commodities produced in each of the LGAs which identified 5 commodities which were produced in higher intensity in the high

prevalence areas: barley, chickpeas, faba beans, lentils, and vetches. With the exception of barley, the identified commodities were all from the pulse family of crops. The authors speculate this association might be linked to natural rotenone production by these crops. This is an unlikely explanation, however. Although rotenone is produced naturally by members of the Fabaceae family which includes the pulses, domesticated food crops have not been identified as producing this secondary metabolite.

Comments. Notably, the areas of Parkinson's disease prevalence clustering in this study are distinctly outside production areas for some other high intensity agriculture, such as viticulture for example, where high use of pesticides at critical points in production is common. Paraquat has applications in vineyards, orchards and market gardens for weed control. The primary significance of this paper is that it illustrates the challenges of identifying causative relationships between such clusters, and any specific agent, without careful exposure analysis involving direct measurement of suspected causative agents, particularly where studies arbitrarily restrict the range of potential causative parameters examined.

Kamel, F., et al., (2007): Pesticide Exposure and Self-reported Parkinson's Disease in the Agricultural Health Study. American Journal of Epidemiology, 2006. 165(4): p. 364-374

This study has previously been considered by the OCS/APVMA in the 2016 review of paraquat neurotoxicity. As the study has been cited by a number of respondents to the request for public comment, illustrates a key source of experimental bias, and aids evaluation of more recent papers from the Agricultural Health Study (AHS), it is briefly reconsidered here.

Study objectives and design. This study investigated the prevalent and incident cases of Parkinson's disease in the AHS cohort and their relationship to self-reported pesticide exposure (prevalent cases 1993-1997, incident cases 1999-2003). Lifetime pesticide exposure of agricultural workers and their spouses was determined at enrolment through structured questionnaires. The study included 83 **prevalent** Parkinson's disease cases and 78 **incident** Parkinson's disease cases, all physician diagnosed. The relationships between total, prevalent and incident cases with general and specific pesticides (including paraquat) exposure were assessed. Adjustments were made for age, state, and type of participant (applicator or spouse), race, education, and smoking.

Results. The paper reports no evidence of a significant positive association of paraquat exposure with prevalent Parkinson's disease (OR = 1.8; 95% CI, 1.0-3.4, n = 14 PQ exposed cases) and no evidence of an association with incident Parkinson's disease (OR = 1.0; 95% CI, 0.5-1.9, n = 11 PQ exposed cases). There was no evidence of a significant positive association of ever use of pesticides in general and Parkinson's disease (OR = 0.5, 95% CI 0.2-1.1, n= 67 pesticide exposed prevalent cases: OR = 1.3, 95% CI 0.5-3.3, n=68 pesticide exposed incident cases).

Comment. A key strength is that the study examined prevalent and incident cases separately. Notably the OR for prevalent cases was substantively higher (OR =1.8 vs 1.0), but still low and non-significant, than that for incident cases, likely reflecting recall bias of prevalent cases that is not present, or less pronounced, for incident cases. Key weaknesses of this study are that exposure was not well defined, respondents knew of the purpose of the study and that pesticide use was a factor of interest in the study, and a substantial proportion of controls had pesticide exposure. Despite the very large cohort in this study only 11 incident cases of Parkinson's disease exposed to paraquat arose during the 5-year follow up. Additionally, the study investigated 50 pesticides introducing issues of multiple comparison.

Krzyzanowski B, Mullan AF, Dorsey ER, et al. (2025): Proximity to Golf Courses and Risk of Parkinson Disease. JAMA Netw Open. 2025;8(5):e259198. doi:10.1001/jamanetworkopen.2025.9198

This reference was reviewed at the paper level as it was published after the paraquat reviews by APVMA.

Study objectives and design. In a case control study involving patients with Parkinson's disease and matched controls from the Rochester Epidemiology Project (REP) from 1991 – 2015, the association between living near to golf courses and Parkinson's disease was examined. Patients were identified retrospectively from medical records. Cases were only required to live in the area of interest (Olmsted County) at the time of symptom onset/diagnosis. Controls were recruited from across 27 counties as covered by the REP. No attempt was made to identify or quantify exposure to any environmental factor other than proximity to a golf course.

Comment. The study is not relevant to a consideration of paraquat human health risk and is not further reviewed here.

