California Environmental Protection Agency
Department of Pesticide Regulation

Preliminary Report of the Potential Human Health Outcomes Resulting from Paraquat Exposure

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Abbreviations

AHS – Agricultural Health Study

BW – Body weight

CI – Confidence interval

DPR –Department of Pesticide Regulation

FIFRA – Federal Insecticide Fungicide and Rodenticide Act

LC50 – Median lethal (air) concentration

LD50 – Median lethal dose

LOAEL – Lowest observed adverse effect level

LOC – Level of concern

MOE – Margin of exposure

NOAEL – No observed adverse effect level

OR – Odds ratio

PEG – Parkinson's Environment and Genes study

PISP – Pesticide Illness Surveillance Program (DPR)

PND – Post natal day

POD – Point of departure

PPE – Personal protective equipment

PUR – Pesticide Use Report data source

RfD – Reference dose

SENSOR – Sentinel Event Notification System for Occupational Risk

SNpc – Substantia nigra pars compacta

UF_A – Uncertainty factor, animal-to-human

UF_H – Uncertainty factor, intra-human

US EPA – US Environmental Protection Agency

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I. Executive Summary

In November 2022, as a part of the annual pesticide registration renewal cycle the Department of Pesticide Regulation (DPR) received public comments from the Center for Biological Diversity (Kylah Staley, Jonathan Evans, and Robert Rutkowski, signatories), the Michael J. Fox Foundation (Ted Thompson, JD, Julia Worcester, JD, signatories), and concerned Parkinson's disease researchers and practitioners (Dr. Beate Ritz and Dr. Jeff Bronstein, signatories, University of California Los Angeles; Dr. Caroline Tanner, signatory, University of California San Franciso; Dr. Ray Dorsey, signatory, University of Rochester Medical Center; Dr. J Timothy Greenamyre, signatory, University of Pittsburgh; and, Dr. Michael S. Okun, University of Florida Health). These comments were accompanied by approximately 80 scientific references or articles. Collectively the comments expressed concern regarding the continued registration of paraquat dichloride as pesticidal active ingredient in California, citing adverse impacts to human health and the environment from use of paraquat-containing products.

In November 2023, additional public comments were received by DPR from the Center for Biological Diversity (Jonathan Evans, signatory) with an additional 25 scientific references or articles again expressing concern for potential human health impacts of paraquat dichloride exposure. Specifically, the letter urged the department begin reevaluation, suspension, and cancellation proceedings for pesticide products containing paraquat dichloride because of the significant adverse impacts of these products citing Title 3 of the California Code of Regulations (§ 6220, 6221) and the California Food and Agriculture Code (§ 12825, 12826).

On November 6, 2024, DPR issued California Notice 2024-20 initiating a reevaluation of paraquat as mandated by Assembly Bill (AB) 1963 (Chapter 688, Statutes 2024). This document, which was prepared in response to public comments received in 2022 and 2023 as part of the annual renewal cycle, is a preliminary report on human health concerns resulting from exposure to products containing paraquat dichloride. This preliminary report does not constitute a determination on whether additional restrictions on the use of paraquat may be necessary as a result of the reevaluation. DPR will issue such a determination in the future.

From the submissions, the Human Health Assessment Branch (HHA) identified 26 documents that are scientific articles whose primary investigations were on paraquat dichloride (human, animal or in vitro). Submitted documents that did not make a clear reference to or association of findings to paraquat were reviewed for relevance but not included in this preliminary report. Editorial, commentary, or published news articles were reviewed but not included in this preliminary report. For a list of submitted documents, see Appendix D.

HHA did not conduct a systematic review of the literature nor a comprehensive human health risk assessment of paraquat for this effort. However, HHA did review US EPA's draft human health risk assessment for paraquat (US EPA, 2019a), the agency's Tier II systematic review of the open literature on potential health effects associated with paraquat exposure (US EPA, 2019b), and US EPA's systematic review of literature that specifically focused on paraquat exposure and Parkinson's disease (US EPA, 2019c). The US EPA draft human health risk assessment for paraquat was also submitted to DPR as part of public comment in November 2022. HHA's review of US EPA's draft human health risk assessment is summarized in this document with a more detailed evaluation in Appendix A.

In addition, HHA independently evaluated the most recent population-based studies and human case studies/case reports published from 2015–2024 that were not previously evaluated by US EPA nor submitted as part of public comment (totaling an additional 48 studies). A synopsis of these findings is included in this document and the literature search criteria and more detailed scientific findings are found in Appendix B and C. An expanded review of in vivo and in vitro data (e.g., animal neurotoxicity models) was also included in this preliminary report. Additionally, HHA reviewed toxicology guideline studies required for pesticide registration, changes in paraquat use and regulation, and Parkinson's disease incidence and mortality data, all in an effort to develop an objective evaluation of the state of the science of paraquat exposure and potential human health effects.

The evaluation process of studies and reports included herein followed DPR's Guidance for Toxicology Study and Data Acceptability in Registration Review and Risk Assessment (DPR, 2023) and/or US EPA's Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides (US EPA, 2016).

A. HHA Conclusions from Study Findings

1. Relationship between Paraquat Exposure and Parkinson's Disease

Parkinson's disease has multifactorial origins requiring combinations of multiple inputs and susceptibilities that become more relevant during aging. While the development of Parkinson's disease is more common under certain genetic, environmental, and lifestyle conditions, the causal factor(s) remain unknown.

Overall, data reviewed from population-based studies focused on Parkinson's disease were consistent with a possible role for paraquat exposure when considered in tandem with other exposures or predisposing factors. Some results were consistent with the multivariate origins of disease onset and development, including studies that showed gene-environment interactions (Ritz et al., 2009), pesticide co-exposures pre-1989 (Costello et al., 2009), and pesticide exposures combined with head injury (Lee et al.,

2012; Shrestha et al., 2020) which appeared to influence the risk of developing Parkinson's disease.

The most recent paraquat-specific study evaluated by HHA (Paul et al., 2024) demonstrated moderate to strong associations between Parkinson's disease and proxy measures of residential paraquat exposure. However, almost all risk estimates were attenuated when examining residential exposure as opposed to workplace exposure. In addition, the authors noted that there were stronger associations between multiple other pesticide exposures and the risk of Parkinson's disease than exposure to paraquat alone (Paul et al., 2024). This latter finding was also emphasized in another publication by the same research group that found increased motor or cognitive decline in Parkinson's patients associated with residential or workplace proximity to the use of numerous pesticides other than paraquat, including 2-methyl-4-chlorophenoxyacetic acid (MCPA) dimethylamine salt, copper sulfate pentahydrate, S,S,S-tributyl phosphorotrithioate, sodium cacodylate, and methamidophos (Li et al., 2023). In a concluding statement, the investigators note that pesticides are not applied in isolation and people are not singly exposed to one agent over a lifetime (Li et al., 2023).

The evidence reviewed herein do not demonstrate a direct causal association with exposure to paraquat and the increased risk of developing Parkinson's disease. As such, HHA's evaluation of the population-based studies largely align with US EPA's conclusion, that:

> "Overall, there is limited, but insufficient epidemiologic evidence at this time to conclude that there is a clear associative or causal relationship between occupational paraquat exposure and PD" (US EPA 2019a, p. 31).

> "Overall, there is insufficient epidemiologic evidence at this time to conclude there a clear associative or causal relationship between non-occupational paraquat exposure and PD. (US EPA 2019a, p. 38).

It is important to note that many population based studies evaluated by both US EPA and HHA spanned several decades, with many that included work or residential histories dating back to the mid-1970s. The retrospective nature of these studies captured potential paraquat use and exposure that are very different than what would occur now. It is expected that the legal label restrictions for paraquat use currently in place at the federal and state level would significantly reduce exposures compared to exposures that study subjects recall experiencing in the past.

As part of the evaluation of the relationship between paraquat exposure and Parkinson's disease, HHA also considered neurotoxic animal models of disease used to investigate the origin and causation of Parkinson's disease. Paraquat only elicits a narrow group of outcomes thought to be involved in Parkinsonism in these animal

models, and does not reproduce all the clinical and pathological features of the human disease (Jackson-Lewis et al., 2012; Zhang et al., 2016). These studies largely relied on intraperitoneal injection or other invasive dosing methods. Most studies that exposed animals to paraquat by inhalation or through feed, which are more representative exposure routes for humans, did not result in the hallmarks of Parkinson's disease (Rojo et al., 2007; Minnema et al., 2014). One dietary study by Anselmi et al. (2018) showed that oral dosing of rats to paraquat combined with lectins (a protein that can enhance absorption of toxins) produced presence of misfolded α-synuclein and neuronal loss in the substantia nigra pars compacta. This study adds to the weight of evidence of the importance of different routes of exposure to paraquat, although it did not result in a complete manifestation of Parkinson's disease. Currently no animal model can fully characterize the complexity of human Parkinson's disease, although such studies may provide insights into the specific processes underlying disease development.

2. Relationship between Paraquat Exposure and Cancer

HHA reviewed four population-based studies that investigated the association between paraquat exposure and cancer. One article submitted as part of public comment investigated thyroid cancer (Omidakhsh et al., 2022). Three studies independently evaluated by HHA considered associations between paraquat exposure and renal cell carcinoma (Andreotti et al., 2020), increased methylation (Alexander et al., 2017), and B-cell lymphoma (Ferri et al, 2017). Briefly, in a case-control study by Omidakhsh and colleagues (2022), investigators found that cases with thyroid cancer in California had 1.46 times the odds of ever having lived within 500 meters of a paraquat agricultural application than controls (95% CI; 1.23, 1.73). In a prospective cohort study as part of the Agricultural Health Study, Andreotti et al. (2020) found a significant risk of developing renal cell carcinoma at the highest exposure estimates following a 20-year lag compared to "never users" (i.e., individuals who report never using paraquat). Alexander et al. (2017) found a significant decrease in Long Interspersed Nucleotide Element 1 (LINE-1) methylation, a possible epigenetic indicator of cancer risk for "ever users" of paraquat (i.e., individuals who report ever using paraquat). Finally, Ferri et al. (2017) conducted a case-control study to explore the relationship between occupational risk and B-cell lymphoma subtypes. The results pointed to a positive association with an odds ratio of 2.8 comparing those with no exposure to those with low exposure, although this was accompanied by an inconsistent dose-response relationship in which those with higher paraquat exposure showed lower risk of developing cancer.

The population-based studies' authors suggest the possibility of a relationship between paraquat exposure and risk of various cancers, however HHA's review of the studies found that the data are not sufficient to support a definitive conclusion. HHA found that each study had limitations that reduce the applicability of findings, such as the use of proxy measures of exposure, investigation of association between the health outcome and multiple pesticides, or self-assessment of exposure via questionnaire. In addition, HHA's evaluation of registrant-submitted toxicological data required as part of pesticide

registration showed limited carcinogenic evidence from chronic studies in experimental animals. US EPA conducted a more exhaustive evaluation of the cancer data and concluded that there was either insufficient epidemiological evidence or non-significance of the relationship between paraquat exposure and risk of various cancers (US EPA, 2019b), a finding which HHA largely agrees with. US EPA has classified paraquat as Category E, or evidence of non-carcinogenicity for humans (US EPA, 2019a).

3. Relationship between Paraquat Exposure and Other Health Outcomes

Submitted and independently-reviewed clinical case reports and population-based studies were also evaluated for other non-cancer health effects that may be associated with acute or chronic exposure to paraquat.

a) Acute Poisoning

Effects of acute exposure to paraquat largely came from reports of accidental or occupational exposure, as summarized in the section on human illness and injury reports below, or from intentional exposure to paraquat as an instrument of self-harm, as summarized in Appendix C. An overwhelming number of human cases were the result of paraquat exposures not associated with legal label use. In countries where paraquat availability has been restricted or made illegal, there appears to be a downward trend in the number of reported self-poisonings (Chang et al., 2021). There is also a downward trend in the number of illnesses and injuries associated with exposure to paraquat alone or in combination with other pesticides in California (DPR, 2019). This decrease in cases parallels a downward trend of legal paraquat use in California over the same period (see Figure 1 later in this document).

b) Acute Lung, Renal and Dermal Injury

Reports of acute exposure are associated with lung, renal and dermal injury. Renal effects and lung toxicity are consistent with animal toxicity studies summarized later in this document. Dermal, renal, and respiratory toxicity were noted by US EPA to be of greater concern than other adverse human effects including neurotoxicity, and more relevant to assessing risk from paraquat exposure from the routine use of pesticidal products in the US (US EPA, 2019a). The lung is the most sensitive organ for paraquat toxicity, with evidence of lung inflammation, scarring and compromised lung function in different experimental species and as a result of both inhalation and oral (systemic) toxic effects. Occupational epidemiological studies show associations between acute paraquat exposure and short-term impacts to the upper respiratory system and other longer-term effects (general lung function, wheeze, allergic rhinitis, difficulty breathing) as well as an association between chronic paraquat exposure and self-reported asthma (Diaz-Criollo et al., 2019). Some studies were conducted outside of the US and verification of the type of PPE used during paraquat applications could not be made.

However, the sensitivity of the lung is reflected in the recent revisions to paraquat use restrictions (US EPA, 2021a). There is also evidence from both experimental animal studies and clinical human case reports that acute exposure to high levels of paraquat is toxic to the renal system. However, associations between paraquat and renal disease from human epidemiological studies were equivocal or not significant (Lebov et al., 2015; Lebov et al., 2016; Holliday et al., 2022). Many of these studies capture paraquat use in the US prior to the implementation of current mitigation and safety measures.

c) Human Birth Outcomes and Animal Developmental Toxicity Data

Two studies independently evaluated by HHA considered the association between paraquat exposure and developmental toxicity or birth outcomes. In a cohort study conducted by Rappazzo et al. (2019), paraquat exposure was significantly associated with several congenital heart defects and lower limb defects, with the latter showing a significant association compared to non-exposed participants (OR = 4.65; CI 1.09, 19.84). Ling and colleagues (2018) found associations between maternal paraquat exposure and preterm births and low birthweights in California, however caution is warranted in interpreting these results as there were multiple differences between the cases and controls and multiple potential exposures were investigated at once and not controlled for statistically. Both studies also utilized proxy measures of exposure based on agricultural pesticide application data and maternal address, which can lead to exposure misclassification. In general, the exposure misclassification resulting from these types of geographic models of environmental exposures can be differential and result in bias away from the null (Chang et al., 2014). In the absence of supporting environmental or biological monitoring data, results from studies with these proxy measures of exposure should be interpreted with caution and results should not be viewed as definitive.

As part of this preliminary report, HHA also reviewed the registrant submitted developmental toxicity studies for consistency of effects between humans and experimental animals. The in vivo animal studies showed that oral paraquat exposure resulted in increased fetal skeletal variations and reduced fetal weight, although maternal toxicity was observed in several of these studies. This, along with other methodological issues, call into question certain in vivo findings according to DPR guidelines (DPR, 2023). However, the consistency of paraquat developmental effects noted in both animal and human studies, coupled with the statistical significance of the findings in Rappazzo et al. (2019), point to the possibility of an association of certain development effects with paraquat exposure that warrant further review.

d) Thyroid Effects

Additional studies evaluated the association between paraquat exposure and perturbations in the thyroid gland. Goldner et al. (2010) focused on first-hand exposure of spouses enrolled in the Agricultural Health Study. Investigators found an association

between “ever-use” of paraquat and a significantly increased odds of developing hypothyroid disease of 1.8 (95% CI: 1.1, 2.8). However, no exposure-response relationship was shown with increasing years of use. Kongtip et al. (2021) studied acute changes in thyroid hormones among Thai sugarcane farmers before and after spray applications. The strength of this study was that it used biomarkers of exposure and effect, thus reducing potential bias. Results showed urinary paraquat levels were significantly associated with reduced total triiodothyronine (T3) (p=0.036) and reduced free triiodothyronine (FT3) (=0.036). The one weakness in the study was that it failed to statistically adjust for co-exposures, as the applicators reported that they often mixed or applied multiple pesticides together. This weakness is mostly overcome by the strength of the biomarker exposure-effect relationship.

The thyroid hormone findings should be taken in context of the importance of thyroid homeostasis during pregnancy. HHA and US EPA have both evaluated other pesticides that impact thyroid function including fipronil[1] and dimethyl tetrachlorophthalate (dacthal or DCPA).[2] In both cases, there have been biologically significant impacts to fetal development when dosing resulted in thyroid changes in pregnant female animals. Thus, the possibility of an association of thyroid hormone effects from paraquat exposure warrant further review.

B. Concluding Statement

HHA has reviewed the submitted studies and additional recent reported impacts of paraquat exposure on human health. The majority of these studies capture paraquat use in the US prior to the implementation of current mitigation and safety measures or were conducted outside of the US in countries which may have different pesticide regulations. Overall, HHA identified data gaps relative to impacts to the thyroid and birth defects that may be linked to adverse impacts from the use of paraquat. With respect to paraquat exposure and Parkinson’s disease, HHA has found that there is currently insufficient evidence to demonstrate a direct causal association with paraquat exposure and the increased risk of developing Parkinson’s disease.

[1] Final Risk Characterization Document for Fipronil. Department of Pesticide Regulation, California Environmental Protection Agency, March 2023. https://www.cdpr.ca.gov/docs/risk/rcd/fipronil_rcd.pdf
[2] Companion Document to the Occupational and Residential Exposure Assessment for the Registration Review of DCPA. US Environmental Protection Agency, Office of Pesticide Programs, May 26, 2023. https://www.regulations.gov/document/EPA-HQ-OPP-2011-0374-0082

II. Introduction

Paraquat dichloride (1,1′-dimethyl[4,4′-bipyridine]-1,1′-diium dichloride) is a non-selective, fast-acting herbicide used to control broadleaf and grass weeds largely under agricultural settings. Common usages of paraquat dichloride (henceforth referred to as paraquat) are to control weeds and grasses in agricultural and non-agricultural areas and as a preplant/preemergent on vegetables, grains, cotton, grasses, sugar cane, peanuts, potatoes, and tree plantation areas. It can be applied postemergence around fruit crops, vegetables, trees, vines, grains, soybeans, and sugar cane. Paraquat can be used as a desiccant or harvest aid on cotton, dry beans, soybeans, potatoes, sunflowers, and sugar cane. The US Environmental Protection Agency (US EPA) has established paraquat tolerances for over 80 crops (Title 40, Code of Federal Regulations (CFR), Section 180.205; https://ecfr.io/Title-40/Section-180.205). Paraquat is also used to control weeds around public airports, electric transformer stations, and commercial buildings. Approved application methods include aerial, groundboom, backpack sprayer, or low pressure handwand. The mechanism of action of paraquat is based on its ability to inhibit photosynthesis by promoting transfer of electrons from photosystem I (ferredoxin) to molecular oxygen, creating cytotoxic reactive oxygen species such as the superoxide anion through redox reactions.

Seven pesticide products containing the active ingredient paraquat are currently registered for use in California including Devour, Drexel Quick-Quat, Gramoxone SL 3.0, Helmquat 3SL, Para-Shot 3.0, Paraquat Concentrate, and Willowwood Paraquat 3SL. All registered products are listed as Restricted Use Pesticides and must only be used by certified applicators. No paraquat containing product is allowed for homeowner use or for application in residential areas.

In response to California Notice 2022-18, the Notice of Proposed Decision to Renew Pesticide Product Registrations for 2023, the Department of Pesticide Regulation (DPR, or department) received public comments requesting that DPR reevaluate, suspend, or cancel products containing paraquat. As part of the public comment process, DPR received submissions from the Center for Biological Diversity, the Michael J. Fox Foundation, and concerned Parkinson's disease researchers and practitioners which were accompanied by approximately 80 scientific references or articles. Collectively the comments expressed concern regarding the continued registration of paraquat dichloride as pesticidal active ingredient in California, citing significant adverse impacts to human health and the environment from use of paraquat-containing products. In November 2023, DPR received public comment in response to California Notice 2023-12, the Notice of Proposed Decision to Renew Pesticide Product Registrations for 2024, again requesting that DPR reevaluate, suspend, or cancel products containing paraquat. The comment submitted by the Center for Biological Diversity contained an additional 25 scientific references or articles again expressing concern for potential human health impacts of paraquat dichloride exposure.

DPR's Human Health Assessment Branch (HHA) conducted an evaluation of the submitted data along with a review of the most recently published findings on associations between paraquat exposure and human health that were not included as part of public comment. Submitted data and information that underwent further scrutiny were human, animal or in vitro studies that contained pertinent human health or toxicology data in peer-reviewed research published in the open literature. HHA's review followed DPR's Guidance for Toxicology Study and Data Acceptability in Registration Review and Risk Assessment (DPR, 2023) and/or US EPA's Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides (US EPA, 2016). In reviewing the submitted data, care was taken to focus on articles whose primary investigations were on paraquat dichloride (human, animal or in vitro); publications that did not make a clear reference or association to paraquat were not reviewed further. Editorial, commentary, or published news articles were reviewed but not discussed further as part of this preliminary report. For a list of documents reviewed, see Appendix D.

After applying inclusion and exclusion criteria, a total of 26 original peer-reviewed scientific publications submitted to the department as part of public comment (17 from the November 2022 submission and 9 from the November 2023 submission) underwent further critical evaluation as part of this preliminary report. Another 48 original peer-reviewed scientific studies published from 2015 through 2024 were independently reviewed by HHA. It was important to survey a representative sample of the literature to provide a more comprehensive and objective evaluation of the current data concerning paraquat and human health effects. Summarized findings are included herein and the literature search criteria, the PECO (Population, Exposure, Comparator, and Outcomes) statement used to define the objectives of the review and data inclusion/exclusion criteria, and more detailed scientific findings are found in Appendix B and C.

A. Regulatory and Registration History of Paraquat

Paraquat was first registered as a pesticide by US EPA in 1964 as a contact herbicide to control or suppress a broad spectrum of emerged weeds. It is unknown when products containing paraquat were first registered for use in California. However, in May 1974 the department designated products containing paraquat dichloride as California restricted materials based on acute toxicity. Once designated a restricted material, the law at the time restricted use to either certified commercial applicators or private applicators who possessed a restricted materials permit. However, no permit was required to possess or use paraquat when possessed and used only for home use in accordance with the registered labeling.

In February 1978, US EPA classified paraquat as a Restricted Use Pesticide (RUP) due to high acute toxicity to animals and people from intentional or inadvertent exposure. In

October 1981, US EPA also established the first pesticide residue tolerances for paraquat used in or on agricultural commodities (Title 40, Code of Federal Regulations, section 180.205). In June 1987, US EPA issued the Guidance for the Registration of Pesticide Products Containing Paraquat Dichloride as the Active Ingredient. As part of that effort, there were strict labeling requirement to maintain the Restricted Use Pesticide designation, as well as changes to certain tolerances and modifications to certain use sites that were instituted because of the tolerance changes. Importantly, no restricted entry interval was required for registered uses of paraquat and products containing 0.276% paraquat. To the last point, one product was registered that was allowed for homeowner and residential use without permit/license. It was designed to kill weeds and grasses around walks, driveways, and in flower beds. This product met the exemption for California's paraquat restricted material permits and was likely available for homeowners to purchase and use without a permit or applicator certification. This product was registered for use in California through January 1, 1987 and federally until 1996. In 1997, the registrant voluntarily cancelled the product because of unacceptable risk to the applicator identified through the federal reregistration process.

In August 1997, US EPA issued its Reregistration Eligibility Decision (RED) for paraquat. The RED reflected a reassessment of all data submitted in response to the 1987 Registration Standard and a subsequent December 1991 data call-in. The RED noted a decline in the number of ingestion incidents, which was presumed to be associated with the safening agents. Several additional mitigation actions required by the RED were specifically intended to lessen the occupational and ecological risks posed by paraquat, including a reduction in maximum application rates, additional PPE for mixers and loaders, reduction of paraquat concentration in backpack sprayers, and spray drift control measures. One item of note is that following the 1997 RED, the accepted paraquat labels no longer required handlers to wear dust/mist filtering respirators (see more about respiratory protection measures below).

In December 2011, US EPA opened the registration review docket for paraquat. The Final Work Plan was issued in on May 29, 2012 and a generic data call-in was noticed on February 20, 2013. To address human health incidents involving paraquat, the agency issued a Proposed Interim Mitigation Decision in March 2016 followed by an amended Human Health Mitigation Decision (HHMD) in January 2017 that proposed the restriction of all paraquat use to certified applicators only. Relative to restricting use to certified applicators, in its 2016 Proposed Interim Mitigation Decision, the agency explained that the number of human health incidents on file indicated that classifying paraquat as an RUP was not enough, thus justifying the restriction of use and handling to certified applicators only. In so doing, US EPA anticipated a decrease in occupational and ingestion risk because of the reduction of paraquat availability and exposure to persons not knowledgeable about the proper use and handling and a general decrease in misuse. The 2016 PID implementation occurred in three phases with completion in March 2017, November 2019, and December 2020, respectively. Restrictions included

the prohibition of use by uncertified persons working under the supervision of a certified applicator, targeted training, and closed-system packaging for all end-use containers of paraquat less than 120 gallons.

In July 2021, US EPA issued the Paraquat Dichloride Interim Registration Review Decision, finalizing certain portions of US EPA's analysis and determining that certain mitigation measures were necessary for paraquat to meet the FIFRA standard for registration and DPR accepted all registrant product labeling changes as of August 2023 (see below).

1. Evolution of Respiratory Protection Requirements for Paraquat in the US

In considering the risk to human health from exposure to paraquat, it is important to evaluate the regulatory history that was in place to protect pesticide handlers from the time paraquat was first registered by US EPA. There has been an evolution of occupational protections over time, both at the federal and state level.

According to the 1974 regulations, an employee had to have the required personal protection equipment (PPE), including a respirator, available on the tractor. The regulations noted that if the weather conditions change to a scenario of potential or eminent exposure, the employee would be required to wear the PPE. Additionally, for most applicators and all flaggers, "if the handler can avoid breathing the spray or dust ... the use is in compliance... If the applicator cannot avoid breathing the mist or dust, the use of ... respiratory protective equipment is required..." Approved labels for paraquat-containing products contained similar language up to and including 1986.

Additionally in 1974, paraquat-containing products were designated as California restricted materials and use was restricted to either certified commercial applicators or private applicators who possessed a restricted materials permit. However, products containing paraquat registered and packaged only for home use were exempt from the permit requirement. From 1974 through 1987, one paraquat product containing 0.276% active ingredient was registered for home use. According to the 1997 US EPA Registration Eligibility Decision, that product was still registered and available for use by homeowners at least until 1997. That changed with the 2021 Interim Registration Review Decision, which stated that there are no paraquat products registered for homeowner or residential use. Also, from 1974 through at least 1987, private applicators in California were defined as (1) persons who use or supervise the use of a restricted material for the purpose of producing an agricultural commodity on property owned or rented by him or his employer, or (2) a householder who uses or supervises the use of a restricted material within the confines of or on property necessary for the maintenance of the householder's residential dwelling. It is unclear what PPE was required during these years for either home use products or for individuals who were working/being supervised by someone holding a permit for the use of restricted materials.

In 1988, regulatory changes to Title 3 of the California Code of Regulations (CCR) Section 6738 required that employees use respirators when required by pesticide product labeling, regulations, or when respiratory protection is needed to maintain employee exposure below an applicable recognized exposure standard. It appears that the same requirements for respiratory protection were in place until the issuance of the US EPA 1997 Registration Eligibility Decision. In 1991 the label for one registered product (Gramoxone Extra Herbicide) required handlers pouring, loading, mixing concentrate or when exposure to concentrate is possible to wear a NIOSH/MSHA-approved pesticide respirator. The respiratory protection requirement evolved in 1994 to more specifically require a “Dust/mist filtering respirator (MSHA/NIOSH approval number prefix TC-21C).” There were exemptions put in place both federally and at the state level for respiratory protection when a closed system or enclosed cab were used.

Following the release of the Registration Eligibility Decision in 1997, US EPA removed all requirements for respiratory protection from the product labels. This was the case through at least 2001. Those requirements were subsequently changed in February 2001 when respiratory protections were reinstated for applicators/other handlers and mixer/loaders (“NIOSH-approved particulate respirator with any N, R, or P filter, NIOSH approval number prefix TC-84A, or a NIOSH-approved powered air-purifying respirator with an HE filter with NIOSH approval number prefix TC-21C"). These requirements were in place until 2018 when even more restrictive respiratory protection requirements were instituted.[3]

2. Current Regulatory Updates

In July 2021, US EPA issued a Paraquat Dichloride Interim Registration Review Decision (US EPA, 2021a) based in part on a preliminary ecological risk assessment and a draft human health risk assessment (US EPA 2019a). The Interim Registration Review decision determined that additional mitigation measures were necessary for paraquat to meet the FIFRA standard for registration including labeling requirements for closed transfer systems, aerial acreage limitations, limitations aimed at minimizing

[3] US EPA Amended Paraquat Dichloride Human Health Mitigation Decision, https://www.regulations.gov/document/EPA-HQ-OPP-2011-0855-0115

Handlers (other than mixers and loaders):

- Wear a minimum of a NIOSH-approved particulate filtering facepiece respirator with any R or P filter; OR a NIOSH-approved elastomeric particulate respirator with any R or P filter; OR a NIOSH-approved powered air purifying respirator with HE filters.

Applicators

- When applying to 80 acres or less in a 24-hour period, if not using an enclosed cab, applicators must wear a minimum of a NIOSH-approved particulate filtering facepiece respirator with any R or P filter; OR a NIOSH-approved elastomeric particulate respirator with any R or P filter; OR a NIOSH-approved powered air purifying respirator with HE filters.
- When applying to more than 80 acres in a 24-hour period, applications must be made using an enclosed cab. Enclosed cabs must have a nonporous barrier that totally surrounds occupant and prevents contact with pesticides outside of the cab.

handler inhalation exposures including closed cab and respirator requirements, increases in restricted entry intervals, prohibitions on use of mechanically pressurized handguns and backpack sprayers, an updated label statement regarding use of gloves, requirements for appropriate rinsing of closed system containers, and human flagger prohibitions. Additional changes were made to residential buffer zones and advisory and mandatory spray drift management language added to address offsite migration following applications. All of these restrictions were undertaken in an effort to mitigate potential exposure and effects of paraquat toxicity. Importantly, the 2021 Interim Decision states that there are no paraquat products registered for homeowner or residential use.

In September 2021, US EPA issued a letter to registrants clarifying certain mitigation requirements. US EPA accepted the registrants' product labeling changes to meet these requirements in August 2022. Most recently in August 2023, DPR accepted the registrants' product labeling changes for paraquat-containing products registered for use in California.

B. Paraquat Use in California

Between 1992 and 2018, annual paraquat use in the US increased more than 3-fold from approximately 3 million pounds in 1992 to approximately 11 million pounds in 2018 (https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2018&map=PARAQUAT&hilo=L&disp=Paraquat). According to DPR's Pesticide Use Reporting (PUR) Database, a similar increase occurred in California over the same period, from approximately 500,000 pounds in 1990 to over 1.4 million pounds in 2018 (https://www.cdpr.ca.gov/docs/pur/purmain.htm). However, since 2018, paraquat use in California has trended sharply downward with current use at or below 1990 levels. A downward trend in acres treated was also noted for the 1998–2023 period (Figure 1). The number of agricultural acres treated, agricultural pounds applied, and non-agricultural pounds of paraquat applied by year are shown in Figure 1. These data represent the most current PUR data for paraquat as of 2023.

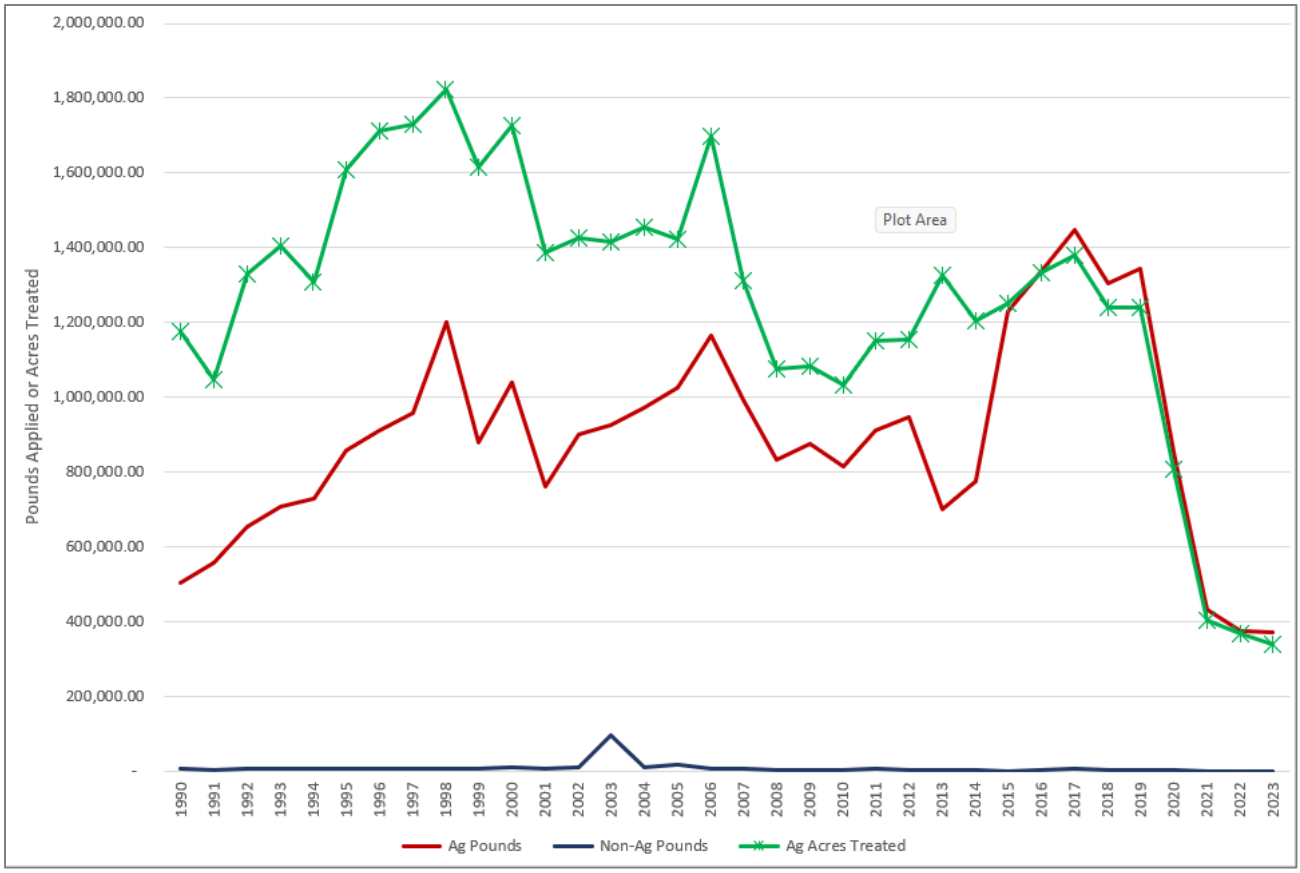

Figure 1. Paraquat dichloride: agricultural acres treated, agricultural pounds applied, and non-agricultural pounds applied in California from 1990–2023

III. Current Knowledge of Paraquat and Human Health Effects

As part of developing this preliminary report, HHA has summarized general toxicology and pesticide illness and injury data for paraquat. Because the focus of public comments centered largely on Parkinson's disease, this section also includes a summary of the etiology of Parkinson's disease and current incidence and mortality statistics for Parkinson's disease, as well as a summary of US EPA's draft human health risk assessment and systematic reviews of the paraquat literature.

A. General Toxicity

The following provides a brief overview of results from contract laboratories conducting guideline and supplemental toxicity studies as part of the federal and state pesticide

registration process as mandated by the Federal Insecticide Fungicide and Rodenticide Act (FIFRA).[4]

Oral exposure to rats and rabbits with paraquat resulted in predominantly fecal excretion within the first week of dosing. Part of the reduced entry of paraquat into urine was likely due to renal toxicity, as evidenced in studies in rabbits and dogs, though a role for enterohepatic recycling has not been investigated. Paraquat's mechanism of toxicity likely involves oxidative stress with resultant mitochondrial damage and lipid peroxidation leading to adverse organ system impacts, notably in lung and kidney.

Paraquat is classified as a Toxicity Category[5] II oral hazard with a median lethal dose (LD50) of 100–350 mg/kg in rats. Clinical signs include decreased activity, dehydration, hypothermia, reduced fecal volume, soft feces, and breathing irregularities. Necropsy revealed liver, intestine and lung discoloration, lung mottling, and renal pelvic dilatation. Paraquat is classified as a Toxicity Category III dermal hazard (LD50 > 2000 mg/kg) and as a Toxicity Category I inhalation hazard, with a median lethal concentration (LC50) of 0.6–1.79 µg/L in rats. Clinical observations following acute inhalation exposure include general debility, salivation, urinary incontinence, respiratory tract irritation, irregular breathing rales and hypoactivity. Necropsy revealed discolored lungs, pulmonary edema, and gaseous intestinal distention. Paraquat is designated as a Toxicity Category I ocular hazard (severe eye irritant) but is not considered a skin irritant. Skin sensitization studies were inconclusive.

Rats and mice showed pulmonary lesions following subchronic (13 week) dietary exposure. Pulmonary lesions were also evident in subchronic inhalation studies in rats. Chronic (1–2 year) oral dosing studies in rats revealed pulmonary lesions similar to those seen in the subchronic studies. In addition, there was evidence for lenticular cataracts and possible neoplastic lesions, although additional data would be needed to confirm this finding. There is some limited evidence of paraquat-induced genotoxicity as shown by chromosomal aberrations. Other genotoxicity results were equivocal. Evidence from guideline studies shows that paraquat is neither immunotoxic nor associated with neurotoxic, reproductive or development effects in laboratory animals.

Acute poisoning in humans including accidental or intentional exposure to large quantities of paraquat has resulted in severe irritation to the eyes and respiratory tract. Ingestion of large amounts of paraquat can affect the lung, kidneys, liver, and

[4] Federal Data Requirements for Pesticides are listed in Title 40 of Code of Federal Regulations, Part 158. Available at https://www.ecfr.gov/current/title-40/chapter-I/subchapter-E/part-158. US Environmental Protection Agency Health Effects Test Guidelines (Series 870) and Supplemental Test Protocols are available at https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances/series-870-health-effects-test-guidelines

[5] US EPA Label Review Manual Chapter 7: Precautionary Statements. US Environmental Protection Agency, Office of Pesticide Programs, Registration Division. Revised March 2018. https://www.epa.gov/sites/production/files/2018-04/documents/chap-07-mar-2018.pdf

cardiovascular system. Victims of poisoning may be at risk of developing lung fibrosis. Contact injuries in workers include skin rashes, burns, eye damage from splashes, nail damage, and nasal bleeding. Paraquat absorption through the skin can contribute to systemic toxicity, especially when contacting damaged or sensitive skin. Ingestion of low doses (< 20 mg/kg) may result in local irritation to oral and GI mucosa. Moderate exposure (20–40 mg/kg) may cause renal, liver and lung damage with respiratory failure developing after 2–3 weeks of exposure. High doses (> 40 mg/kg) may result in cause pulmonary fibroplasia, respiratory failure, and even death. A summary of case reports and case series for human poisoning incidents can be found in Appendix C of this document.

B. Human Illness and Injury Reports

A variety of illnesses and injuries associated with occupational or accidental exposure to paraquat have been identified at the state level through DPR's Pesticide Illness Surveillance Program (PISP) and from the Sentinel Event Notification System for Occupational Risk (SENSOR) Pesticides program through the National Institute for Occupational Safety and Health (NIOSH).