Lee PC, B.Y., Bronstein J, Ritz B, (2012): Traumatic brain injury, paraquat exposure, and their relationship to Parkinson disease. Neurology, 2012. 79(20): p. 2061-6

Although this study was published prior to both the APVMA and EPA paraquat reviews and is not therefore new, it is briefly reconsidered here as the findings are relevant to the recent Shrestha et al (2020) paper, reviewed below, that also investigated the impact of traumatic brain injury (TBI) on any relationship between paraquat exposure and Parkinson's disease.

Study objectives and design. This study investigated associations between TBI, paraquat exposure, and Parkinson's disease in the California Parkinsons Environment and Genes (PEG) study incorporating 357 cases and 754 controls. TBI was defined as self-identified loss of consciousness for 5 min or longer. Paraquat exposure within 500 m of residences and workplaces was inferred from a geographic information system (GIS) based on records of pesticide applications to agricultural crops in California.

Results. The authors found a positive association between Parkinson's disease and paraquat exposure with OR = 1.36 (95% CI 1.02-1.81, n = 169 PQ exposed cases). No evidence of a significant positive interaction between paraquat exposure and TBI was found. The association between TBI and Parkinson's disease was 1.70 (95% CI 0.95-3.04) for subjects who had never been exposed to paraquat and was 3.01 (95% CI: 1.51-6.01) for subjects ever exposed to paraquat. The increased association between TBI and Parkinson's disease from paraquat exposure was not statistically significant however (OR for interaction = 1.29, 95% CI: 0.52-3.19).

Comment. The study analysis was not stratified to paraquat only exposure, and exposure assessment was not quantitative. Although the use of GIS data for inferring exposure avoids recall bias it suffers from the "ecological fallacy", is too imprecise to be used quantitatively and does not avoid information bias. Studies such as this may be unable to distinguish between factors associated with geographic proximity to agricultural land and living, pesticide use in general, and specific pesticides. There were important differences in demographic factors between the subjects with Parkinson's disease and the control subjects. The control subjects on average were younger, more likely to be female, and more likely to be non-white. These factors were controlled for statistically in the logistic regression models, however there is a potential for error with this approach. Matching control subjects based on these demographic factors would have reduced selection bias and confounding.

McGwin G and Griffin RL. (2022): An ecological study regarding the association between paraquat exposure and end stage renal disease. Environmental Health 21, 127

Study objectives and design. Using an ecological study design this study examined the association between end stage renal disease (ESRD) and inferred paraquat exposure based on the agricultural pesticide use estimates for counties across the USA maintained by the National Water Quality Assessment Program. Cases were obtained from the US renal data system.

Results. The study concluded that:

> **"The incidence of ESRD increased with increasing paraquat density. Based on a 20-year exposure lag, those in the highest paraquat density quartile had a 21% higher rate of ESRD compared to the lowest quartile whereas for a 15-year lag the increase was 26%. Adjusted associations were attenuated though still followed an increasing linear trend across quintiles"**

Comment. The assignment of exposure based on application rates across counties of case residence at the time of diagnosis is tenuous at best. The study provides no basis for dose response considerations and the link between "exposure" as defined by paraquat use "density" and ESRD is weak. The study outcomes appear to be discordant with findings in the AHS where mortality for private pesticide applicators associated with renal disease is generally comparable to controls (Shrestha et al. 2019, not stratified for paraquat however).

Paul KC, Cockburn M, Gong Y, Bronstein J, Ritz B. (2024): Agricultural paraquat dichloride use and Parkinson's disease in California Central Valley. Int J Epidemiol. 2024 Feb 1;53(1):dyae004

Study objectives and design. This case-control study drew data from the PEG study which comprised 829 Parkinson's disease patients and 824 community controls across 3 Californian counties in a case control design. This data was then used for an ecological study where the authors estimated residential and workplace proximity to commercial agricultural applications in the 3 counties since 1974 using the California pesticide use reporting (PUR) data and land use maps. "Exposure" was expressed in terms of use of paraquat and the duration and average intensity of use, in pounds/active constituent/year of paraquat across 4 time periods.