1. Pesticide Illness Surveillance Program (California)

Illnesses or injuries associated with exposure to paraquat were identified through DPR's Pesticide Illness Surveillance Program (PISP).[6] PISP maintains a database of pesticide-related illnesses and injuries reported in California. Case reports are received from physicians and workers' compensation records. The DPR database indicated that 133 cases of illness were linked to paraquat from 1992 to 2019 including 80 cases due to paraquat exposure alone, 3 cases due to exposure to paraquat combined with an adjuvant, and 49 cases to paraquat exposure in combination with other active ingredients. A year-to-year enumeration of the illness/injury cases reported to PISP, 1992–2019, appears in Figure 2.

[6]DPR's Pesticide Illness Surveillance Program (PISP) is tasked with collecting and evaluating pesticide illness and injury reports and assisting California county agricultural commissioners in investigating the exposure circumstances. California PISP Annual Reports are available at https://www.cdpr.ca.gov/docs/whs/pisp.htm

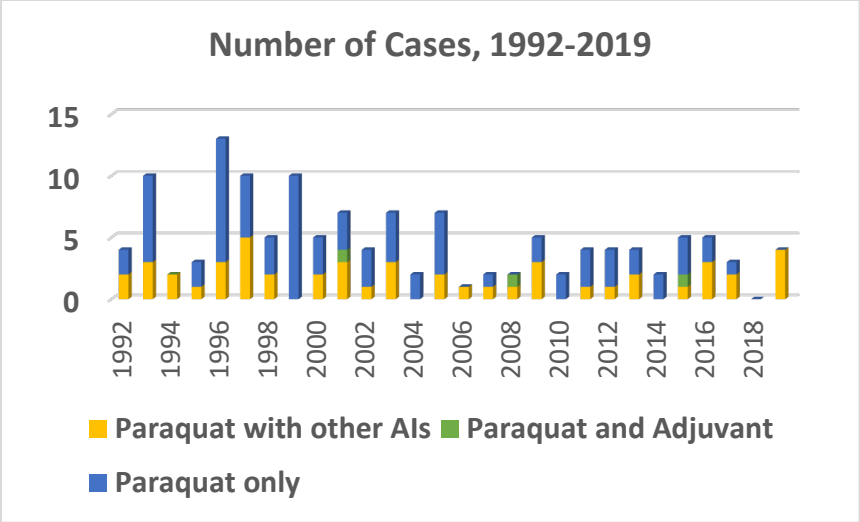

Figure 2. Number of illness / injury cases associated with paraquat by year reported in California through the DPR Pesticide Illness Surveillance Program (1992–2019)

Acute symptoms reported include headache, chest pain, rapid breathing, nausea, vomiting, abdominal pain, dizziness, chest congestion, coughing, uncoordinated gait, disorientation, dermal irritation and burning, upper respiratory irritation, and mucosal tissue and eye irritation. Of the very few incidences of accidental or intentional ingestion reported, the patients experienced multiple organ failure, pulmonary fibrosis, or renal failure, which were fatal in certain instances. The overwhelming proportion of reported cases included injury to the eye (26 cases; 33%) or skin (22 cases, 28%).

Handlers accounted for 75% of the illness/injury cases involving paraquat alone (60 cases) and 69% of the cases involving paraquat in combination with other active ingredients (91 cases). Ground applications including groundboom and pressurized hose-line sprayers accounted for most of the application methods associated with the reported illnesses/injuries. A majority of reported cases resulted from use on farms (109 of 132 cases). Forty four percent (35 cases) of the illness/injury cases resulted from exposure to paraquat alone via direct contact (spray/squirt, spills, or other forms of direct contact) and 38% (49 cases) of the direct contact cases resulted from paraquat alone and in combination with other active ingredients or adjuvants. A majority of paraquat-related injuries did not result in any disability or hospitalization days, although there were some cases resulting in disability for as long as 40 days and reports of indefinite hospital stays.

There has been a decrease in the number of reported illnesses and injuries associated with exposure to paraquat alone or in combination with other pesticides. Data for the most recent year (DPR, 2019) shows only 4 cases, all reporting exposure to paraquat in combination with other active ingredients. This decrease in reported illness and injuries

shows a similar downward trend as paraquat use in California over the same period, as shown above in Figure 1.

2. Sentinel Event Notification System for Occupational Risk (US)

Illnesses and injuries associated with occupational paraquat exposure are tallied for 13 states[7] through the Sentinel Event Notification System for Occupational Risk (SENSOR-Pesticides) administered by the National Institute for Occupational Safety and Health (NIOSH). There were 260 illness/injury cases from paraquat exposure from 1998–2020 reported to SENSOR-Pesticides, of which 190 were from paraquat-only formulations. For paraquat-only cases, the states with the highest number of cases include Washington (67 cases), California (41 cases), North Carolina (41 cases), and Texas (15 cases). For all SENSOR data, the majority of cases occurred in agricultural settings, with handlers reporting the most injuries. Applicators accounted for 43% of the cases (82), while pesticide handlers or pesticide equipment operators accounted for 3% of the cases (6) and mixer/loaders accounted for 7% of the cases (13). Injuries were recorded for skin (41%), nervous system (40%), eye (39%), respiratory system (33%), gastrointestinal system (30%), cardiovascular system (17%), and kidneys (5%).

C. Overview of Parkinson's Disease

Parkinson's disease a multi-system multi-symptomatic neurodegenerative disorder and the second most common neurodegenerative condition diagnosed in the US. It has been recognized as a syndrome in medical literature since the early 1950s. While not completely elucidated, the pathophysiology of Parkinson's disease involves death of nigral dopaminergic neurons, significant dopamine loss, the widespread formation of Lewy bodies, and intracytoplasmic deposits α-synuclein and ubiquitin (Constantinidis et al., 1983; Duvoisin, 1986; Erwin and Turco, 1986; Graybiel et al., 1990; Marsden, 1990). Pathologically, Parkinson's is the result of selective degeneration of dopaminergic neurons in the substantia nigra which causes decreased levels of dopamine in the striatum and leads to abnormal motor control (Nalls et al., 2015). The motor symptoms include bradykinesia, muscle tone rigidity, resting tremor, and postural instability. In addition, patients also display several non-motor symptoms such as sleep disorders, dementia, sensory abnormalities, and autonomic dysfunctions. Parkinson's disease is considered to have a multifactorial origin requiring combinations of multiple inputs and susceptibilities that become more relevant during aging. (Nalls et al., 2015). While the development of Parkinson's disease is more common under certain genetic, environmental, and lifestyle conditions, the causal factor(s) remain unknown.

[7] States participating in SENSOR include California, Florida, Illinois, Iowa, Louisiana, Michigan, Nebraska, New Mexico, New York, North Carolina, Oregon, Texas, and Washington. For more information about NIOSH's Pesticide Surveillance Program and the SENSOR-Pesticides, visit https://www.cdc.gov/niosh/topics/pesticides/overview.html

1. The Etiology of Parkinson's Disease

Investigations into the pathogenesis of Parkinson's disease have largely focused on degeneration of the dopaminergic neurons populating a region of the midbrain known as the substantia nigra pars compacta (SNpc). Onset of overt Parkinson's disease typically occurs after 60–80% of SNpc dopaminergic neurons are lost. Despite the widely recognized importance of dopaminergic systems to disease expression, neuronal systems employing other neurotransmitters such as acetylcholine, norepinephrine, and serotonin are also implicated in Parkinson's disease development and likely account for aspects of disease symptomology.

Underlying Parkinson's disease's motor pathophysiology is the accumulation of abnormal forms of the protein α-synuclein in the SNpc. Normally, α-synuclein plays a role in the storage and release of neurotransmitters. For people with Parkinson's disease, dopaminergic cell death is likely initiated by α-synuclein degradation, starting with protein misfolding and proceeding to aggregation, fibril formation, and eventual emergence of Lewy bodies. Lewy bodies, histologically visible proteinaceous aggregates that develop within nerve cells, are specifically characteristic or indicative of Parkinson's disease.

Genesis of Parkinson's disease may involve dysfunction in one or several cell and tissue processes. A current hypothesis involves mitochondrial dysfunction leading to overproduction of cytotoxic free radicals and subsequent damage to cellular macromolecules and structures. Damage to the ubiquitin-proteasome system that targets proteins for degradation within proteosomes may also be indicated. Evidence for cell-to-cell transmission of misfolded proteins (including α-synuclein) is a third such process active in Parkinson's disease.

While the causes of Parkinson's disease are not known with precision, genetic factors may play a role. Family history is evident in 15–25% of cases. Genetics appears to be a more prominent factor in early-onset forms of the disease, although these forms represent only about 10% of the total disease burden. According to the National Institutes for Neurological Diseases and Stroke, 28 loci have been associated with Parkinson's disease susceptibility, with many more tentatively identified. Prominent among these are mutations in the genes for α-synuclein, leucine-rich repeat kinase 2, several genes coding for proteins that maintain mitochondrial integrity, and β-glucocerebrosidase. Parkinson's disease is considered a multifactorial disease requiring combinations of multiple inputs and susceptibilities that become more relevant during aging. And while Parkinson's disease development is more common under certain genetic, environmental, and lifestyle conditions, the causal factor(s) remain unknown (reviewed in Chen and Ritz, 2018; Ball et al., 2019).

2. *Parkinson's Disease Incidence and Mortality in California and the US*

A study published in Nature investigated the prevalence of Parkinson's disease in the US by gathering data from a few states and projecting estimates from those data (Marras et al., 2018). Baseline information was collected from 2010 and estimated that 680,000 individuals in the US 45 years old and older were living with Parkinson's disease, which equals approximately 572 cases per 100,000 individuals in the US. From these data, the authors projected a prevalence for the year 2020 of 930,000 cases in the US for individuals over the age of 45 (Marras et al., 2018). In publishing incidence estimates from Medicare records in 2012, the same research group found 15,250 incident cases of Parkinson's disease in people over the age of 65, which equals a rate of 212 per 100,000 people (Willis et al., 2022).

The earliest US population disease estimates found for this report were from 1978. The overall prevalence of Parkinson's disease was reported as 1 in 1000, with a steep increase in prevalence for individuals over 50 years old of 1 in 100 (Pearce, 1978). If this is the case, then prevalence rates have not increased much in over 50 years. However, other publications indicate that many individuals go undiagnosed or are misdiagnosed, such that the actual number is likely to be much higher, with some estimations of as many as 1 million individuals in the US having the disease.[8]

In California, the Parkinson's Disease Registry collected data for a total of 107,601 unique cases in 2022, which is the best prevalence estimate available currently.[9] Unfortunately, the Registry's historical data is much less complete, so it is difficult to consider trends over time. When the registry's data collection system was in development, it recorded slightly over 50,000 unique cases in 2020. This would equal a rate of about 275 per 100,000 people based on a state population of 39.03 million in in 2022. This is lower than what would be expected using other estimated prevalences. Mortality rates provide the most reliable statistics available for Parkinson's disease. The Centers for Disease Control and Prevention (CDC) collect Parkinson's disease rates by state.[10] The mortality rates for Parkinson's disease in California from 2005–2022 are summarized in Table 1, below.

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[8] Parkinson's Disease: Challenges, Progress, and Promise. National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD. September 30, 2015. NIH Publication No. 15-5595 https://www.ninds.nih.gov/current-research/focus-disorders/parkinsons-disease-research/parkinsons-disease-challenges-progress-and-promise

[9] California Parkinson's Disease Registry (CPDR). Program Summary, March 2024. https://www.cdph.ca.gov/Programs/CCDPHP/DCDIC/CDSRB/CDPH%20Document%20Library/CPDR/CPDR_Program_Summary_MAR%202024_Final_v3.2.pdf

[10] QuickStats: Age-Adjusted Death Rates for Parkinson Disease Among Adults Aged 65 Years or Older. Centers for Disease Control and Prevention, National Center for Health Statistics, National Vital Statistics System, Mortality Data 1999–2017. https://blogs.cdc.gov/nchs/2019/09/06/4576/

Table 1. Mortality rate for Parkinson's disease in California from 2005 – 2022

Year	Parkinson's disease mortality rate	# of deaths	Year	Parkinson's disease mortality rate	# of deaths
2005	6.1 per 100,000	1,905	2018	8.2 per 100,000	3,519
2014*	6.8	2,641	2019	8.8	3,874
2015	7.2	2,896	2020	9.3	4,147
2016	7.6	3,088	2021	9.5	4,044
2017	8.1	3,370	2022	9.4	4,289

*Data for the years 2006–2013 were not available from the NCHS website.

From 1999 to 2017, age-adjusted death rates for Parkinson's disease among adults aged 65 years or older increased from 41.7 to 65.3 per 100,000 population. While not specifically reflective of the prevalence of Parkinson's disease in California, these statistics give an indication of the impact of mortality in the state's population from the disease. It appears that there is a trend of increasing Parkinson's disease related mortality in California over the past decade. However, it is unclear is this is due to an increasing rate of the disease, or if it reflects better definition, recognition, and diagnosis.

Parkinson's disease-related mortality and incidence in California and nationwide may be increasing. This may be due to better recognition and diagnosis. However, one of the factors in increasing incidence, and one of the biggest risk factors, is age (Willis et a., 2022). As the population ages, there will be a natural trend for higher rates of Parkinsonism in the population (Willis et al., 2022).

D. US EPA Evaluation of the Paraquat Literature

As part of its issuance of a Proposed Interim Mitigation Decision in March 2016, US EPA initiated an update to its human health risk assessment of paraquat. Because of the enormity of the database, the Health Effects Division in US EPA's Office of Pesticide Programs also initiated a systematic review of the open literature of both population-based studies and toxicology studies involving paraquat and putative health effects.

1. US EPA's Systematic Review of the Open Literature

Five hundred and seventy-six (576) unique peer-reviewed open literature scientific articles were identified across multiple search engines. A supplemental search was specifically conducted of publications from the Agricultural Health Study (AHS), an ongoing effort spearheaded by the National Institutes of Health to follow health outcomes in a cohort of approximately 52,000 agricultural workers and 32,000 spouses from Iowa and North Carolina (https://aghealth.nih.gov/). Articles were screened according to specific inclusion/exclusion criteria with a goal of identifying all possible

human health outcomes reported in the open literature including lung and respiratory effects, cancer, thyroid effects, oxidative stress, Parkinson's disease and a number of other outcomes. A total of 74 peer-reviewed population based studies were considered relevant an underwent further review. US EPA published its findings in Paraquat Dichloride: Tier II Epidemiology Report (US EPA, 2019b).

From this systematic review of population based studies, US EPA concluded that there was insufficient epidemiologic evidence for a clear causal relation between the occurrence of general lung function and respiratory symptoms, wheeze, allergic rhinitis, asthma, and chronic bronchitis and occupational paraquat exposure and insufficient evidence to support a relationship between any paraquat exposure and lymphoma. Although not significant, odds ratios of >1 were observed for other cancer types. US EPA also concluded that there was insufficient evidence of an association between paraquat exposure and diabetes, myocardial infarction, renal/liver function, thyroid disease, and aplastic anemia, and limited but insufficient evidence for a causal relationship between occupational paraquat exposure and end-stage renal disease based on the results from AHS.

2. US EPA's Systematic Review of the Association between Paraquat Exposure and Parkinson's Disease

Of the 74 relevant articles, 26 investigated the association between paraquat exposure and Parkinson's disease. US EPA evaluated findings in these studies along with a review of the current toxicological database specific to paraquat and Parkinson's disease and published its findings in Paraquat Dichloride: Systematic Review of the Literature to Evaluate the Relationship between Paraquat Dichloride Exposure and Parkinson's disease (US EPA, 2019c).

US EPA reviewed 26 human studies that evaluated data from three agricultural cohorts, nine hospital-based populations, and one Parkinson's disease registry in Nebraska. Study populations were evaluated for occupational and/or non-occupational exposure pathways that varied in magnitude, frequency, and duration. In its review, the agency assumed that occupational study populations were more likely to be exposed as a direct result of handling/using/applying paraquat. With respect to occupational exposure, US EPA determined that there was limited but insufficient epidemiologic evidence of a clear associative or causal relationship between occupational exposure and the development or risk of Parkinson's disease. With respect to non-occupationally exposed study populations, US EPA determined that there was insufficient epidemiologic evidence of a clear associative or causal relationship between paraquat exposure and the development or risk of Parkinson's disease. This conclusion was based on the small number of studies on non-occupational populations, lack of consistent evidence of a positive association, and the potential for bias (US EPA 2019c).

US EPA also evaluated the paraquat/Parkinson's relationship in 11 neurotoxic animal model studies. One animal study in particular was cited in which motor impairment in male mice was connected to dopaminergic neuron degeneration and neurochemical disruption, two hallmarks integral to the pathology of Parkinson's disease in humans. While US EPA recognized other evidence deriving from toxicokinetic, in vitro, and mechanistic analyses to be consistent in some cases with a Parkinsonism-type etiology, overall, the agency considered that the animal evidence was inadequate with respect to consistency, dose-response, or temporal concordance (US EPA, 2019c).

3. Draft Human Health Assessment for Registration Review

As part of the proposed interim mitigation decision, US EPA also initiated a human health risk assessment in support of registration review (US EPA, 2019c). This risk assessment was also submitted to DPR as part of the public comments received in November 2022. A summary of the findings from the risk assessment follows and a more detailed description of findings is in Appendix A of this document.

The 2019 draft US EPA human health risk assessment contains a hazard analysis, proposed points of departure (PODs), exposure estimates (including assumptions on default body weight, unit exposure, transfer coefficient, acreage treated, and amounts used), uncertainty factor determinations, and the risk calculations associated with paraquat use in the US. The risk assessment also documents human pesticide-related illness and injuries through 2014. US EPA found no dietary risks when paraquat was used according to the label instructions. However, there were potential risks identified for mixer/loaders/applicators as well as reentry workers. The agency also identified potential risks from spray drift to bystanders.

Shortly after the release of the 2019 systematic review, a newer AHS prospective cohort study was released that investigated Parkinson's disease (Shrestha et al., 2020). The investigators stated that there was no association between paraquat exposure alone and Parkinson's disease. However, when co-exposures were evaluated, results indicated a strong association for combined paraquat exposure and head trauma and an increased risk of developing Parkinson's disease. After the publication of this study, US EPA updated its review and provided a detailed evaluation of Shrestha et al., 2020 in its Response to Comments on the Proposed Interim Decision for Registration Review and updated Occupational Handler Exposure and Risk Estimates (US EPA, 2021b). The results underscored US EPA's earlier finding that there is insufficient evidence of a direct association with paraquat alone and the increased risk of Parkinson's disease, however noting the exception of the sub analysis for head injury (US EPA, 2021b).

As summarized in the Regulatory and Registration History of Paraquat section earlier in this document, US EPA released the Paraquat Dichloride Interim Registration Review Decision in July 2021 (US EPA, 2021a). This finalized certain portions of the human health assessment, determining that additional mitigation measures were necessary for

paraquat to meet the FIFRA standard for registration. These mitigation measures included paraquat labeling requirements for closed transfer systems, aerial acreage limitations, limitations aimed at minimizing handler inhalation exposures including closed cab and respirator requirements, increases in restricted entry intervals, prohibitions on use of mechanically pressurized handguns and backpack sprayers, an updated label statement regarding use of gloves, requirements for appropriate rinsing of closed system containers, and human flagger prohibitions. Additionally, changes were made to residential buffer zones and advisory and mandatory spray drift management language to address offsite migration following applications, as well as the prohibition of any paraquat products registered for any homeowner or residential use. All of these restrictions were undertaken in an effort to mitigate potential exposure and effects of paraquat toxicity.

IV. Submitted Population-Based Studies

A. Background

In 2022 and 2023, DPR received a combined 105 documents as part of public comment in response to the annual pesticide registration renewal cycle. Of these, HHA identified and evaluated 11 original peer-reviewed population based studies according to DPR's Guidance for Toxicology Study and Data Acceptability in Registration Review and Risk Assessment (DPR, 2023) and/or the Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides from the US Environmental Protection Agency (US EPA, 2016). Nine studies investigated the association between paraquat exposure and the development of Parkinson's disease. Seven of these studies were cohort, case-control or cross sectional studies (Kamel et al., 2007; Costello et al., 2009; Ritz et al., 2009; Gatto et al., 2010; Tanner et al., 2011; Lee et al., 2012; Caballero et al., 2018), and 2 were meta-analyses (Tangamornsukan et al., 2019; and Vaccari et al., 2019). Of the studies submitted to DPR, US EPA also reviewed Kamel et al., 2007, Costello et al., 2009, Ritz et al., 2009, Gatto et al., 2010, Tanner et al., 2011, and Lee et al., 2012 in its systematic review of Parkinson's disease (US EPA, 2019c). Two population based studies were submitted to DPR on the association between paraquat exposure and other health effects, specifically thyroid hormone effects and thyroid cancer (Goldner et al., 2010; Omidakhsh et al., 2022).

Study populations of interest included the Agricultural Health Study (AHS), an ongoing effort to follow health outcomes in a cohort of approximately 52,000 agricultural workers and 32,000 spouses from Iowa and North Carolina (https://aghealth.nih.gov/) and the Parkinson's Environment and Genes (PEG) study, conducted among residents of farming regions in the California's southern Central Valley. The PEG study examined Parkinson's disease incidence among more than 2000 residents of Kern, Fresno, and Tulare counties since 2001 (https://www.ritzenvironmentalepi.com/?page_id=267).

The results of these studies were expressed as odds or hazard ratios accompanied by 95% confidence intervals. Ratios greater than 1.0 suggested that paraquat may have a role in the development of the health outcome under study. However, the reliability of the findings is dependent on several factors including the confidence interval range (which relates closely to statistical significance), the total number of cases and controls, the accuracy and specificity of the case determinations, the adequacy of the exposure determinations, and the identification and control of other potential confounders. Individual evaluations of submitted studies follow.

B. Summaries of Submitted Population-Based Studies (by year of publication)

Kamel et al. (2007)

Kamel et al. (2007) used data from the Agricultural Health Study (AHS), a cohort study, to assess the association between Parkinson's disease and pesticide exposure. At enrollment (1993–1997) and at five years of follow-up (1999–2003) AHS gathered information from pesticide applicators and their spouses using a structured questionnaire about pesticide use and Parkinson's disease diagnosis. More than 99% of applicators were male, and 96% of spouses were female, so this was used as a proxy measure for gender in the study. To assess pesticide exposure to individual chemicals, investigators asked about frequency of use of 50 pesticides including paraquat and utilized hierarchical logistic regression models to help control for the correlation among use and function of different chemicals. The results were categorized as prevalent or incident, depending on if the participant reported Parkinson's disease diagnosis at enrollment or during follow-up, respectively. There were 48,938 applicators assessed for prevalent Parkinson's disease, and 33,076 assessed for incident Parkinson's disease. There were 30,702 spouses assessed for prevalent Parkinson's disease, and 22,933 spouses assessed for incident Parkinson's disease. There were 83 prevalent Parkinson's disease cases, and 79,557 prevalent controls. There were 78 incident cases and 55,931 incident controls. Prevalent Parkinson's disease was not positively associated with pesticide exposure in general, although there was a significant association with paraquat exposure (OR=1.8, CI=1.0, 3.4). The association between incident Parkinson's disease and paraquat exposure was null (OR=1.0, CI=0.5, 1.9).

<u>Strengths</u>

The prospective design can avoid the potential for recall bias when relying on self-reporting for assessing exposure. Additionally, sophisticated hierarchical models were used to avoid confounding. However, because 99% of the applicators and 56% of the spouses in the study used pesticides in some capacity, the chances of collinearity and confounding remained high.

<u>Weaknesses</u>

- Despite the large cohort, there were only 11 incident cases at 5 years of follow-up who were exposed to paraquat. This small sample size leaves the analysis open to error due to unmeasured variability in the association.

- The analysis was exploratory in nature due to the fact that they investigated 50 pesticides at one time. This increases the chances of finding a significant result, making any results found less meaningful. This design is beneficial for hypothesis generation, but one should use caution in inferring causality.
- The assessment of exposure and Parkinson's disease status both relied on self-report. This may potentially lead to misclassification and bias.
- Study enrollment was 1993–1997, with some participants likely using paraquat before this time. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.

DPR Conclusion

This was a hypothesis generating study. The results do not indicate an association between Parkinson's disease and paraquat exposure because of the null result in the analysis of the incident cases. While the prevalent results do indicate a correlation between Parkinson's disease and paraquat exposure, those particular data are more prone to bias and do not establish that exposure occurred before the disease. Moreover, for the prevalent results it is not known if the exposure or the disease happened first, nor if the fact that they had Parkinson's disease affected their interest in agreeing to participate in a study about Parkinson's disease and pesticide exposure. Neither of those factors apply to the incident results.

Costello et al. (2009)

The objective of this case-control study was to examine the joint effects of paraquat and maneb exposures on the development of Parkinson's disease in human subjects. To do this the investigators recruited participants between 1998 and 2007 who were diagnosed with Parkinson's disease within three years of enrollment and lived in central California for at least the five years prior. Control subjects were recruited from Medicare lists and tax assessor records using a clustered sampling design. There were 368 cases (161 females, 207 males) and 341 controls (165 females, 176 males). Exposure to paraquat and maneb was assessed through a geographical analysis identifying who lived within 500 meters of an application of paraquat/maneb using the PUR database and a detailed residential and work history obtained from interviews with the subjects. For most of the reported results, no distinction was made between paraquat and maneb exposure. Participants living or working within 500 meters of a paraquat/maneb application were considered to be exposed. The investigators also examined differences between time periods of exposure (1974–1989 and 1990–1999) and age at Parkinson's disease diagnosis (above or below 60 years old) as potential effect modifiers. The overall odds ratio between exposure to paraquat alone (with no maneb exposure) and Parkinson's disease was 1.01 (0.71, 1.43). The investigators assessed the interaction of paraquat/maneb and time period and age group on Parkinson's disease using logistic regressions and presenting the stratified analyses. Overall, there

was an increased odds of paraquat/maneb exposure for Parkinson's disease cases with an odds ratio of 1.75 (1.13, 2.73). The association was stronger in the earlier time period (OR=2.14, CI=1.24, 3.68 for 1974–1989), and for younger participants (OR=5.07, CI=1.75, 14.71 for participants aged 60 years and younger).

Strengths

Using geographical methods for exposure assessment reduces the likelihood of error due to recall bias (although recall was relied upon for residential and occupational history which was used to assess exposure), a particularly problematic source of bias in retrospective studies. Unlike many studies utilizing this design, the hypothesis was specific to a single combination of pesticides and one endpoint.

Weaknesses

- Despite the complexity of the recruitment process, there were important differences in demographic factors between the subjects and the controls. On average, the controls 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.
- Using geographical location as a proxy measure of exposure is an imprecise method for determining who was exposed and to what level. This approach avoids recall bias of the subjects. However, it is too imprecise a measure of exposure to avoid information bias.
- The investigators did not control for co-exposure of paraquat with other pesticides. While the investigators adjusted for likelihood of occupational exposures using information on past employment, this does not alleviate the potential for confounding.
- Study enrollment was 1998 and 2007, with some participants asking to recall work-related exposures back to 1974. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.

DPR Conclusion

Results indicate that paraquat/maneb exposure may be associated with the development of Parkinson's disease. This was particular evident in early onset cases of Parkinson's disease (before age 60) and for participants with potential paraquat/maneb exposure prior to 1989. It is unclear why the two work history time periods (1974–1989 and 1990–1999) were chosen for this study, although US EPA and DPR instituted significant changes to paraquat use protections in 1988. Given differing exposure conditions and other limitations in the study design, caution is warranted in applying these results beyond this study population.

Ritz et al. (2009)
The objective of this case-control study was to build on findings from Costello et al., (2009) to consider genetic variants in Parkinson's disease patients. Ritz and colleagues analyzed the same study participants as Costello et al. (2009), thus had the same recruitment (1998–2007) and exposure assessment methods. The study did not distinguish between exposure to paraquat and maneb. There were 324 cases (145 females, 179 males), and 334 controls (166 females, and 168 males). Participants living or working within 500 meters of a paraquat/maneb application were considered to be exposed. The investigators estimated how long participants were exposed and compared participants above and below the median of that exposure rather than identifying those who were ever/never exposed. The study assessed how genetically susceptible participants were to Parkinson's disease by determining the presence of specific alleles. Genetic susceptibility was defined as the presence of dopamine transporter (DAT) locus clades and 3' variable number of tandem repeats . The odds ratios for the association of these different genetic variations and Parkinson's disease ranged from 1.15 (0.82, 1.86) to 1.86 (0.96, 3.57) with the DAT-A diplotype being significant (OR=1.66, CI=1.08, 2.57). The interaction of paraquat/maneb and genetic susceptibility on development of Parkinson's disease was assessed using logistic regressions and the stratified analysis was presented. Residential and occupational exposure to paraquat/maneb were both positively associated with Parkinson's disease, with odds ratios of 2.32 (1.23, 4.40) and 1.56 (0.95, 2.56), respectively. The stratified results indicated the presence of an interaction with odds ratios of 2.83 (1.01, 7.92) and 4.53 (1.70, 12.09) for those with both paraquat/maneb exposure and 2+ susceptibility alleles compared to those with neither exposure nor any susceptibility alleles among occupational and residential populations respectively.

Strengths
Using geographical methods for exposure assessment reduces the likelihood of error due to recall bias (although recall was relied upon for residential and occupational history which was used to assess exposure), a particularly problematic source of bias in retrospective studies. Unlike many studies utilizing this design, the hypothesis was specific to a single combination of pesticides and one endpoint, and focused on replicating previous results, which is important because it checks the reliability of the previous study results.

Weaknesses
- Despite the complexity of the recruitment process, there were important differences in demographic factors between the subjects and the controls. On average, the controls 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.

- Using geographical location as a proxy measure of exposure is an imprecise method for determining who was exposed and to what level. This approach avoids recall bias of the subjects. However, it is too imprecise a measure of exposure to avoid information bias.
- The investigators did not control for co-exposure of paraquat with other pesticides. While the investigators adjusted for likelihood of occupational exposures using information on past employment, this does not alleviate the potential for confounding.

DPR Conclusion

The results indicate that Parkinson's disease may be more likely to develop in people with paraquat/maneb exposure and genetic susceptibility. However, the associations were only for co-exposure of paraquat and maneb. It is unclear how participants with exposure to only one of the pesticides were evaluated. In addition, the odds ratio for occupationally exposed participants was lower than for residentially exposed participants, which is opposite of what would be expected. As such, caution is warranted in the applicability of the results beyond this study population.

Gatto et al. (2010)

Gatto and colleagues analyzed the same study participants as Costello et al. (2009) and Ritz et al. (2009) including the recruiting (1998–2007) and the approach of using median exposure to distinguish participants as either having "high" exposure or "low/no" exposure. However, the focus of this study was on paraquat only. This case-control study also assessed how susceptible participants were to developing Parkinson's disease depending on the presence or absence of two single nucleotide polymorphisms which have previously been implicated in Parkinson's disease susceptibility. There were 333 cases (149 females, 184 males) and 336 controls (167 females, 169 males). The interaction between paraquat exposure and genetic susceptibility on development of Parkinson's disease was assessed using logistic regressions, with the results presented as a stratified analysis. The odds of paraquat exposure and genetic susceptibility were elevated in Parkinson's disease patients compared to controls. When further stratified by age group, the odds ratios ranged from 0.67 (0.36, 1.24) to 3.15 (0.74, 13.37), although none were significant.

Strengths

Using geographical methods for exposure assessment reduces the likelihood of error due to recall bias (although recall was relied upon for residential and occupational history which was used to assess exposure), a particularly problematic source of bias in retrospective studies. Unlike many studies utilizing this design, the hypothesis was specific to one pesticide (paraquat) and one endpoint.

Weaknesses

- Despite the complexity of the recruitment process, there were important differences in demographic factors between the subjects and the controls. On average, the controls 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.
- Using geographical location as a proxy measure of exposure is an imprecise method for determining who was exposed and to what level. This approach avoids recall bias of the subjects. However, it is too imprecise a measure of exposure to avoid information bias.
- The investigators did not control for co-exposure of paraquat with other pesticides. While the investigators adjusted for likelihood of occupational exposures using information on past employment, this does not alleviate the potential for confounding.

DPR Conclusion

The results did not strongly indicate an interaction between genetic susceptibility and paraquat exposure in the development of Parkinson's disease. This combined with the limitations in the design of the study indicate that no definitive conclusions should be drawn from this research.

Goldner et al. (2010)

This cohort study explored the connection between use of specific pesticides and thyroid disease in spouses enrolled in the AHS cohort. Enrollment was from 1993–1997 with follow-up 5 years after initial enrollment (1999–2003). This study was limited to female spouses of applicators, including a total of 14,486 spouses with no thyroid disease, 369 with hyperthyroid disease, 1114 with hypothyroid disease, and 560 with other thyroid disease included in the analysis. Spouses indicated their overall pesticide exposure by reporting ‘‘years lived or worked on a farm,’’ ‘‘ever personally mixing or applying any pesticide during their lifetime,’’ ‘‘total years of personally mixing or applying pesticides,’’ ‘‘days per year of mixing or applying pesticides,’’ and “ever use” of 50 itemized herbicides, insecticides, fumigants, and fungicides. This exposure information was focused on first-hand exposure of the spouse. There were insufficient participants with hyperthyroid disease to calculate an odds ratio for paraquat. However, the investigators found an association between “ever-use” of paraquat and increased odds of hypothyroid disease of 1.8 (95% CI: 1.1, 2.8). This study investigated the association between spouses' own pesticide exposure and thyroid disease, not the husband's pesticide use. And while an association was found for “ever-use” of paraquat, no exposure-response relationship was shown with increasing years of use, from less than 5 years to greater than 17 years of reported use.

Strengths

The prospective design is an important factor in avoiding information bias from recall and other factors related to exposure assessment. A strong model selection process was used to include the covariates that were most likely to confound the associations being examined.

Weaknesses

- Despite the large cohort, there were only 21 reported cases of hypothyroid disease and 8 reported cases of other thyroid disease at 5 years of follow-up. This small case size reduces the statistical power of the study to find a true association and leaves the analysis open to error due to unmeasured variability in the association.
- The analysis was exploratory in nature due to the fact that they investigated 44 pesticides at one time. This increases the probability of finding a significant result by chance alone, making any results found less meaningful.
- Study enrollment was 1993–1997, with a questionnaire of spouse use/exposure back to 17 years prior to enrollment (e.g., as early as 1976). Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.
- Importantly, while as association between "ever-use" of paraquat was found with hypothyroidism, there was no difference in odds of developing hypothyroidism with increasing years of potential paraquat exposure, up to 17 years. This calls into question the veracity of any association with this health outcome and paraquat.

DPR Conclusion

The results indicate that there may be an association between hypothyroid disease and paraquat exposure, but the weaknesses inherent in the ascertainment of exposure and disease status warrant further research before conclusions should be drawn.

Tanner et al. (2011)

The intent of this study was to determine if specific pesticides are associated with Parkinson's disease or clinical symptoms of Parkinson's disease. This was a follow up study to an earlier study of associations between different occupations and occupational exposures and risk of Parkinson's disease which found that of 71 pesticide users, cases were more likely to have ever used pesticides (in general) (Tanner et al., 2009). However, paraquat was rarely used among the study population and detailed associations could not be made. In this nested case-control study within AHS, participants were looked at nine years after initial enrollment (1993–1997). Parkinson's disease was assessed with in-person visits from neurologists or identified on death certificates as a contributing cause of death. There were 110 Parkinson's disease cases (30 females and 80 males) and 358 controls (93 females and 265 males). Control

subjects were selected from a stratified random sample of AHS participants without Parkinson's disease or cognitive impairment. To assess exposure to pesticides, the investigators relied on self-reported use of pesticides as determined in the questionnaires at enrollment and in additional interviews during the nine-year follow-up. Exposure was summarized as ever or never use of 31 pesticides including paraquat, and lifetime days of use. Logistic regression found an odds ratio of 2.5 (1.4, 4.7) for paraquat exposure with Parkinson's disease, indicating that Parkinson's disease patients had 2.5 times the odds of having been exposed to paraquat as control subjects. In addition, a very similar odds ratio of 2.5 (1.3, 4.7) was established for rotenone, raising the possibility that co-exposure to these two pesticides played a role in Parkinson's disease incidence.

Strengths

The nested case-control design allowed the researchers to ensure that exposure happened before Parkinson's disease diagnosis, unlike a traditional case-control. Additionally, this study had a strong methodology for assessing Parkinson's disease diagnosis with neurologists doing in-person interviews.

Weaknesses

- The statistical analysis was not hierarchical and did not account for the potential for confounding due to exposure to multiple pesticides. This was likely due to the small sample size, which limited the options for analysis.
- Even though this was a follow-up study, it was still exploratory in nature. The researchers investigated 31 different chemicals for potential associations with Parkinson's disease. This increases the chances of finding a significant result, making any results found less meaningful. This was a follow-up study from the same study population as the previous analysis of Kamel et al. (2006). While this study helps add to the weight of evidence, it should not be viewed as independent from the other AHS analyses.
- The assessment of exposure relied on self-report. This could result in misclassification and bias.
- Study enrollment was 1993–1997, with some participants likely using paraquat before this time. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.

DPR Conclusion

Tanner et al. (2011) was a hypothesis generating study. The results suggest an association between Parkinson's disease and paraquat exposure in light of the significant odds ratio of 2.5. With the limitations inherent to the methods of the study, this finding in isolation does not merit action beyond recognition of a need for additional research.

Lee et al. (2012)
The objective of this case-control study was to examine the evidence of the independent and joint effects of traumatic brain injury and paraquat exposure on the development of Parkinson's disease. The same study participants and recruiting (1998–2007) were used in this study as in Costello et al. (2009), Ritz et al. (2009), and Gatto et al. (2010). Exposure to paraquat was assessed through a geographical analysis identifying who lived within 500 meters of the application of paraquat using the PUR and a detailed residential and work history obtained from interviews with the subjects. People living or working within 500 meters of a paraquat application were considered to be exposed. There were 357 cases (152 females, 205 males) and 754 controls (402 females, 352 males). The interaction of paraquat and traumatic brain injury on Parkinson's disease was assessed using logistic regressions for the multiplicative scale, and relative excess risk due to interaction (RERI) for the additive scale. A significant but relatively weak association between paraquat and Parkinson's disease was found with an OR of 1.36. The combination of paraquat exposure and traumatic brain injury combined compared to neither exposure resulted in an OR of 3.01 (1.51–6.01) indicating that there may be an interaction between the two in the development of Parkinson's disease. However, on the additive scale, the RERI was weak and not significant.

Strengths
Using geographical methods for exposure assessment reduces the likelihood of error due to recall bias, a particularly problematic source of bias in retrospective studies. Additionally, unlike many studies utilizing this design, the hypothesis was specific to one pesticide and one endpoint.

Weaknesses
- Despite the complexity of the recruitment process, 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.
- The geographical exposure assessment is a proxy measure of exposure and is imprecise in who was exposed and how much exposure there was. This approach avoids recall bias of the subjects. However, it is too imprecise a measure of exposure to avoid information bias.
- Study enrollment was 1993–1997, with some participants likely using paraquat before this time. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely

experienced, such that paraquat during the study period does not reflect current paraquat use.
- Little was done in this study to attempt to control for co-exposure of paraquat with other pesticides. This co-exposure could be a source of vulnerability of the analysis to confounding, making the results possibly invalid.

DPR Conclusion

While the interaction results were not conclusive, they do suggest that there may be increased risk of Parkinson's disease from people with both paraquat exposure and a history of traumatic brain injury. However, the vulnerabilities to bias inherent in the study design are too strong to draw any definitive conclusions from this research.