Results. The authors conclude that their study demonstrated Parkinson's disease was associated with paraquat "exposure" at both residence and workplace and summarised their results as follows:

> **"The Parkinson's disease patients from the PEG study both lived and worked near agricultural facilities applying greater amounts of the herbicide than community controls. For workplace proximity to commercial applications since 1974, working near paraquat applications every year in the window [odds ratio (OR) = 2.15, 95% confidence interval (CI) = 1.46, 3.19] and a higher average intensity of exposure [per 10 pounds (4.54 kilograms), OR=2.08, 95% CI=1.31, 3.38] were both associated with increased odds of Parkinson's disease. Similar associations were observed for residential proximity (duration: OR=1.91, 95% CI=1.30, 2.83; average intensity: OR=1.72, 95% CI=0.99, 3.04). Risk estimates were comparable for men and women, and the strongest odds were observed for those diagnosed at or before 60 years of age."**

The authors concluded that their study "provides further indication that paraquat dichloride exposure increases the risk of Parkinson's disease".

Comment. A major weakness of this study is that while the ecological study design using GIS pesticide use data for exposure inference removes recall bias, it does not provide quantitative or qualitative data on exposure for any individual study participant (the "ecological fallacy") [4]. At best the design provides evidence of potential exposure at unknown levels. The distance between a subject's residence and workplace and the location of paraquat application and amount applied were the only evidence of potential exposure. Important differences between subjects and controls in this study included controls being younger, more likely to be female and non-white. While these factors were controlled for statistically using the logistic regression models, there is a potential for error with this approach.

Conversely a strength of the study is that Parkinson's disease diagnoses were confirmed by movement disorder physicians. The authors also adjusted for several other potential pesticide exposures but used the same ecological approach with the same weaknesses. Adjustments were also made for age, gender, race, study wave and index year. Notably while the authors documented around 190 odds ratios (and their 95% CI estimates) they focussed their abstract and discussion on only 4 of these with odds ratios greater than 1.0 and 95% confidence intervals that did not include 1.0. The ORs for 77 (41%) of the total number of comparisons had 95% confidence intervals that did include 1.0 and the paper does not correct for multiple comparisons for reasons not explained in the paper.

Notably 2 of the authors of the paper are involved in paraquat-Parkinson's disease litigation as expert witnesses for the plaintiffs, a substantive potential conflict of interest, which is acknowledged in the paper.

Sanders, L., Paul, K., Howlett, E., Lawal, H., Boppana, S., Bronstein, J., Ritz, B. and Greenamyre, J. (2017): Base Excision Repair Variants and Pesticide Exposure Increase Parkinson's Disease Risk. Toxicological Sciences, 158(1), 188–198

Study objectives and design. This paper is one of a number by various authors reporting analysis arising from the Californian PEG study. The PEG study used a case control design, recruiting rural Parkinson's disease patients (diagnosed by clinicians), from 3 California counties, to investigate the interaction between genetics and environmental susceptibility to Parkinson's disease. Cases were required to have lived in Californian for 5 years prior to their Parkinson's disease diagnosis. This paper reports analysis of 619 patients and 854 population based controls used to investigate the relationship between paraquat exposure and single nucleotide polymorphisms (SNPs) in base excision repair (BER) genes. Exposure inference was derived from GIS-based paraquat usage data and considered both residential and occupation address. The study considered pesticides identified mitochondrial complex 1 inhibitors and and/or oxidative stressors as reported in Tanner at al. (2011). Study subjects provided blood and saliva samples in order to determine the genetic data.

Results. The study reports evidence of a positive association between paraquat residential/ workplace exposure and Parkinson's disease (OR = 1.54, 95% CI: 1.23-1.93, n = 245 exposed cases). For the interaction between

[4] Chang, E. T., Adami, H. O., Bailey, W. H., Boffetta, P., Krieger, R. I., Moolgavkar, S. H., & Mandel, J. S. (2014). Validity of geographically modeled environmental exposure estimates. Critical Reviews in Toxicology, 44(5), 450–466. https://doi.org/10.3109/10408444.2014.902029

paraquat exposure and genetic susceptibility, the study reports no evidence of a significant positive association between paraquat exposure in subjects with no more than 1 risk allele (OR = 1.13, 95% CI: 0.75-1.70, n = 48 exposed cases) compared to those with 1 or fewer risk alleles and a strong positive association in subjects with 2 or more risk alleles (OR = 2.38, 95% CI: 1.44-3.95, n = 22 exposed cases).