Caballero et al. (2018)

This case-control study examined the relationship between residential exposures to agricultural chemicals and Parkinson's disease-related mortality in Washington State. The study population consisted of people for whom Parkinson's disease was identified as an underlying cause of death in state mortality records between 2011 and 2015. The investigators compared subjects who were under 75 years old when they died (cases) to those who were 75 and older when they died (controls). Exposure to four different agricultural chemicals (including paraquat) was evaluated using a geographical analysis that identified those who lived within 1 km of the application of these pesticides from 2011–2015. Additionally, the potential for well water contamination was assessed if the case or control's groundwater well was within 500 meters of an application of these pesticides. There were 659 cases (189 females, 470 males) and 3932 controls (1555 females, 2377 males) Logistic regressions indicated that the odds of exposure to paraquat was 22% higher among participants who died of Parkinson's disease before age 75 compared to those who died of Parkinson's disease after age 75. However, this was not a significant finding.

Strengths

Using mortality data for diagnosis of Parkinson's disease and geographical methods for exposure assessment reduces the likelihood of error due to recall bias, a particularly problematic source of bias in retrospective studies.

Weaknesses

- The study assessed exposure during the same time frame as mortality. Because life expectancy from the onset of Parkinson's disease is around ten years, the associations found should not be viewed as necessarily causal.
- Using geographical location as a proxy measure of exposure is an imprecise method for determining who was exposed and to what level. This approach avoids recall bias of the subjects. However, it is too imprecise a measure of exposure to avoid information bias.

DPR Conclusion

Because of the potential vulnerabilities and weaknesses stated above, the design of this study is not appropriate for drawing conclusions about a potential causal relationship between paraquat and Parkinson's disease.

Tangamornsuksan et al. (2019)

Tangamornsuksan and colleagues conducted a meta-analysis to evaluate the possibility of an association between paraquat exposure and Parkinson's disease. A total of 13 studies were included in the final meta-analysis from a pool of over 7000 studies that resulted from a systematic literature review conducted in 2018. All 13 studies used self-report to determine exposure to paraquat. The authors used a random-effects model to calculate the pooled effect size so that correlation within studies was accounted for. There was a total of 3231 cases and 4901 controls in these studies. The overall odds ratio for the 13 studies was 1.64 (1.27, 2.13), indicating a significant positive association between paraquat exposure and Parkinson's disease. This result had an I^2 test of 24% indicating a low likelihood of heterogeneity. A sensitivity analysis of study quality, types of statistical adjustments used in the studies, study design, potential publication bias, and criteria for cases selection did not reveal any flaws in the meta-analysis.

Limitations

- Many of the studies included in the meta-analysis did not provide adjustment for confounders which could potentially change the results.
- The authors note that their findings support previous findings of the association of paraquat use with Parkinson's disease, however that underlying (primary) studies lacked objective measurement of paraquat exposure was inadequate.

DPR Conclusion

Overall, limitations in the methods used in the included (primary) studies for exposure assessment and the lack of control for confounding weakens the findings.

Vaccari, et al. (2019)

Vaccari and colleagues conducted a meta-analysis of a possible association between paraquat exposure and Parkinson's disease. A total of 11 studies were included in the final meta-analysis from a pool of over 4021 studies that resulted from the systematic literature review conducted in 2019, eight of which were common to both this analysis and that of Tangamornsuksan et al. (2019). Nine studies relied on self-report for exposure assessment and two studies relied on geographical analysis. A random-effects model was used to calculate the pooled effect size so that correlation within studies was accounted for. There was a total of 2466 cases and 62,279 controls included in the analysis. The overall odds ratio for the 11 studies was 1.43 (1.06–1.91), with a moderately high degree of heterogeneity (I^2 = 56%). When one study was removed for being an outlier, the odds ratio was 1.24 (1.03, 1.49) with an I^2 = 23%, indicating low likelihood of heterogeneity. The authors conducted a sensitivity analysis

for different exposure durations, different co-exposures with paraquat, and study quality, the results of which demonstrated a variety of effect sizes and inconsistencies in statistical significance. Finally, the authors performed a qualitative causality analysis of the association between paraquat and Parkinson's disease, which provided equivocal results.

Limitations

- The authors evaluated the likelihood of publication bias affecting their results with an Egger's test and a funnel plot. The latter produced evidence of publication bias. It is not clear how the authors addressed that bias.
- There were no adjustments for possible confounders in the analysis because of the inconsistency in which variables were used in the included studies. This could potentially alter the results.
- The authors state that with the relatively low estimates of risk (meta-analytic result of 1.25 (95 % CI: 1.01–1.55) do not enable one to propose a definitive conclusion regarding a causal relationship between paraquat and Parkinson's disease.
- The authors also caution that a dose-response relationship has not been established between paraquat exposure and Parkinson's disease.

DPR Conclusion

Overall, limitations in the methods used in the included (primary) studies for exposure assessment, the lack of an apparent exposure-effect relationship, and lack of control for confounding weakens the findings.

Omidakhsh et al. (2022)

Omidakhsh et al. (2022) was a case-control study that hypothesized that pesticide exposure may be related to increasing rates of advanced thyroid cancer in California. Participants with thyroid cancer were recruited from the California Cancer Registry. Population controls were selected from Medicare, randomly mailed residences, and tax assessor parcels (Parkinson's Disease Environment and Gene study). Participants were only selected if they were age 35 years or older. Exposure assessment was done using the PUR database and a geographic analysis estimating whether pesticide was applied within 500 meters of the residence of participants (for disease cases this represented the address at the time of diagnosis). The investigators estimated the association of exposure to 29 different pesticides individually with thyroid cancer. For paraquat, cases had 1.46 (1.23, 1.73) times the odds of ever having lived within 500 meters of an application than controls. Investigation of co-exposure of paraquat and 12 other pesticides was also carried out. Results were noted for paraquat alone, with no increase in risk when co-exposures to additional pesticides were considered. This means that additive exposures or co-exposures did not result in an increased odds of developing thyroid cancer, and that the increased odds could be attributed to paraquat exposure alone.

Strengths
Using geographical methods for exposure assessment reduces the likelihood of error due to recall bias (although recall was relied upon for residential and occupational history which was used to assess exposure), a particularly problematic source of bias in retrospective studies. Additionally, extracting the endpoint data from the California Cancer Registry is a reliable method of case ascertainment.

Weaknesses

- Despite the complexity of the recruitment process, there were differences in important demographic factors between the subjects with thyroid cancer and the controls. The controls on average were older and more likely to be male. While these factors were controlled for statistically in the logistic regression models, this was not without potential for error. Matching control subjects based on these demographic factors would have decreased selection bias and confounding.
- Using geographical location as a proxy measure of exposure is an imprecise method for determining who was exposed and to what level. This approach avoids recall bias of the subjects. However, it is too imprecise as a measure of exposure to avoid information bias.
- The analysis was exploratory in nature due to the fact that 29 pesticides were monitored in this single study. This increased the chances of finding a significant result, making any results found less meaningful. This design is beneficial for hypothesis generation, not conclusions of causality.

DPR Conclusion
The results suggest that there may be a relationship between paraquat exposure and thyroid cancer. However, due to the exploratory nature of the study design, and the relatively attenuated association found, no definitive conclusions can be drawn from the results. In addition, the lack of thyroid cancer in chronic animal studies using high doses of paraquat lessen the impact of this finding.

C. HHA Conclusions from Submitted Population-based Studies

Overall, data from the population-based studies focused on Parkinson's disease indicated that (the overwhelming majority of studies submitted), paraquat exposure alone was not responsible for increased risk. Many findings were not statistically significant. Likewise, the association of paraquat with Parkinson's disease in many study populations could not be precisely determined because the subjects had other environmental exposures that were not always controlled for statistically by the investigators. However, some studies pointed to a possible role for paraquat exposure when considered in tandem with other predisposing factors. There were some compelling results from studies showing gene-environment interactions (Ritz et al., 2009), pesticide exposures pre-1989 (Costello et al., 2009), and pesticide exposures combined with traumatic brain injury in increasing risk of Parkinson's disease

development (Lee et al., 2012). This is consistent with the multivariate inputs suspected to influence Parkinson's disease onset and development. However, the evidence reviewed herein do not demonstrate a direct causal association with exposure to paraquat and the increased risk of developing Parkinson's disease.

Exposure determination can be a particularly problematic aspect of population-based studies. For studies reviewed here, many relied upon self-reported exposures (e.g., pesticide and chemical use) in occupational and/or residential setting. Attributing exposure from these methods introduces recall bias, which can be particularly problematic for studies whose participants have been recruited because of their health status, such as with case-control studies, or in retrospective cohort studies where participants are asked to provide information on exposures from memory. Exposure determination based on geolocation of address/location can avoid recall bias. However, the methods used to estimate residential ambient pesticide exposure make numerous assumptions about the off-site migration of pesticides regardless of application type, meteorological conditions, or physico/chemical properties of the pesticide. These methods also ascribe a uniform estimation of exposure in a subpopulation without accounting for migration or movement in and out of the identified study area. Such methods are more precise when the smallest geographic area is used (Kelsey et al., 1996; Rull and Ritz, 2003). However, studies using a geolocation methodology for proxy exposure attributions tend to use larger and larger geographic areas to increase the probability of capturing enough prevalent cases (i.e., to have enough power to determine a statistical relationship). This can dilute the meaningfulness of an exposure attribution and lead to exposure misclassification. When modeling exposures with methods such as this, the strength of the model can be increased by ground truthing the model estimates with empirical data, such as air monitoring data (Chang et al., 2014). In none of the studies reviewed here attempted to correlate or corroborate population-attributed exposure levels with monitoring data. Caution is warranted in application of results from such studies outside of the defined study population, and especially is assuming that a proxy assumption of exposure means that individuals were actually exposed.

In considering the risk to human health from exposure to paraquat, it is also important to evaluate historical uses versus restrictions that are currently in place to protect pesticide handlers and the general public. The epidemiological cohorts examined in the submitted studies largely reflect farmworkers and pesticide handlers who may have worked with paraquat from the 1970s through the 1990s. Questionnaires used in these studies retrospectively assess exposure from 1974–1999. Occupational study populations are largely located in Iowa and North Carolina, states which may not have the same restrictions and protections as California. In addition, the California-based cohorts from the Parkinson's Environment and Genes (PEG) Study enrolled subjects from 2001 to 2015 and retrospectively assessed disease status starting in 1984 and proxy pesticide

exposure from county agricultural commission records starting in 1982 and by work histories back to 1974.

There has been an evolution of occupational and bystander protections to paraquat exposure over the same time period, both at the federal and state level. These regulations have labeling requirements have progressively increased the protections afforded pesticide handlers, reentry workers, and bystanders. US EPA and California have both recently instituted additional requirements for paraquat labeling and use restrictions (see earlier in this document). The interpretation of findings from the studies reviewed is that the types of paraquat exposures that may have been reported in the past would not occur under the current regulatory climate in California or elsewhere in in the US. Likewise, there is an overall decrease in paraquat use since 2018, with an overall trend of decreasing number of acres treated since 1998 (see Figure 1 earlier in this document). Increased restrictions on paraquat use coupled with decreased overall use are occurring temporally with an increased incidence of Parkinson's disease. Even with a 30-year lag in potential exposure to disease onset or diagnosis, a one-to-one relationship between paraquat exposure and Parkinson's disease is unsupported.

V. Submitted Studies using Neurotoxin-based Animal Models to Investigate the Pathogenesis of Parkinson's Disease

A. Background

As part of the review of articles or submissions received by DPR, HHA also conducted an evaluation of animal (in vivo) and in vitro data investigating the neurotoxic mode of action of paraquat in the pathogenesis of Parkinson's disease. Again, care was taken to focus on articles whose primary investigations were on paraquat dichloride; publications that did not make a clear reference to or association of findings to paraquat were not reviewed further.

DPR received 9 original peer-reviewed experimental studies using paraquat-based neurotoxic models (McCormack and Di Monte, 2003; Peng et al., 2004; McCormack et al., 2005; Prasad et al., 2007; Rudyk et al., 2015; Anselmi et al., 2018; Duan et al., 2023; Nuber and Selkoe, 2023; and Zuo et al., 2023). These studies were in vivo animal studies, in vitro studies, or a combination of the two. Evaluation followed DPR's Guidance for Toxicology Study and Data Acceptability in Registration Review and Risk Assessment (DPR, 2023).

B. Summaries of Submitted Animal Studies (by year of publication)

McCormack and Di Monte (2003)
This study was designed to determine if paraquat-induced nigrostriatal degeneration and α-synuclein aggregation in male C57BL/6 mice can be blocked by competition with

amino acids using the same neutral amino acid transporter (the system L carrier) into the brain. The investigators considered that passive diffusion of paraquat into the brain is unlikely due to its hydrophilicity and membrane impermeability. Instead, they hypothesized that the blood-brain barrier neutral amino acid transporter may mediate paraquat entry. Intraperitoneal injection into mice (animal numbers not stated) of L-valine or L-phenylalanine (200 mg/kg for each amino acid) 30 minutes before injection of paraquat (30 mg/kg) reduced the immunofluorescence generated by an anti-paraquat antibody, indicating that paraquat entry into the brain was reduced under the specified conditions. L-valine also blocked dopaminergic nigral cell loss (assayed by tyrosine hydroxylase-immunoreactive staining) and halted the formation of intracellular aggregates (assayed by thioflavine S staining) following 2 or 3 weekly intraperitoneal injections of paraquat (10 mg/kg) into mice (4/condition), suggesting that α-synuclein pathology was avoided. L-dopa (100 mg/kg), which is also transported into the brain via the system L carrier, had a similar effect to L-valine in preventing the nigral neuron loss.

DPR Conclusion

This study demonstrated that the ability of paraquat to induce nigrostriatal neuron loss in mice could be ameliorated by blocking its transport into the brain with neutral amino acids (L-valine or L-phenylalanine) or L-dopa via the neutral amino acid carrier.

Peng et al., (2004)

This was a mixed in vitro and in vivo histopathological and immunohistochemical investigation using eight-week-old male C57BL/6 mice treated with paraquat or saline (control) and the N27 dopaminergic cell line derived by SV40 immortalization of rat midbrain neurons from isolated mesencephalic cultures. The investigators proposed that c-Jun N-terminal kinases (JNK) play a role in paraquat induced cell death (apoptosis) in midbrain dopaminergic neurons. First in the in vivo investigation, the authors assessed midbrain sections immunostained with an antibody to the dopamine-synthesizing enzyme tyrosine hydroxylase. Paraquat treatment resulted in significant depletion of tyrosine hydrolase-positive neurons in the substantia nigra pars compacta at day 8 after the last injection. The investigators compared JNK activation in the substantial nigral SNpc neurons between paraquat-treated and control mice using immunofluorescent labeling. Midbrain tissues from control mice showed weak and diffuse immunostaining whereas the same region in treated mice, cytoplasmic immunolocalization within dopamine neurons exhibited a bright speckled appearance that became progressively more intense with duration of paraquat treatment. Also evidenced in treated mice was the activation of cytosolic capcase-3 dopaminergic neurons by antibody-staining, confirmed by Western blot. For the in vitro results, the investigators used N27, a dopaminergic cell line that produces dopamine and expresses the dopamine-synthesizing enzyme tyrosine hydroxylase and dopamine transport. Paraquat reduced cell viability in a dose-dependent manner, likely through a caspase c moderated mechanism (hypothesized from the Western blot results). The investigators

also conducted a series of MAPK experiments, with results that collectively suggested that both superoxide production and JNK activation are required for paraquat-induced caspase-mediated cell death of N27 cells in vitro. The investigators suggest that the results point to paraquat-induced cell death is at least partly through an apoptotic mediated mechanism.

DPR Conclusion

This investigation continued earlier investigations of the mode of action for paraquat neurotoxicity using both animal (C57BL/6 mice) and in vitro (N27 cell line) models. The data provide evidence that paraquat induces cell death of dopaminergic neurons through capcase-3 activation and neuronal death. These data add to the knowledge base of how dopaminergic neurons function and survive and that paraquat neurotoxicity is partially mediated by an oxidative stress adverse outcome pathway. Dopaminergic neuronal cell death is only one aspect of Parkinsonism and other neurodegenerative syndromes.

McCormack et al. (2005)

McCormack et al. (2005) designed a study to elucidate the role of oxidative damage in paraquat-induced neurotoxicity. 8-week-old male C57BL/6 mice (≥4 per group for each experiment) and transgenic mice overexpressing the human H ferritin gene were treated with 10 mg/kg paraquat dichloride or saline intraperitoneally once per week for 3 weeks. Assessments for death of nigral dopaminergic neurons using cell counting and Nissl-staining[11] were performed after every injection. 4-Hydroxynonenal (a breakdown product of the decomposition of polyunsaturated fatty acid peroxides) and nitrotyrosine were measured in neurons as markers of lipid peroxidation and oxidative damage. Mice injected once with paraquat did not exhibit significant neurodegeneration at one or two weeks post injection. Mice injected twice with paraquat, showed 8% and 15% decreases in nigral dopaminergic neuron numbers at one and two days, respectively, and a 25–30% decrease at four and seven days following the second injection. Maximum cell loss occurred four days after the second exposure. No further decrease was observed after the third injection. Lipid peroxidation in the midbrain increased 2-fold after a single paraquat injection and 5- to 6-fold after two injections. The third injection produced no additional changes. Three injections into transgenic mice overexpressing ferritin (where iron is less available for reactions such as decomposition of hydrogen peroxide that generates hydroxyl radicals) produced no significant cell loss.

DPR Conclusion

McCormack et al. (2005) demonstrated that intraperitoneal paraquat exposure in mice results in lipid peroxidation and neurodegeneration. Inhibition of oxidative reactions in mice overexpressing the human ferritin gene resulted in neuroprotection from paraquat.

[11] Nissl stains label rough endoplasmic reticulum and free ribosomes found in neuronal somata and dendrites (https://en.wikipedia.org/wiki/Nissl_body).

Prasad et al. (2007)
This study examined aspects of the toxicokinetics of paraquat in regions of the C57BL/6J mouse (sex not stated) brain. A single intraperitoneal injection of 10 mg/kg paraquat (4 mice/time point) resulted in a roughly linear decrease in paraquat concentration in the ventral midbrain (the location of the substantia nigra) starting at one week post dosing and continuing over the course of 4 weeks, though never coming to zero concentration ($t_{1/2}$=4 weeks). This was in contrast to the liver, where complete elimination occurred within 4 days. Intraperitoneal administration of 1, 3, or 5 doses every 2–3 days resulted in a linear increase in ventral midbrain paraquat with number of doses as measured 1 week after the final dose (the only time point measured). This suggested a form of dose-responsiveness for paraquat build-up following intraperitoneal exposure. Similar results were obtained after oral administration. Levels of brain lipid peroxides increased over the course of a week after a single intraperitoneal dose, with the ventral midbrain exhibiting the highest levels, followed by striatum and frontal cortex. Three or five doses did not result in increased lipid peroxide levels compared to a single dose in any of the 3 areas examined, in contrast to the increasing paraquat levels observed after multiple doses. Proteasome 20S[12] was also elevated, but as with lipid peroxide levels, not as responsive to multiple dosing.

DPR Conclusion
The results of this study confirmed that paraquat has access multiple brain regions including the striatum after intraperitoneal exposure. It also showed that paraquat residence in the in the mouse brain could be prolonged depending on the number of doses received by the animal.