Comment. The primary strength of this and other PEG studies is the recruitment of cases with clinically confirmed Parkinson's disease diagnosis and the use of GIS data to infer exposure not subject to recall bias. Controls however were recruited using a population-based approach that relied on Medicare enrolee lists and residential tax-collector records which may have introduced selection bias if cases and controls represent populations with different demographics, lifestyle factors, potential for exposure, and willingness to participate in the study. Additionally, while GIS based data is not subject to recall bias it does not provide reliable quantitative exposure estimates and cannot isolate paraquat exposure specifically as opposed to general residential/workplace proximity to agricultural land. No published information on the measurement of paraquat residue levels in residential or workplace environments is available for the counties of residence or workplace for study participants. In the absence of exposure validation, the relationship between being present at addresses within 500 m of agricultural land and actual paraquat exposure is indeterminate.

Shrestha S, Parks CG, Keil AP, Umbach DM, Lerro CC, Lynch CF, Chen H, Blair A, Koutros S, Hofmann JN, Beane Freeman LE, Sandler DP. (2019): Overall, and cause-specific mortality in a cohort of farmers and their spouses. Occup Environ Med. Sep;76(9):632-643. doi: 10.1136/oemed-2019-105724

Study objectives and design. This paper reports a cohort mortality study in the AHS study population where Parkinson's disease is one of many outcomes (n = 113) analysed. Subjects were followed for 16 years, from 1999 through 2015. Study subjects were enrolled from pesticide applicators applying for or renewing a license to use restricted pesticides between 1993–1997, whether private or commercial [more than 50000 mostly male subjects]. Married spouses (mostly women) of private applicators were also invited to participate for a total of approx. 85,000 subjects. Deaths were identified through state death registries and the National Death Index through December 31, 2015. There were 13,104 deaths with 153 cases of Parkinson's disease in the studied cohorts.

The study calculated 3 relative outcome ratios for each outcome, causal ratio (CMR), standardised mortality ratio (SMR) and relative SMR (rSMR) with 95% confidence intervals provided for each. Adjustments were performed for age, calendar year, race, and state.

Results. Studies have shown that farmers experience lower overall mortality from natural causes as compared to the general population. This finding has been attributed to farmers' healthier lifestyle and a potential "healthy worker effect". Regardless of the mechanism, this overall pattern was reconfirmed in this study. Among private applicators overall mortality rates, as well as many cause- specific mortality rates, were significantly less than expected (SMRoverall=0.69, 95% CI: 0.67–0.70). There were no cases of Parkinson's disease identified in commercial operator decedents.

For pesticide applicators the observed number of Parkinson's cases (153) was less than expected (156). When these raw figures were adjusted for "person-time" accrued the study provided some evidence of a slightly elevated CMR (OR 1.47, 95% CI 1.25-1.71) and rSMR (OR 1.42, 95% CI 1.21-1.66) for pesticide application and standardised mortality ratio, but the SMR was not elevated in the private applicators OR 0.98, 95% CI 0.83-1.14). There was no evidence for increased standardised mortality ratio related mortality and pesticide exposure for

spouses of private applicators, with all confidence intervals extending below 1. Although substantive pesticide exposure of participants is unequivocal given their occupations involving regular application of agricultural chemicals, the exact nature of the pesticide exposure (identity and quantity) was not determined in this study.

Comment. The observed number of Parkinson's disease cases in pesticide applicators was less than that expected from the referent population incidence. The study did not attempt to investigate any association with specific pesticides or pesticide classes with Parkinson's disease although data on ever/never use of various pesticide classes is reported. As the all-cause mortality of pesticide applicators was significantly lower than the referent population adjustment for "person-time" increases the ORs for Parkinson's disease (and all other conditions reported) as observed for CMR and rSMR for Parkinson's disease. This study does not provide substantive support for a relationship between pesticide exposure (as a collective class) and risk of Parkinson's disease.

Shrestha, S., et al. (2020): Pesticide use and incident Parkinson's disease in a cohort of farmers and their spouses." Environ Res 191: 110186

Study objectives and design. The authors investigated the relationship between incident Parkinson's disease cases and 50 specific pesticides, including paraquat. Detailed information on both duration and frequency of use was collected at enrolment or in take home questionnaires. Study participants (from the Agricultural Health Study), farmers and commercial pesticide contractors, completed an additional questionnaire asking about pesticide use in the most recent year prior to the study. This analysis used information collected at Phase 2 (conducted 2 to 10 years after initial enrolment, with a 5-year average). Parkinson's disease cases were self-identified as well as by reference to the National Death Index and state death registries. In Phase 2 of the study self-identified Parkinson's disease cases were assessed by movement disorder specialists for confirmation of diagnosis (84% confirmation rate of self-reported Parkinson's disease).

Exposure estimations utilised a range of information to provide more robust estimates than are possible from more common approaches such as GIS based use rates for example. Commercial contractors and farmers generally maintain records of chemicals use, both for farm management and accounting purposes.

Exposure intensity weights were derived using a previously described algorithm that incorporates information on; mixing practices, application methods, equipment repair status, and personal protective equipment use (as described in Coble et al., 20115). The study then used intensity-weighted lifetime days (IWLD) of pesticide use (i.e., the product of years of use and days used per year weighted by exposure intensity) as a measure of cumulative exposure for applicators. IWLD days were categorised using cut-points based on the exposure distribution of the full sample and number of Parkinson's disease cases (i.e., at least 5 cases) in each exposure category, creating a four-category exposure variable (never use and 3 categories among users with cut-points at tertiles of IWLD). As only applicators were asked about duration and frequency of use of specific pesticides in Phase 1, the IWLD analyses were limited to the male applicators due to the small number of females. Adjustments were made for age, state of residence, smoking status, and education.

[5] Coble, J., Thomas, K.W., Hines, C.J., Hoppin, J.A., Dosemeci, M., Curwin, B., Lubin, J.H., Beane Freeman, L.E., Blair, A., Sandler, D.P., Alavanja, M.C., 2011. An updated algorithm for estimation of pesticide exposure intensity in the Agricultural Health Study. Int. J. Environ. Res. Publ. Health 8, 4608–4622. https://doi.org/10.3390/ ijerph8124608.

Results. There was no association for ever use of paraquat and Parkinson's disease (HR = 1.09, 95% CI: 0.84-1.19). When analysed for lifetime days of use, there was again no evidence of an association and no evidence of a trend across lifetime use days. The hazard ratios through enrolment were 1.03 (0.58 – 1.81), 1.42 (0.86 – 2.33), and 0.74 (0.37 – 1.49) for the first, second, and third tertile of use respectively indicating no dose-response based on increasing exposure. The same pattern was observed through phase 2: 0.92 (0.51 – 1.63), 1.49, (0.92 – 2.41), 0.69 (0.34 – 1.38) for the first, second, and third tertile of use respectively again indicating no dose-response based on increasing exposure. Notably, the study found a significant interaction between occupational paraquat exposure and head injury (HI) with the development of Parkinson's disease, with a hazard ratio for those with head injuries being 3.2 (95% CI,1.38–7.45).

Comments. Overall, the study was well designed and suggests, but does not prove, a potential interaction between head injury and paraquat exposure with the development of Parkinson's disease. There were only 23 participants with both Parkinson's disease and a history of prior HI across the entire study population and co-exposure of Parkinson's disease and HI cases with multiple pesticides confounds attribution of Parkinson's disease to any specific combination of HI and a specific (or class of) pesticide exposure with any confidence.

The absence of any association between paraquat exposure and Parkinson's disease in the absence of HI is notable given the unequivocal and substantive exposure of participants to paraquat and the large number of exposed participants (~3000), unexposed controls with otherwise directly comparable working environments (~15000) and the substantive number of Parkinson's disease cases across the participants (n=228). Exposure analysis was more robust than GIS approaches, being based on actual frequency of use of pesticides over the previous year and calculating likely exposure based on a previously developed algorithm. This approach however has the weakness of using chemical use data over the previous year and assuming it to be representative of the 2-10 years since enrolment. The investigation of 50 pesticides in the one study introduces the problem of multiple comparisons and increases the potential for random statistically significant outcomes. The assessment of exposure and Parkinson's disease status both relied on self-report which introduces recall bias and misdiagnosis. Only 4 other chemicals were controlled for statistically to reduce the likelihood of confounding in the study. Given the number of different exposures that may or may not be related, this is a significant weakness in the design of the analysis.