Rudyk et al. (2015)
Rudyk et al. (2015) attempted to determine the impacts of chronic intermittent stressors on paraquat-induced neurotoxicity. Male C57BL6/J mice (8–9 weeks old; 10–12 per group) were exposed to 10 mg/kg paraquat by intraperitoneal injections twice per week for 6.5 weeks. For 30 min prior to each injection, the mice were either socially defeated[13] (half of the group) or physically restrained (the other half of the group), following an alternating schedule (chronic intermittent restraint/social defeat stressor challenge). Relationships between various cytokines were assessed using Pearson product moment correlations. Paraquat injections increased both the plasma corticosterone and hematopoietic colony stimulating factor (GMCSF) concentrations, but not other cytokines. It also increased the frequency of significant cytokine correlations from 3 (of a possible 66 correlations) to 17 in a non-stressed mice group, including IL-10 and GMCSF. In stressed mice paraquat reduced the frequency of

[12] Proteasomes are protease complexes designed to carry out selective, efficient and processive hydrolysis of client proteins.
[13] Social defeat involved introducing experimental mice into the homecage of a significantly larger and more aggressive mouse without allowing significant fighting.

significant paraquat associated cytokine correlations decreased from 17 to 2. Paraquat also increased norepinephrine concentrations within the locus coeruleus and paraventricular nucleus of the hypothalamus in both stressed and nonstressed mice. Moreover, this treatment decreased dopamine levels and increased the rate of turnover (i.e., the ratio of dopamine metabolites to parent dopamine) in the nucleus accumbens, which is located in the basal forebrain. Paraquat also increased levels of the dopamine metabolite homovanillic acid and increased dopamine turnover in the dorsal striatum of stressed mice and reduced the animals' preference for a palatable sucrose-containing solution after 4 weeks, and more so in a stressed animal.

DPR Conclusion

Paraquat affects the hypothalamic–pituitary–adrenal axis, peripheral cytokine levels, and mesolimbic and nigrostriatal dopamine turnover. In addition, the imposition of psychological or physical stress modulates these effects.

Anselmi et al. (2018)

Anselmi and colleagues investigated the neurological responses in male Sprague-Dawley rats (5–20 per group) following seven daily oral gavage treatments with paraquat (1 mg/kg), both with and without lectin (0.05%). Lectins are carbohydrate binding proteins that help defend plants from predators, but can also enhance absorption and/or transport of toxins. There were no deaths following oral gavage dosing with paraquat and lectin. However, this treatment resulted in the formation of misfolded α-synuclein, a major contributor to the proteinaceous aggregations that underlie Lewy body formation in nerves of Parkinson’s sufferers. Motor impairments were also noted following dosing in the vibrissae (whiskers), in stepping, and in rotational behavior tests. These motor impairments were not observed after subdiaphragmatic vagotomy, suggesting visceral vagal involvement in the Parkinson-like motor responses. The normal motor responses were also absent following administration of paraquat or lectin alone, suggesting that diet can modulate the paraquat effect, at least in the short term. Central nervous involvement following paraquat plus lectin exposure was indicated by reduction in tyrosine hydroxylase-positive (i.e., dopaminergic) neurons in the SNpc.

DPR Conclusion

These results were consistent with the “Braak’s staging” hypothesis now in use to describe Parkinson’s disease progression following exposure to exogenous agents in humans. Braak’s staging evolves as follows: a) spread of synucleinopathy (α-synuclein fibrillation and aggregation) upon ingestion of a pathogen; b) entry into myenteric nerves; c) retrograde transport to the central nervous system through the vagus nerve leading to; d) effects in the dorsal motor vagal horn in the absence of neuronal loss, the gastrointestinal system through the loss of fine vagal modulation of motility, and ultimately; e) effects in higher neural areas including the dopaminergic neurons of the SNpc with consequent motor impairment. A notable

study weakness is that investigators only tested a single dose (1 mg/kg), making it impossible to determine a dose-response relationship between paraquat exposure and the observed motor impairments.

Duan et al. (2023)

Duan et al. (2023) measured the extent of paraquat-induced upregulation of the mitochondrial calcium uniporter, a possible contributor to paraquat-induced neuropathology in the mouse brain. It was conducted both in vivo and in cell culture. In the in vivo part of the study, 8- to 9-week-old male and female CD1 mice (14 per group) were injected with 0, 5 or 10 mg/kg paraquat intraperitoneally twice per week for 6 weeks. Mitochondrial calcium uniporter knock-out (MCU KO) mice were also injected with 0 or 10 mg/kg paraquat intraperitoneally using the same regiment. Paraquat exposure in wildtype mice resulted in motor deficits, decreased levels of dopamine and its metabolites homovanillic acid and DOPAC, and reduced counts of dopaminergic neurons in the substantial nigra (SN) and striatum (STR). Paraquat also increased the levels of the mitochondrial calcium uniporter (MCU) in the SN and STR and imbalanced processing of optic atrophy 1 (OPA1) by increasing short OPA-1 (S-OPA1) content while decreasing in the ratio of long to short forms (L/S-OPA1) in SN and STR tissue. Furthermore, imbalance in OPA1 processing was accompanied by an increased number of abnormal mitochondria, including those displaying ruptured outer membranes and decreased or absent cristae. In MCU KO mice treated with paraquat, the counts of dopaminergic neurons, L-OPA1 expression and the L/S-OPA1 ratio were restored and behavioral test scores were improved, demonstrating a neuroprotective effect of MCU deletion on dopaminergic neuron loss. However, the reduction of dopamine and its metabolites was maintained.

In the in vitro experiments, Neuro-2a cells (a mouse cell line derived from the neural crest) were incubated with 125, 250, 500 µM paraquat for 24 hours. MCU levels were increased in a dose-dependent manner, while Ca^{2+} homeostasis was disrupted. Incubation with paraquat also resulted in mitochondrial dysfunction and elevated mitochondrial reactive oxygen species (mtROS). MCU inhibition restored Ca^{2+} homeostasis and mitochondrial function, abolished the mtROS elevation, and improved cell viability. As in whole mice, paraquat exposure imbalanced OPA1 processing leading to increased S-OPA1 content, decreased L/S-OPA1 ratio, and swollen or fragmented mitochondria. Inhibition of MCU ameliorated this mitochondrial fragmentation and restored L-OPA1 and S-OPA1 expression.

DPR Conclusion

This study demonstrated that paraquat exposure enhanced the function of mitochondrial calcium uniporter (MCU), which imbalanced optic atrophy 1 (OPA1) processing and triggered mitochondrial fragmentation and dysfunction, resulting in dopaminergic neuron loss and motor deficits.

Nuber and Selkoe (2023)

Nuber and Selkoe (2023) evaluated the effects of paraquat on α-synuclein cleavage by the calcium-activated protease, calpain, both in vitro (using primary cell culture) and in vivo (using transgenic mice). In the in vitro phase, rat primary cortical neuronal cells were cultured from embryos and suspended, followed on days 4 and 8 by the addition of astrocyte-conditioned medium to promote synaptogenesis. Cultures were exposed to 0 to 50 μM paraquat for 12, 24 or 48 hours. At 14 days after plating, neurites projecting from neuronal cell bodies formed a dense neuropil network. In the in vivo phase, three mouse transgenic strains useful for evaluating α-synuclein function in Parkinson's disease (including human WT α-synuclein transgenic mice, E46K (1K) α-synuclein mutant mice, and 3K mutant α-synuclein mice) were analyzed for calpain-cleaved C-terminally truncated α-synuclein using mass-spectrometry (the ability of calpain to digest recombinant α-synuclein was demonstrated in vitro). Cytotoxicity observed after neuronal cell exposure to 30 μM paraquat for 24 hours was prevented by cotreatment with calpeptin, a selective inhibitor of calpain 1. The authors confirmed calpain activation by detecting calpain-specific α-spectrin cleavage, a reaction that also was prevented by calpeptin. Paraquat induced α-synuclein punctate deposits and reduced the number of intersections of neurites after 24 and 48 hours of exposure. These processes were prevented by calpeptin, suggesting that paraquat-induced α-synuclein inclusions were associated with degeneration of neuritic fibers. Twelve hours of incubation with 10 or 20 μM paraquat resulted in decreased levels of α-synuclein tetramer and monomer. Thirty (30) or 50 μM paraquat resulted in decreased in monomer and increased proteolytic α-synuclein truncation. These data suggest that paraquat induces an initial decrease in α-synuclein tetramers and accumulation of α-synuclein monomers that undergo calpain-induced truncation, producing α-synuclein oligomers that aggregate into cytoplasmic deposits. Calpain truncates recombinant α-synuclein resulting in oligomerization ex vitro. The authors analyzed the brains of 1K and 3K mutants, detecting truncated α-synuclein and thus confirming the role of this truncation in the pathogenesis of Parkinson's disease.

DPR Conclusion

Paraquat-induced a tetramer-monomer α-synuclein shift, increasing α-synuclein-containing aggregates and decreasing the connectivity of neuronal fibers. This study identified a pathway by which α-synuclein fibrillation and misfolding may occur under the influence of paraquat.

Zuo et al. (2023)

Zuo et all. (2023) determined both the long-term effects of paraquat on non-motor neurobehavior and the effects of early-life exposure and re-exposure at adulthood. C57BL/6 mice 5 days old pups divided into six groups as follows:

1. NS (11-15 F mice/group, 9-18 M mice/group) received saline intraperitoneally.

2. PQ (14-16 F/group and 8–23 M/group) received 0.8 mg/kg paraquat starting on PND5 for 15 consecutive days intraperitoneally. The mice were assessed with behavioral testing at 22 months.

Re-exposure experiments:

3. NS+NS (9-18 M mice/group) treated with saline only.
4. NS+PQ (8-15 M mice/group) treated with saline PND 5-19 and 10 mg/kg intraperitoneal paraquat at 8 months delivered every other day for 10 days.
5. PQ+NS (8-23 M mice/group) treated with 0.8 mg/kg paraquat daily PND 5-19 and saline at 8 months.
6. PQ + PQ (15-18 M mice/group) treated with 0.8 mg/kg paraquat daily PND 5-19 and 10 mg/kg paraquat at 8 months. Behavioral assessments were conducted at 22 months.

Exposure to 0.8 mg/kg paraquat on PND 5-19 affected short-term memory in male but not female mice as determined by a reduction in alternation and an increase in total number of arms entered in male but not female mice in the Y-maze test). Increased latency time, test latency and train were also observed in males. In females only increased latency time was significantly decreased indicating more impact on cognitive behavior in male mice. The elevated plus maze test did not show differences from controls suggesting paraquat did not cause excessive anxiety.

In the re-exposure experiment, the NS+PQ, PQ+NS and PQ+PQ groups all showed impairment of spatial working memory, with the PQ+PQ showing the largest effect. In the passive avoidance test, cognitive impairment was demonstrated in NS+PQ, PQ+NS and PQ+PQ. In the elevated plus maze test PQ+PQ showed significant reduction in the number of open arm entries, time spent in the open arm, and increased time spent in closed arm, indicating increased anxiety.

DPR Conclusion

Male mice exposed to intraperitoneal paraquat displayed impairments of cognitive behavior and spatial working memory regardless of whether exposure occurred during early life or adulthood. Greater impairments of non-motor neurobehaviors and greater anxiety were observed in those animals re-exposed to paraquat. Paraquat exposure at an early life stage can produce progressive and irreversible non-motor neurobehavioral impairments and enhance susceptibility to subsequent paraquat insults in animals. However, with most toxicological studies, the doses received by experimental animals can be several orders of magnitude higher than documented environmental or occupational exposures (Borgert et al., 2021).

C. HHA Conclusions from Submitted In Vivo and In Vitro Studies

Numerous experimental animal models have been designed to investigate the origin and causation of Parkinson's disease and to provide insight into therapeutic treatments. To best represent Parkinson's disease, experimental animal models should ideally exhibit motor and non-motor changes that are evident in Parkinson's disease in humans. Rodents are often chosen as the experimental model of choice for Parkinson's disease because either genetic or neurotoxic-induced degeneration of the nigrostriatal dopaminergic activity directly correlates with motor deficits observed in the animals (Chia et al., 2020). This makes it possible to design animal studies where specific changes in movement, grip strength, grip coordination, righting behavior, akinesia, and bradykinesia, tremor response and posture can be quantitatively measured. Non-motor symptoms can also be measured, such as food and water consumption, sleeping duration, weight loss, grooming and other behavior changes, and inactivity (reviewed in Guimarães et al., 2024; Jackson-Lewis et al., 2012; McDowell and Chesselet, 2012).

Animal disease models for Parkinson's disease to date have largely been categorized as neurotoxic models or genetic models, although there is some progress in the development of mixed models to better capture the multi-factorial pathophysiology of Parkinson's disease in humans (reviewed in Chia et al., 2020; El-Gamal et al., 2021; Guimarães et al., 2024; Lal et al., 2024; Zhang et al., 2016). Four major compounds have been used in neurotoxic animal models, all of which are considered analogs of dopamine, a neurotransmitter at the heart of Parkinson's disease etiology. These compounds include the dopamine analogs 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and paraquat. 6-OHDA and MPTP have long been used as the neurotoxic models for Parkinson's disease, as their administration creates lesions in dopaminergic neurons that result in the motor and non-motor observations noted above. However, neither analog perfectly recreates Parkinsonism in animals and both have certain drawbacks.

To create Parkinsonism-like dopaminergic effects, 6-OHDA has to be injected directly into specific regions of the brain. This is because 6-OHDA does not cross the blood brain barrier and systemic dosing (like though gavage or dietary exposure) does not induce Parkinsonian-type symptoms (reviewed in Chia et al., 2020; El-Gamal et al., 2021; Lal et al., 2024). The working hypothesis is that 6-OHDA injection causes death of dopaminergic neurons in substantia nigra in the midbrain, specifically in the pars compacta region. Oxidative stress and caspase activation contribute to the 6-OHDA-induced apoptotic cell death of dopaminergic neurons. This neurotoxic model is an imperfect recreation of Parkinsonism in that dosing can result in severe symptoms in the animals and does not result in the formation of Lewy Bodies (Schober, 2004).

MPTP is also commonly used in neurotoxic Parkinson's disease models (Petroske et al., 2001). It is lipophilic, easily crossing the blood brain barrier where it oxidizes to 1-methyl-4-phenylpyridinium ion (MPP+), a potent dopaminergic neurotoxin. Systemic

dosing can lead to loss of dopaminergic neurons in the substantia nigra and a decrease in striatal dopamine levels, as well as a cascade of cell death-associated effects that results from impacted mitochondrial respiration (Petroske et al., 2001). This neurotoxic model replicates the pattern of dopaminergic cell loss in the striatum similar to that of Parkinson's disease. However, MPTP model lacks the most important neuropathological feature of Parkinson's disease which is the formation of Lewy bodies (Schober, 2004), although intraneuronal inclusions similar to Lewy bodies have been described (Fornai et al. 2005).

Another neurotoxicant used in animal models for Parkinson's disease is paraquat (reviewed in Chia et al., 2020; El-Gamal et al., 2021; Lal et al., 2024; Zhang et al., 2016). Paraquat is identified as a neurotoxicant based on its structural similarity to MPP+. Intraperitoneal injection of paraquat up to 3 weeks has resulted in dose-responsive motor activity changes as well as dopaminergic changes including significant decline in dopamine levels within the striatum, loss of dopaminergic neurons, degeneration of the nigrostriatal dopamine system, and aggregate formation in the substantia nigra pars compacta containing α-synuclein, and formation of Lewy bodies (Brooks et al., 1999; Manning-Bog et al., 2002).

Paraquat is an imperfect representation of the causative factors of Parkinson's disease, in that it only elicits a narrow group of outcomes thought to be involved in Parkinsonism but fails to reproduce all the clinical and pathological features of the human disease (Jackson-Lewis et al., 2012; Zhang et al., 2016). The mode of action of paraquat neurotoxicity seems to be different from the dopamine analogs MPP+ or 6-OHDA. Once within the brain, paraquat acts as a redox cycling compound at the cytosolic level, leading to indirect mitochondrial toxicity and reducing the ability of cells to protect against oxidative stress (reviewed in El-Gamal et al., 2021; Zhang et al., 2016). It is also evident that the paraquat animal models do not reproduce all the clinical and pathological features of Parkinson's disease in humans (Zhang et al., 2016). Jackson-Lewis et al. (2012) reviewed 13 animal studies and noted that while paraquat can reduce motor activity and impact the substantia nigra pars compacta neurons, the nigrostriatal dopamine system seems to be unaffected. While the paraquat model can induce certain pathological features of clinical Parkinson's disease, it lacks many of the hallmarks of the human disease. These studies largely relied on intraperitoneal injection or other invasive dosing methods. Most studies that exposed animals to paraquat by chronic inhalation or through feed, which are more representative exposure routes for humans, did not result in the hallmarks of Parkinson's diseases (Rojo et al., 2007; Minnema et al., 2014). One dietary study by Anselmi et al. (2018) showed that oral dosing of rats to paraquat combined with lectins (a protein that can enhance absorption of toxins) produced presence of misfolded α-synuclein and neuronal loss in the substantia nigra pars compacta. This study adds to the weight of evidence of the importance of different routes of exposure to paraquat, although it did not result in a complete manifestation of Parkinson's disease. Currently no animal model can fully

characterize the complexity of human Parkinson's disease, although such studies may provide insights into the specific processes underlying disease development.

VI. Independent Review of the Current Literature on Paraquat

HHA conducted a literature review to summarize the most current research on paraquat and human health effects published in the open literature from June 2015 through August 2024. A systematic review of the published literature was outside the scope of this document. However, it was important to survey a representative sample of the literature to provide a more comprehensive and objective overview of human-based studies beyond those received by DPR as part of public comment. The studies fell into three major groups: population based studies on the association of paraquat exposure and Parkinson's disease, population based studies on the association of paraquat exposure and other human health effects, and clinical case reports or case series on acute paraquat poisoning. Studies included here were not duplicates of those submitted through public comment or ones that US EPA included in its systematic reviews (US EPA 2019b; 2019c).

A. Independent Review of Population Based Studies (2015–2024)

An initial literature search was conducted using the PubMed database on June 30, 2023 (https://pubmed.ncbi.nlm.nih.gov/). This resulted in 554 potential articles of interest. Of these 58 articles met the inclusion criteria (see Appendix B). Of those, 6 studies were duplicates of those examined by US EPA in its systematic review (US EPA, 2019c), 4 were originally submitted to DPR with public comments and were reviewed as part of that effort, 1 article was retracted, 1 study did not specifically investigate paraquat, and 33 were case reports/case series that are summarized in Appendix C. A final literature search was conducted using the PubMed database on August 1, 2024. This resulted in 51 articles, of which 14 studies were considered relevant and not duplicates from previous searchers. Of these, 6 studies focused on the potential mode of action of paraquat in terms of Parkinson's disease, of which 3 articles (Zhang et al., 2016, Chia et al., 2020, El-Gamal et al., 2021) were summarized in the Neurotoxin-based Animal Model section later in the document. The remaining 3 articles were not paraquat specific and not reviewed here. Eight studies were population-based studies of paraquat exposure and associated human health effects. Of these, 3 were reviewed in the US EPA systematic review, 1 was a literature review and not considered primary data, and 2 did not investigate paraquat exposure specifically, and therefore not included here. All totaled, 15 studies (13 from the initial literature search and 2 from the final literature search) underwent comprehensive evaluation (see Appendix B). HHA's independent review of the population based studies is summarized below.

1. Summary of Findings from Parkinson's Disease-Related Studies

There were 5 observational studies found during this literature review that evaluated the relationship between paraquat and Parkinson's disease (Cheng et al., 2017; Shrestha et al., 2020; Tomenson et al., 2021; Yuan et al., 2023; Paul et al. 2024). Findings from Yuan et al., 2023 were difficult to associate with the development of Parkinson's disease from the measured outcome (dream-enacting behaviors). Tomenson et al. (2021) did not find evidence of an increased risk of Parkinson's disease-related mortality among the United Kingdom workforce who manufactures paraquat when compared to the expected national mortality. While Cheng et al., 2017 found a significant association between ambient exposure to paraquat and the development of Parkinson's disease, the authors assumed that there was a uniform airborne concentration of paraquat available to the entire study population, an averaging methodology that is not realistic with point/area source emissions or flux following pesticide applications. The results and specifically the exposure determination, were called into question by Travis et al., 2018. The limitations of the exposure assessment in Cheng et al. (2017) are too great for DPR to consider as weight of evidence of the association of paraquat exposure and Parkinson's.