Tomenson JA, Campbell C. (2021): Mortality from Parkinson's disease and other causes among a workforce manufacturing paraquat: an updated retrospective cohort study. J Occup Med Toxicol 16: 20

Study objectives and design. The aim of this retrospective cohort study was to update information on the risk of Parkinson's disease and mortality from major causes of death among a UK workforce who manufactured paraquat at any of the 4 plants (at Widnes, UK) manufacturing paraquat (between 1961 and 1995), by extending the follow-up by 7 and a half years. The study followed 926 male and 42 female workers through to 2017 and compared the rate of mortalities and cause of death for males with national and local rates. Exposure data was available from 1330 static monitoring results at one site between 1979 and 1993 and 100 personal monitoring results were collected between 1983 and 1993. There was insufficient sampling information available to perform a quantitative exposure assessment, however. A limited qualitative exposure assessment of male workers based on their highest level of exposure to 11 substances including paraquat and its manufacturing precursor was also available. Approximately 300 of the 729 male workers included in the initial mortality investigation were assessed in the mid-1980s to have had high or medium exposure to paraquat.

Results. Of 394 males who had died by the end of follow up, 4 death certificates mention Parkinson's disease (as cause of death or present prior to death combined) compared to the expected number of 6 giving a SMR for Parkinson's disease of 0.67 (95% CI 0.18 - 1.72).

Unlike many case-control studies where the exposure estimates of cases is somewhat tenuous, a strength of this study is the likely higher exposure of workers engaged in paraquat production, particularly before the 1980s, as evidenced by overt symptoms of significant exposure recorded by workers. As the paper notes "A document describing handling precautions during manufacture at Widnes in the early 1970s noted that ingestion of a high dose of paraquat led to liver or kidney failure within 2 or 3 days, but that smaller doses could result in a progressive pulmonary insufficiency leading to death". The primary hazard at the plants was identified as dust particles and it was noted that "inhalation of dust particles causes nose bleeding which ceases on removal from exposure".

Many of the workers at 2 plants reported skin lesions and nose bleeds following exposure to dust and the prevalence of nose bleeds was considerably higher than that seen in cross-sectional surveys of paraquat sprayers. These symptoms reflect substantial exposure of workers, at markedly higher levels, on a daily basis, than is plausible for residents living in rural areas near to agricultural land or for applicators of diluted sprays applied on a seasonal (infrequent) basis. Improved OHS requirements after the 1970s substantially lowered exposure of workers and personal monitoring after 1983 yielded estimated daily intakes of 25.8 mg of PQ ion. The authors estimate that the daily exposure of workers in the plants from the mid-1980s onwards was comparable to that of an agricultural sprayer, although factory worker exposure was more frequent and prolonged.

Comments. A key strength of this study is participants were followed over a protracted period of time from exposure to outcome, which, when compared to other observational studies, provides a clearer temporal sequence and strengthens causal inferences about the relationship between exposure and outcome, or lack thereof. The study established temporality and used biomarker and environmental monitoring measurements of exposure, avoiding self-reporting of paraquat exposure and recall bias. The authors conclude that "The study provided no evidence of an increased risk of Parkinson's disease, or increased mortalities from other causes among paraquat production workers whose exposure to paraquat on a daily basis was at least comparable to that of a paraquat sprayer or mixer/loader." There was also no evidence of increased mortality from other causes including cancer. As the authors note, although other studies have determined that approximately 30 to 50% of death certificates for a deceased person with Parkinson's disease do not mention Parkinson's disease, this does not bias the SMR as the same degree of underreporting applies to national and local mortality rates.

Yuan Y, Shrestha S, Luo Z, Li C, Plassman BL, Parks CG, et al. (2022): High Pesticide Exposure Events and Dream-Enacting Behaviors Among US Farmers. Mov Disord. 2022;37(5):962-71

Study objectives and design. Based on the participants of the AHS study the authors identified individuals reporting high pesticide exposures from 1983 to 1997 and examined self-reported "dream enacting behaviours" (DEB) from 2013 to 2015. Dream-enacting behaviour is a characteristic feature of rapid eye movement sleep behaviour disorder (RBD), the most specific prodromal marker of synucleinopathies. As diagnosis of RBD requires video-polysomnography recorded episodes of motor behaviours or vocalisation during rapid eye movement sleep, which was beyond the capabilities of the current study, dream enacting behaviour was used in this study as a surrogate for RBD.