The strongest findings for an association between paraquat exposure and the development of Parkinson's disease came from Shrestha et al., 2020. Shrestha et al. (2020) found a non-significant increase in risk of development Parkinson's disease among pesticide applicators in the Agricultural Health Study. But when broken down by the lifetime number of days, the hazard ratio was 1.03, 1.42, and 0.74 for the first, second, and third tertile of use respectively indicating no dose-response based on increasing exposure. The importance of this study, however, was the 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 (1.38–7.45).

The most recent study investigating potential exposure to paraquat and the risk of Parkinson's disease was Paul et al., 2024 (part of the PEG study). Investigators assessed exposure to paraquat was assessed through a geographical analysis identifying which participants lived within 500 meters of an application of paraquat using the PUR database and a detailed residential and work history for the years 1974–2015 obtained from interviews with the subjects. Associations between paraquat exposure and Parkinson's disease diagnosis were found across exposure locations (residential and workplace exposure), exposure scheme (ever/never use, count, and average exposure per year), and "overall" versus "lagged" exposure. Significant odds ratios for this association ranged from 1.19 (CI=1.03, 1.38) to 2.15 (CI=1.46, 3.19). While there were some moderate to strong associations, almost all risk estimates were attenuated when examining residential exposure as opposed to workplace exposure. In addition, the authors noted that there were stronger associations between multiple pesticide exposures and risk estimates of Parkinson's disease than exposure to paraquat alone

(Paul et al., 2024). The investigators used a combination of work history and geolocational methods for determining exposure status. One benefit of using both methods to determine potential exposure is that a more comprehensive pattern of residential movement/migration for study subjects could be developed. However, caution is warranted in making the assumption that pesticide application records equate to actual exposure of individuals. As described in the Regulatory and Registration History of Paraquat section earlier in this document, there have been significant changes in restrictions and personal protective equipment requirements for paraquat use over the years. Participants in the study provided residential and work histories including addressed for 1974–2015. Exposures that may have occurred in California during this timeframe would likely not occur with the numerous restrictions and protections currently in place at both the state and federal level.

2. Summary of Findings from Non-Parkinson's disease Related Studies

Ten observation studies were reviewed that evaluated the association between paraquat exposure and various human health outcomes including thyroid hormone effects, respiratory effects, renal disease, birth defects, and cancer.

Kongtip et al., (2021) studied acute pesticide exposures and acute changes in thyroid hormones among Thai sugarcane farmers before and after spray applications of pesticides including paraquat. The strength of the study was that it used biomarkers of exposure and effect, thus reducing potential bias. Results showed urinary paraquat levels were significantly associated with reduced total triiodothyronine (T3) (p=0.036) and reduced free triiodothyronine (FT3) (p=0.036). However, the study failed to adjust for co-exposures, as the applicators reported that they mixed and/or applied multiple pesticides together over 77% of the time. Diaz-Criollo and colleagues (2019) investigated associations between chronic exposure to pesticide mixtures and respiratory outcomes among Colombian farmers. Urine samples were used to determine the level of paraquat exposure in conjunction with reported use. Most participants had no internal paraquat exposure (n=147/217) even if they reported using the herbicide. Chronic exposure was found to be associated with self-reported asthma (prevalence ratio 1.06; 95% CI: 1.00, 1.13). It's important to note that both of these studies were conducted outside of the US where they may be different occupational protection requirements for the use of paraquat or other pesticide.

Four studies investigated the link between paraquat exposure and renal disease or renal cell carcinoma. Lebov et al. (2015) initially reported an association between private pesticide applicator exposure to paraquat prior to 1993 and the incidence of end-stage renal disease in the applicators' spouses who did not apply the herbicide themselves. This highlighted the potential role of take-home exposures among the study subjects in the Agricultural Health Study. However, the same association was not found for spouses who reported applying paraquat themselves (and therefore higher exposure than spouses who were not pesticide applicators). In a follow up study that adjusted for

correlation between other pesticide use, no association could be made between end-stage renal disease and paraquat, even at the highest reported uses (Lebov et al., 2016). Holliday and colleagues (2022) investigated chronic renal disease in a population of Latin American immigrants. An association was initially identified with paraquat exposure. But when adjusted for additional covariates, the findings were no longer significant.

The strongest findings of renal disease were from a prospective AHS study by Andreotti et al. (2020). They investigated the association between lifetime use of individual pesticides and the incidence of renal cell carcinoma. The authors used a lagged analysis in which they estimated cumulative exposure for each year of follow-up until cancer diagnosis or other events, and then discounted exposure during the most proximal years. Results indicated an exposure-response association with paraquat and the development of renal cell carcinoma (relative risk = 1.95; 95% CI: 1.03, 3.70). AHS Study participants (private pesticide applicators from Iowa and North Carolina) were recruited between 1993–97, and likely worked applying pesticides for many years prior to enrollment. As described earlier, there have been significant changes in restrictions and personal protective equipment requirements for paraquat use over the years. Study enrollment was 1993–1997, with some participants likely using paraquat before this time. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.

Two additional population-based studies with cancer outcomes were reviewed. Alexander et al. (2017) evaluated the association between paraquat exposure and Long Interspersed Nucleotide Element 1 (LINE-1) methylation, a possible epigenetic indicator of cancer risk. For “ever users” of paraquat among male private pesticide applicators in AHS, there was a significant association (beta: -0.45 (SE: 0.23)) in reduction of LINE-1 methylation. Caution is warranted in interpreting these findings in that the authors investigated 57 different pesticides at one time without statistical controls, thus increasing the probability of finding a significant result by chance alone. Ferri et al. (2017) conducted a case-control study to explore the relationship between occupational risk and B-cell lymphoma subtypes. The results pointed to a positive association between paraquat exposure and risk of B-cell lymphoma with an OR of 2.8. However, this was accompanied by an inverse dose-response relationship, meaning those with higher paraquat exposure showed lower risk of developing this cancer.

Finally, two studies investigated the possible link between paraquat exposure and birth defects. Ling et al. (2018) examined whether prenatal exposure to agricultural pesticides contributes to the risk of preterm birth or term low birth weight. After adjusting for covariates, the investigators found that paraquat exposure in mothers during gestation was significantly associated with preterm birth in the first and second

trimester. Caution is warranted in interpreting these results as there were multiple differences between the cases and controls and multiple potential exposures were investigated at once and not controlled for statistically. Rappazzo et al. (2019) conducted a cohort study in North Carolina in which data on birth defects from live births were gathered from 2003–2005. Paraquat was significantly associated with several congenital heart defects and especially with lower limb defects, with the latter showing a significant association compared to non-exposed participants (OR = 4.65; CI 1.09, 19.84). Both studies described above utilized a proxy measures of exposure based on agricultural pesticide application data and maternal address, which can lead to exposure misclassification. However, registrant submitted developmental toxicity studies showed increased fetal skeletal variations along with a reduction in fetal body weight in experimental animals. Even with methodological issues with several of the animal studies (such as high maternal toxicity), the consistency of effects noted in both animal and human studies, coupled with the significance of the findings in Rappazzo et al. (2019), point to the possibility of an association of paraquat with certain developmental effects.

B. Independent Summary of Human Clinical Case Reports (2015–2024)

The purpose of this literature review was to summarize human clinical case reports and case series of acute paraquat exposure or poisoning to provide additional context for the paraquat injury and illness reports received by the department as part of public comment. The studies summarized herein were published from 2015 through July 2024. The result can be considered with the weight of evidence of paraquat associated health effects as DPR considers mitigation or other control measures surrounding the registration and use of this paraquat in California.

A search of the PubMed data source (https://pubmed.ncbi.nlm.nih.gov/) resulted in 33 case reports and case series of the clinical signs, symptomology and treatment of paraquat poisoning cases published in the open literature since 2015. The case reports largely involve patients that ingested paraquat as a suicide attempt resulting in serious hospitalization or mortality. Pesticide ingestion is a leading method for suicide worldwide (Chang et al., 2021). There is evidence that bans on production or import of paraquat results in a predictive reduction in the use of this herbicide in cases of self-harm or suicide because of increased difficulty in obtaining the chemical (Chang et al., 2021). Fifteen articles investigated acute symptomology and sequelae resulting from paraquat poisoning, a summary of which is found in Appendix C of this document.

VII. Conclusions and Recommendations

This report is not intended to be a comprehensive human health risk assessment of paraquat dichloride, nor is it full systematic review of the database of paraquat

associated effects. Such a review is outside the scope of this document. Rather, this report was designed to evaluate submitted information relevant to human health concerns as well as evaluating the most recently published findings on associations between paraquat exposure and human health that were not included as part of public comment. To determine this, the department reviewed articles submitted in November 2022 and November 2023 in response to Notices of Proposed Decision to Renew Pesticide Product Registrations for 2023 and 2024 (California Notice 2022-18 and 2023-12, respectively). To provide a more comprehensive overview of the current paraquat literature, three US EPA documents were also reviewed and an independent search of human related studies was conducted. A summary of paraquat toxicology, pesticide illness and injury reports, an overview of Parkinson's disease, and the regulatory and registration history of paraquat were included for completeness.

Overall, data reviewed from the population-based studies focused on Parkinson's disease (were consistent with a possible role for paraquat exposure when considered in tandem with other exposures or predisposing factors. There were some compelling results from studies showing gene-environment interactions (Ritz et al., 2009), pesticide exposures pre-1989 (Costello et al., 2009), and pesticide exposures combined with head injury (Lee et al., 2012; Shrestha et al., 2020). This is consistent with the multivariate original of Parkinson's onset and development.

However, the evidence reviewed herein are insufficient to demonstrate a direct causal association with exposure to paraquat and the increased risk of developing Parkinson's disease. This includes the most recent and most paraquat-specific study evaluated by HHA to date (Paul et al., 2024). Findings from the study showed that while there were moderate to strong associations of increased risk of developing Parkinson's disease with proxy measures of residential paraquat exposure, almost all risk estimates were attenuated when examining residential exposure as opposed to workplace exposure. In addition, the authors note that stronger associations were found between exposures to multiple pesticides and the risk of developing Parkinson's disease than for paraquat alone (Paul et al., 2024). This finding was also emphasized in another publication by the same research group which found increased motor or cognitive decline in Parkinson's patients associated with residential or workplace proximity to the use of pesticides other than paraquat, including 2-methyl-4-chlorophenoxyacetic acid (MCPA) dimethylamine salt, copper sulfate pentahydrate, S,S,S-tributyl phosphorotrithioate, sodium cacodylate, and methamidophos (Li et al., 2023). In a concluding statement, the authors noted that pesticides are not applied in isolation and people are not singly exposed to one agent over a lifetime (Li et al., 2023).

HHA's evaluation of the population-based studies largely align with US EPA's conclusion that there is insufficient epidemiological evidence to conclude that there is a clear associative or causal relationship between non-occupational paraquat exposure and Parkinson's disease but that there is limited but insufficient evidence to conclude

that there is a clear associative or causal relationship between occupational paraquat exposure and Parkinson's disease (US EPA, 2019b).

HHA's evaluation of a limited number of population based studies investigating paraquat exposure and cancer showed some strength of associations with thyroid cancer, renal cell carcinoma, and decreased methylation, a possible epigenetic indicator of cancer risk. However, each study had limitations that reduce the applicability of findings, such as the use of proxy measures of exposure, investigation of association between the health outcome and multiple pesticides, or self-assessment of exposure via questionnaire. In addition, HHA's evaluation of toxicology data required as part of pesticide registration showed limited carcinogenic evidence from chronic studies in experimental animals. US EPA conducted a more exhaustive evaluation of the cancer data and concluded that there was either insufficient epidemiological evidence or non-significance of the relationship between paraquat exposure and risk of various cancers (US EPA, 2019b), a finding which HHA largely agrees with. US EPA has classified paraquat as Category E, or evidence of non-carcinogenicity for humans (US EPA, 2019a).

Studies independently evaluated by HHA considered the association between paraquat exposure and other non-cancer health outcomes. Two studies considered the association between paraquat exposure and developmental toxicity or birth outcomes, both of which showed significant associations. These studies utilized proxy measures of exposure based on agricultural pesticide application data and maternal address which can lead to exposure misclassification. However, the consistency of effects noted in both human and registrant-submitted developmental and reproductive toxicity studies (not reviewed here), coupled with the significance of the findings in Rappazzo et al. (2019), point to the possibility of an association of certain development effects with paraquat exposure that warrant further review. Additional studies were evaluated the association between paraquat exposure and perturbations in the thyroid gland. The strength of one study (Kongtip et al., 2021) came from the use of biomarkers of exposure and effect, thus reducing potential bias and providing verification of study participant of paraquat exposure. The thyroid hormone findings should be taken in context of the importance of thyroid homeostasis during pregnancy and warrant further review.

Population-based studies are difficult to conduct well. The strength of associations are often tied to the sample size, methods of statistical analysis, and the assessment or assignment of exposure. Such limitations can restrict the applicability of findings beyond the study population and make it difficult to generalize the associations more broadly. Exposure assessment within epidemiological studies can be especially problematic. Numerous studies evaluated as part of this preliminary report used an exposure estimation methodology based on a combination of pesticide application data and geolocation of study participants. These models often lack validation against empirical

data from environmental, area, ambient, or personal monitoring. Older studies exist for detection of pesticide drift of paraquat during an era of very different paraquat regulations and restrictions than those currently in place (Ames et al., 1993; Scarborough et al., 1989). Two more recent studies that utilized personal monitoring in California did not include paraquat in the analyzed samples (Harley et al., 2019; Bennett et al., 2024). In general, the use of unvalidated models can lead to exposure misclassification, resulting in bias away from the null. Without supporting environmental or biological monitoring data for specific pesticides, geographically modeled estimates of pesticide exposure cannot be assumed to be valid surrogates of personal exposure to pesticides (Chang et al., 2014).

It is important to put the population-based data into the context of historical paraquat use. The epidemiological cohorts examined in the submitted studies largely reflect farmworkers and pesticide handlers who may have worked with paraquat from the 1970s through the 1990s. Questionnaires used in these studies retrospectively assess exposure from 1974–1999. Occupational study populations were largely located in Iowa and North Carolina, states which may not have the same restrictions and protections as California. In addition, the California-based cohorts from the Parkinson's Environment and Genes (PEG) Study enrolled subjects from 2001 to 2015 and retrospectively assessed disease status starting in 1984 and proxy pesticide exposure from county agricultural commission records starting in 1982. There has been an evolution of occupational and bystander protections to paraquat exposure over the same time period, both at the federal and state level. US EPA and DPR recently instituted numerous additional requirements for paraquat use aimed at mitigating potential exposure and effects of paraquat toxicity.

Finally, for Parkinson's disease specifically, mortality and incidence in California and nationwide appears to be increasing. This may be due to better recognition and diagnosis. However, one of the biggest risk factors in the development of Parkinson's disease is age (Willis et a., 2022). As the population ages, there will be a natural trend for higher rates of Parkinsonism in the US (Willis et al., 2022). This contrasts with a significant decrease in annual paraquat use in California since 2018 and an overall trend of decreasing number of acres treated since 1998.

In conclusion, DPR has identified data gaps relative to impacts to the thyroid and birth defects that may be linked to adverse impacts from the use of paraquat. However, the evidence reviewed herein are insufficient to demonstrate a direct causal association with exposure to paraquat and the increased risk of developing Parkinson's disease.

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Appendix A.

Summary of the 2019 US EPA Draft Human Health Risk Assessment for Paraquat

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Summary of the 2019 US EPA Draft Human Health Risk Assessment for Paraquat

Pharmacokinetic handling, oral exposure. Because no guideline pharmacokinetic study was available, US EPA relied on studies from the open literature to characterize pharmacokinetic handling. Those studies indicated that paraquat "is poorly absorbed and efficiently eliminated" in rats. Data from one study indicated 6% of low gavage dose (4–6 mg/kg-BW) and 8–14% of a higher dose (50 mg/kg/bw) was absorbed. As fecal excretion was very high (~95% of the administered dose) and biliary excretion was low, the vast majority of the dose passed through the rat system unabsorbed. Ninety-five percent of the dose was excreted by 48 hours. Similar results were obtained in rabbits, though compromised renal and fecal function may have reduced elimination at higher doses (30 mg/kg/bw). In contrast to the situation after oral dosing, subcutaneous or intraperitoneal dosing resulted in >80% urinary excretion by 24 hours, further supporting the conclusion that paraquat is poorly absorbed through the digestive tract.
With 70% of an oral dose appearing as the parent compound in the feces, it appeared that there is little internal metabolism of paraquat. Any alterations of paraquat structure found in the remaining 30% were attributed to microbial degradation.

Pharmacokinetic handling, dermal exposure. Paraquat is poorly absorbed by the dermal route, at least at low acute doses. A study in humans in which paraquat was delivered at a dermal dose of 8.6 μg/cm^2 (~0.008 mg/kg for an 80-kg person; considered a low dermal dose based on lack of discernable effects) and urine subsequently collected resulted in absorption estimates of 0.23–0.30%. However, higher doses and continual redosing will damage the epidermal/dermal barrier and very likely result in systemic absorption and toxicity. Because of the likelihood of progressive harm either to human subjects or animals, high-dose dermal toxicity studies (> 6 mg/kg-BW) have not been done.

Toxicological effects. US EPA cites the lungs, kidneys, and skin as the primary targets for paraquat-induced toxicity. Mortality occurred across species at oral doses as low as 3 mg/kg-BW/day and inhalation concentrations as low as 1.3 μg/L/day. As with humans, death upon acute oral exposure was not immediate, occurring up to a week following dosing. Lung was considered the primary target regardless of species, exposure route and duration. Inflammation, scarring, and decreased pulmonary functionality were commonly observed. While serious impacts were noted in rats after both oral and inhalation exposure, the inhalation route appeared to be the more sensitive, at least after acute exposure (Toxicity Category I vs. Category II). Tissue level responses in the upper respiratory tract following repeated inhalation exposures included squamous metaplasia and hyperplasia of the larynx epithelium. Kidney toxicity occurred in mice at doses lower than those causing pulmonary toxicity. In rabbits, oral

exposure to paraquat resulted in progressive proximal tubule degeneration accompanied by reduced urine flow and fecal output.

Despite minimal skin irritation following acute exposure in rodents (LD50 > 2000 mg/kg/BW), repeated exposures can produce serious pathology ("scabbing, hyperkeratosis, epidermal erosion/ulceration, surface exudation, acanthosis, and inflammation"). Dermal impacts in humans are reported more than any other signs. On the other hand, paraquat does not appear to be a sensitizer.

Studies in rodents were negative for reproductive toxicity, neurotoxicity, immunotoxicity, and oncogenicity. Developmental toxicity studies did not show offspring effects at doses lower than those inducing maternal toxicity, despite the evidence from the open literature for increased oral sensitivity (i.e., lower LD50s) of younger animals following acute or subchronic exposure. Despite its ability to induce sister chromatid exchange in Chinese hamster lung fibroblasts, negative tests in several in vivo and in vitro systems led US EPA to consider paraquat to be non-genotoxic.

Points of departure and uncertainty factor determinations

US EPA carried out a series of dose-response assessments deriving from laboratory animal studies for the purpose of determining points of departure for risk assessment. The PODs are listed in the following bullets:

Acute dietary (all populations)

- POD = 5 mg/kg-BW (LOAEL = 10 mg/kg-BW based on clinical signs and mortality in a rat developmental study)
- RfD = 0.05 mg/kg-BW (UF_A = 10x; UF_H = 10x; FQPA = 1x)

Incidental oral (all populations)

- POD = 0.5 mg/kg-BW (LOAEL = 1.5 mg/kg-BW/day based on increased lung weight and alveolitis in a dog subchronic study ***and*** LOAEL = 0.93 mg/kg-BW/day based on chronic pneumonitis, lung lesions, and pulmonary granulomas in a dog chronic study)
- Level of concern, MOE = 100 (UF_A = 10x; UF_H = 10x; FQPA = 1x)

Dermal short-term (1-30 days for all populations; 1 day–6 months for occupational populations)

- POD = 6 mg/kg-BW/day (LOAEL = 6 mg/kg-BW/day at the highest dose tested in a 21-day rabbit dermal study)
- Level of concern, MOE = 100 (UF_A = 10x; UF_H = 10x; FQPA = 1x)

Inhalation short-term (1-30 days for all populations; 1 day–6 months for occupational populations)

- POD = 0.01 µg/L/day (LOAEL = 0.1 µg/L/day based on squamous metaplasia and hyperplasia of the larynx epithelium in a 21-day rat inhalation study)

- AED (animal equivalent dose) = 0.0026 mg/kg-BW/day
- Level of concern, MOE = 100 (UF_A = 10x; UF_H = 10x; FQPA = 1x)

Cancer (non-occupational and occupational)

- Category E (evidence of non-carcinogenicity for humans)

Risk calculations

Risk calculations for paraquat were based on the PODs summarized above and on exposure estimates generated by standard approaches (which were not reviewed for this document).