The study used multivariable logistic regression analyses to identify correlations between high pesticide exposure events and DEB among 11,248 pesticide applicators (farmers, age 47 + 11 years). In the initial (phase 1) enrolment participants farmers reported whether they had ever had an incident resulting in unusually high pesticide exposures and provided details about only the highest exposure incident. High pesticide exposure events were again surveyed in 1999-2003 (phase 2), 2005 to 2010 (phase 3) and 2013-2015 (phase 4) and any high exposure incidents identified. On each occasion, details of only the most recent incident with respect to the time of questioning were obtained.

Occurrence of DEB was self-identified by participants in response to specific questions regarding typical symptoms. The authors state that the specific question asked was designed for use in large epidemiological studies to screen for probable RBD and has shown high sensitivity (93.8%) and specificity (87.2%) in a clinical validation study they cite. The authors also cite a previous paper in which they demonstrate that the presence of dream-enacting behaviours is associated with an approximately 8 times higher odds of having Parkinson's disease.

Results. Of a total of 11,248 eligible participants 939 reported DEB in the phase 4 survey. Compared with farmers without DEB, those who reported such behaviours were more likely to be current smokers, married or living as married, had histories of head injury and depression, and reported more nonspecific symptoms and major chronic conditions at baseline. A history of high pesticide exposure events was reported by 1847 farmers. Farmers reporting high pesticide exposures were significantly more likely to also report DEB, OR = 1.74 (95% CI 1.49-2.05).

For paraquat specifically, farmers reporting a high exposure event were significantly more likely to also report DEB, OR = 3.48 (95% CI 1.37 – 8.81) but the absolute number of high exposure subjects was small at 26 with only 6 of these reporting DEB.

Comment. The study examined the identity and nature of the high exposure events only for the event most recent with respect to the time of surveying the participants, which may also distort the outcomes observed. Whether the occupational behaviours of individual participants during pesticide use that lead to the high exposure events is potentially reflective of poor occupational hygiene in those groups, and therefore indicative of above average exposures throughout their working history is not explored.

Acronyms and abbreviations

Shortened term	Full term
ABS	Australian Bureau of Statistics
ADI	Acceptable Daily Intake
AHS	(US) Agricultural Health Survey
APVMA	Australian Pesticides and Veterinary Medicines Authority
ARfD	Acute Reference Dose
BBB	Blood Brain Barrier
BER	Base Excision Repair
CI	Confidence Interval
CV	Average Coefficient of Variation
DA	dopamine
DDR	DNA Damage Response
DEB	Dream Enacting Behaviours
DNA	Deoxyribonucleic Acid
DPR	Californian EPA Department of Pesticide Regulation
ESRD	End Stage Renal Disease
FAME	Farming and Movement Evaluation study
GIS	Geographical Information System
GLP	Good Laboratory Practice
h-α-syn	human-α-synuclein
HHRA	Human Health Risk Assessment
HBGV	Health Based Guidance Values (e.g. ADI, ARfD)
HI	Head Injury
ip	Intraperitoneal injection
ISO	International Organisation for Standardisation
IWLD	Intensity-Weighted Lifetime Days

Shortened term	Full term
LDH	Lactate dehydrogenase
LGA	Local Government Area
NOAEL	No Observed Adverse Effect Level
MII	Metaphase II Stage
MCPA	2-methyl-4-chlorophenoxyacetic acid
MEF	Mouse Embryonic Fibroblast
MPTP	1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI	Magnetic Resonance Imaging
OCS	Office of Chemical Safety
OECD	Organisation for Economic Cooperation and Development
OHS	Occupational Health and Safety
OR	Odds Ratio
PBS	Pharmaceutical Benefits Scheme
PD	Parkinson's Disease
PEG	Parkinson's Environment and Genes study
PET	Positron Emission Tomography (scan)
PFF	Pre-formed fibrils
POD	Point of Departure
PPE	Personal Protective Equipment
PQ	Paraquat
PRD	Proposed Regulatory Decision
PUR	California Pesticide Use Reporting Data
REP	Rochester Epidemiology Project
RBD	Rapid Eye Movement Sleep Behaviour
ROS	Reactive Oxygen Species
RTR	Paraquat Review Technical Report
SMR	Standardised Mortality Ratio

Shortened term	Full term
SNP	Single Nucleotide Polymorphism
SNpc	Substantia nigra pars compacta
TG	(OECD) Test Guidelines
TH	Tyrosine hydroxylase
US EPA	United States Environmental Protection Agency

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