Non-occupational risks

Acute dietary risk

- All commodity residues were set at tolerance (equivalent to Tier 1 in DPR assessments)
- Acute assessment assumed 100% crop treated
- The highest exposure subpopulation, children 1–2 years old, was at 38% of the population adjusted dose (the PAD is a dose considered by US EPA to be the maximum acceptable dose; a theoretical risk exists when exposure exceeds 100% of the PAD)

Chronic dietary risk

- All commodity residues were set at tolerance
- Percent crop treated varied with commodity, ranging between 1% and 50%
- The highest exposure subpopulation, children 1-2 years old, was at 25% of the PAD

Cancer dietary risk

- Analysis not performed due to paraquat's status as a Category E chemical (see above)

Residential risk

- Analysis not performed due to paraquat's status as a restricted use pesticide

Spray drift risk, residential – children 1–2 (highest risk subpopulation), post-application, dermal and incidental oral

- US EPA expressed their results in terms of distances from the field edge to obtain an MOE of 100 (equal to the level of concern)
- Inhalation was not considered likely, presumably because (1) there are no residential uses for paraquat and (2) this was defined as post-application scenario
- Dermal distances ranged from the field edge to 150 feet depending on application scenario (spray type, nozzle configuration, and application rate)

- Incidental oral distances ranged from the field edge to 10 feet depending on application scenario (spray type, nozzle configuration, and application rate)
- Distances less than those indicated for specific scenarios require mitigation

Spray drift risk, residential – adults, post-application, dermal only

- Dermal distances ranged from the field edge to 75 feet depending on application scenario (spray type, nozzle configuration, and application rate)
- Incidental oral exposure was not considered likely
- Inhalation – no assessment

Spray drift risk, bystanders – post-application, inhalation

- US EPA relied on an ambient air monitoring study in Fresno County conducted by the California Air Resources Board in 1987
- Because all 318 samples were below the minimal detection limit of 0.022 $\mu g/m^3$, US EPA concluded that no bystander post-application inhalation exposures would be expected from volatilization following applications of paraquat to cotton [highest use commodity in CA in 2000] in CA
- US EPA recognized major uncertainties in this analysis including that the CA study may not reflect current agricultural practices, locales, and crops
- Because of these uncertainties, US EPA is committed to further air monitoring

Cumulative risk

- Because paraquat does not belong to a common mechanism group, a cumulative risk estimation was deemed unnecessary

Occupational risks

Because the dermal and inhalation PODs were equal for both the short- and intermediate-term, the risk calculations are applicable to both exposure durations.

Handlers, dermal and inhalation

- Mixer/loader, inhalation: Inhalation MOEs were lower than dermal MOEs for all scenarios; MOEs were <100 (i.e., the LOC) for 21/26 scenarios when engineering controls (EC; e.g., closed system applications) were used; when PPE were used (e.g., single and double clothing layers and APR10 respirators), the number of risk scenarios was reduced to 13/26; no data were provided if both (PPE + EC) were used
- Mixer/loader, dermal: in contrast to inhalation, ECs were more effective in limiting the risk than PPE (4/26 scenarios vs. 8/26 scenarios MOEs<LOC, respectively)
- Applicator, inhalation: 13/21 scenarios exhibited MOEs<LOC when ECs were used; there were no data for PPEs
- Applicator, dermal: none of the 21 scenarios examined exhibited MOEs<LOC

- Flagger, inhalation: 5/5 scenarios examined exhibited MOEs<LOC when PPEs were used; there were no data for ECs
- Flagger, dermal: 1/5 scenarios examined exhibited MOEs<LOC when PPEs were used: there were no data for ECs

- Mixer/loader/applicator, inhalation: 5/8 scenarios examined exhibited MOEs<LOC when PPEs were used: there were no data for ECs
- Mixer/loader/applicator, dermal: 5/8 scenarios examined exhibited MOEs<LOC when PPEs were used; there were no data for ECs

- Loader/applicator, inhalation: 1/1 scenario examined exhibited MOEs<LOC when PPEs were used: there were no data for ECs
- Loader/applicator, dermal: 1/1 scenario examined exhibited MOEs<LOC when PPEs were used: there were no data for ECs

Handler biomonitoring study: An occupational biomonitoring study that assessed absorbed paraquat in 17 applicators was also used to estimate handler risks. Several uncertainties were noted with the study, including the use of different attire and protective gear in the individuals studied, different mixing/loading durations, different amounts of formulated product used, inability to differentiate dermal and inhalation exposure, the inhalation endpoint was based on portal of entry effects. Even so, the MOEs were similar to those obtained using the deterministic handler values above. Thus, for the single scenario examined, MOEs ranged between 13 and 97, thus below the LOC of 100.

Occupational post-application, dermal: Using updated transfer coefficients and dislodgeable foliar residue values, US EPA determined that there were several re-entry scenarios (scouting in particular) with MOEs less than 100 for alfalfa and cotton on day 0 post application. Those scenarios would require re-entry times of 11–27 days before MOEs of 100 or more would obtain. Other crops, including guar, corn, grasses, forage crops, clary, peanut, potato, soybean, sugarcane, and sunflower showed MOEs of 100 or greater on day 0.

Occupational post-application, inhalation: The US EPA did not publish risk estimates for this scenario, but instead intend to use a volatilization screening tool and analysis to determine the necessity for future studies.

Public health incident data review

US EPA used several data tracking systems to identify paraquat-associated incidents. The health effects noted in this search included dermal, ocular, and neurological effects of low or moderate severity, though high severity incidents and deaths were also noted. Most incidents occurred under occupational scenarios involving leaks, spills, splashes, or equipment malfunctions. Dermal symptoms were the most frequent. These included

“welts, hives, peeling skin, chemical burns, swelling, blisters, [and] lesions”. Ocular symptoms included “blurred vision, ocular pain, chemical conjunctivitis, corneal abrasion, [and] vision problems”. Sixty-three incidents were identified through the Main-Incident Data Service 2012–2018 and 140 through SENSOR-Pesticides (data through 2014).

Summary of the general epidemiology review

US EPA’s review of 74 articles was carried out to assess relationships between paraquat exposure and various health outcomes, including but not restricted to Parkinson’s disease, pulmonary conditions, and cancer. US EPA’s review resulted in a report (D449108, A. Niman, 6.26.2019) and conclusions summarized within the present HHRA. Those conclusions are summarized here.

Parkinson’s disease under occupational scenarios. A conclusion of limited, but insufficient epidemiologic evidence (page 27) for a clear causal relationship between Parkinson’s disease and occupational paraquat exposure was based on mixed results from seven studies. US EPA’s reluctance to make a stronger connection was due to concerns about “weaker study designs, more limited exposure assessment approach[es], and potential for recall bias.” Further details on a paraquat-Parkinson’s disease etiology appear in the systematic review section on Parkinson’s disease below.

Parkinson’s disease under non-occupational scenarios. US EPA states that there was insufficient epidemiologic evidence (page 27) for a clear causal relationship between Parkinson’s disease and non-occupational paraquat exposure, with three studies highlighted. This was based on a “lack of consistent evidence of a positive association [to Parkinson’s disease], and the potential for bias in the available studies”. In addition, US EPA expressed concern that a specific link to paraquat could in some cases be obscured by co-exposure to other pesticides. Further details on a paraquat-Parkinson’s disease etiology appear in the systematic review section on Parkinson’s disease below.

Lung function under occupational scenarios. Based on US EPA’s review of 17 articles (nine study populations), there was insufficient epidemiologic evidence for a clear causal relation between the occurrence of general lung function and respiratory symptoms, wheeze, allergic rhinitis, asthma, and chronic bronchitis and occupational paraquat exposure. US EPA based this conclusion on two main issues: (1) a cross-sectional design that could not evaluate the temporal association between paraquat exposure and onset of the health outcomes of interest; and (2) studies that originated outside of the USA, thus raising the possibility of agricultural practices, demographics, and lifestyles not relevant to this country.

Cancer outcomes. Following examination of eight separate studies from the original report by US EPA (US EPA, 2019b), US EPA concluded that there was (1) insufficient

epidemiological evidence to support a relationship between lymphoma incidence and paraquat, although stating that an apparent association in one study may warrant re-evaluation in AHS and further investigation in other study populations that may experience chronic exposure (page 63, US EPA (2019b)); and (2) other cancer types with odds ratios > 1 in one or more studies, but never achieved statistical significance. In combination with the observation that these cancers only appeared in single study population, US EPA concluded that there is no epidemiological evidence for a causative relation to paraquat at this time.

Other health outcomes. US EPA examined 25 articles for evidence of an association between paraquat exposure and other health outcomes and found no association between general mortality, suicide, and infant birth weight and exposure. The agency also found insufficient evidence of an association between diabetes, myocardial infarction, eye disorders, injury mortality, renal/liver function, oxidative stress, abnormal skin pigmentation, actinic keratosis, depressive symptoms, thyroid disease, and aplastic anemia and exposure to paraquat. For end-stage renal disease, US EPA concluded that there was limited, but insufficient evidence for a causal occupational relationship based on the results of two AHS studies.

Summary of the Parkinson's disease systematic review

US EPA undertook a systematic review of the paraquat/Parkinson's disease literature to evaluate the significance and environmental relevance of the postulated association between paraquat exposure and Parkinson's disease (US EPA, 2019c; page 4)). Literature was gathered from the Office of Pesticide Programs (OPP) paraquat registration database, the OPP paraquat epidemiology review, and the National Toxicology Program scoping review covering the Parkinson's disease-paraquat relation. The systematic review was divided into human, animal, and in vitro evidence categories, consisting of 28, 217, and 244 studies, respectively. Subsequent study exclusions were based on use of a non-relevant animal exposure route (injection, in particular), over-representation in the in vitro group of result types and outcomes irrelevant to the emerging weight of analysis, and data quality issues. A final total of 26, 11, and 34 studies, respectively, were subjected to analysis.

Human studies

The 26 human studies encompassed 13 populations (three agricultural cohorts, nine hospital-based populations, and one Parkinson's disease registry). Exposure determinants such as amount, frequency, and duration varied, with higher exposure doses associated with occupational populations. Equivocal results from the AHS cohort and others population-based studies led US EPA to a finding of limited, but insufficient epidemiologic evidence of a clear associative or causal relationship for occupational populations. A smaller number of studies (three), combined with inconsistent results and potential study bias, led to a finding of insufficient epidemiologic evidence of a clear associative or causal relationship for non-occupational populations.

Animal and in vitro studies

US EPA concluded that the evidence for Parkinson's-like symptomology in response to following paraquat exposure was weak in the 11 animal studies reviewed. Only one animal study was cited in which motor impairment in male mice was connected to dopaminergic neuron degeneration and neurochemical disruption – two hallmarks integral to the pathology of Parkinson's disease in humans. While US EPA recognized other toxicokinetic, in vitro and mechanistic evidence were consistent in some cases with a Parkinson's etiology, US EPA concluded on the whole that the animal evidence was inadequate with respect to consistency, dose-response, or temporal concordance.

In conclusion, US EPA considered the human, animal, and in vitro evidence provided only limited support for a role for paraquat in the development of Parkinson's disease in humans. Further, the agency stated that the regulatory targets when combined with the standard uncertainty factors and when used according to the legal label were adequate to protect the public from Parkinson's disease development Resulting from paraquat exposure.

Appendix B.

Summary of Population Based Studies Published from 2015 – 2024

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I. Purpose

The purpose of this literature review was to summarize population based studies that investigated associations between exposure to the herbicide paraquat dichloride (paraquat) and human health effects published from 2015 through July 2024. A systematic review of the published literature was outside the scope of this document. However, it was important to survey a representative sample of the literature to provide a more comprehensive and objective overview of human-based studies beyond those received by the Department of Pesticide Regulation (DPR) as part of public comment. In addition to supplementing studies submitted to the department, the studies included here were either published after the US Environmental Protection Agency (US EPA) concluded its systematic review of the relationship between paraquat exposure and Parkinson's disease (US EPA, 2019) or are studies that were excluded from US EPA's review due to relevance or data quality.

The summary of the study findings, their strengths and weakness, and notable associations can be considered with the weight of evidence of paraquat associated health effects as DPR considers mitigation or other control measures surrounding the registration and use of this paraquat in California.

Note that numerous case studies and case reports involving acute paraquat exposure were captured as part of the literature search. A summary of relevant case studies and case reports in included in Appendix C.

II. Methods

As stated above, a systematic review of the literature was outside the scope of this document. However, the intention was to search for human based studies using stated search terms in a common database and then stratify the resulting studies using a PECO statement for more in-depth review (see below).

A. Search terms

PubMed was used as the database of interest for this literature review. The search was limited to original research articles (reviews/comments/addendums were excluded). The search was also limited to research articles published between 2015 and June 30, 2023 with a final search conducted August 1, 2024. The search was limited to English language articles.

Search terms for the initial search included:
("paraquat" OR "paraquat dichloride") AND ("human health effects" OR "human health" OR "Parkinson's disease" OR "respiratory" OR "acute exposure" OR "chronic exposure" OR "cancer")

Search terms for the final search included:
((paraquat) AND (("2016/01/01"[Date - Publication] : "3000"[Date - Publication]))) NOT (poisoning) Filters: Humans, Humans

B. PECO Statement

Population (including animal species), Exposure, Comparator, and Outcomes (PECO) statements define the objectives of the review as well as informing the data inclusion and exclusion criteria and the interpretation of the directness of the findings in answering the original review objective.

For human-based paraquat studies, the PECO statement used to stratify search results was as follows:

Population of interest: Population studied must be humans with no restrictions, including no restrictions on age, life stage, sex, country of residence/origin, race/ethnicity, lifestyle, or occupation.

Exposure: Exposure studied must be to paraquat in any application via any route of exposure.

Comparator: Exposed or case populations must be compared to a population with low/no exposure or to non-cases to arrive at a risk/effect size estimate of a health outcome associated with paraquat exposure.

Outcome: All reported human health effects, with no restrictions on human system affected (effects could be based on survey or other self-report, medical records, biomarkers, publicly available health data, or measurements from human sample populations).

III. Search Results

Using the search terms specified above, a literature search was conducted using the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) on June 30, 2023. This resulted in 554 potential articles of interest. Of these 58 articles met the inclusion criteria stated above. Of those, 6 studies were duplicates of those examined by US EPA in its systematic review (US EPA, 2019), 4 were originally submitted to DPR with public comments and were already reviewed in the main preliminary report, 1 article was retracted, 1 study did not specifically investigate paraquat, and 33 were case reports/case series that are summarized in Appendix C. Therefore, 13 studies from the initial literature search underwent comprehensive review for this Appendix. The final search was conducted using the PubMed database on August 1, 2024. This resulted in 51 articles, of which 14 studies were considered relevant and not duplicates from previous searchers. Of these, 6 studies focused on the potential mode of action of paraquat in terms of Parkinson's disease, of which information from 3 (Zhang et al., 2016, Chia et al., 2020, El-Gamal et al., 2021) was summarized in the main preliminary

report. The other 3 were not paraquat specific, and not reviewed here. Eight studies were population-based studies of paraquat exposure and associated human health effects. Of these, 3 were reviewed in the US EPA systematic review, 1 was a literature review and not considered primary data, and 2 did not investigate paraquat exposure specifically, and therefore not included here. A new meta-analysis was included in the search results (Aravindan et al., 2024) which evaluated evaluate environmental factors known or suspected to be associated with an increased risk of developing Parkinson's disease. When organized into qualitative categories, occupational exposure to industrial toxins and dyes had the highest association with developing Parkinson's disease, followed by exposure to food contaminants and residential and/or occupational exposure to pesticides. This meta-analysis did not report results on specifically paraquat, and was not reviewed further. Therefore, 2 studies from the final literature search underwent comprehensive review for this Appendix. The literature search also netted several studies that were previously submitted to the department for review as part of public comment, and which have already been reviewed as part of the main preliminary report. Table 1 summarizes the results from the literature searches and Table 2 displays the study type breakdown for the relevant articles reviewed in this Appendix.

Table 1. Summary of Paraquat Literature Search

Initial Search – January 1, 2015 through June 30, 2023					
Total studies	Population-based studies of interest	Duplicates of studies submitted by public comment	Duplicates of studies reviewed by US EPA[a]	Relevant studies reviewed	Year of publication
554	58	4	6	13	2015 – 2023
Final Search – January 1, 2015 through August 1, 2024					
Total studies	Population-based studies of interest	Duplicates of studies submitted by public comment	Duplicates of studies reviewed by US EPA[a]	Relevant studies reviewed	Year of publication
51	8	0	3	2	2020 – 2024

[a]US EPA, 2019. Paraquat Dichloride: Systematic review of the literature to evaluate the relationship between paraquat dichloride exposure and Parkinson's disease. US Environmental Protection Agency, Office of Pesticide Programs, Health Effects Division, Washington DC. EPA-HQ-OPP-2011-0855-0125. June 26, 2019. https://www.regulations.gov/document/EPA-HQ-OPP-2011-0855-0125

Table 2. Study Types Reviewed in Appendix B (n=15)

Study type	AHS cohort	Non-AHS cohort	Residential/ Environmental Exposure	Occupational Exposure
Number of studies	6	2	5	2

AHS – Agricultural Health Study (https://aghealth.nih.gov/)

IV. Review of Population Based Studies

This review of population studies is divided by cohort studies conducted as part of the Agricultural Health Study (AHS), cohort studies conducted outside of AHS, studies that evaluate residential/environmental exposures or occupational exposures to paraquat, studies that evaluate occupational exposure. Individual study summaries follow.

A. Agricultural Health Study (AHS) Cohort Studies

The most common cohort studies involving paraquat and human health use the Agricultural Health Study (AHS) cohort. AHS aims to investigate how pesticides and other agricultural exposures may affect the health of farming populations. Briefly, 52,394 private pesticide applicators (mostly farmers) from Iowa and North Carolina were enrolled in the AHS for Phase 1 between 1993 and 1997 by completing an enrollment questionnaire about demographics, lifestyle, farming practices, ever/never use of 50 pesticides, and duration and frequency of 22 pesticides. Of these, 22,916 (44%) applicators also completed a take-home questionnaire to provide additional details on duration and frequency of 28 pesticides and "ever use" of other pesticides. Also, 32,345 spouses were enrolled into the study at baseline and were surveyed on "ever use" of 50 pesticides. Following the baseline surveys (Phase 1), participants updated their exposure and health status every 5–6 years via telephone interviews or mailed surveys at Phase 2 in 1999–2003, Phase 3 in 2005–2010, and Phase 4 in 2013–2015. During these surveys or interviews, applicators and spouses were asked to provide names of pesticides used in the last year or the most recent year pesticides were used. Participants consented to the study by returning the enrollment questionnaires or participating in the telephone or mailed follow-up surveys.

There were 7 AHS cohort studies found during this literature review exploring links between paraquat exposure and human health outcomes, 1 of which (Furlong et al., 2015) was reviewed by US EPA in the 2019 systematic review and not included here. Of the remaining 6 studies, 1 study evaluated the association between paraquat exposure and Parkinson's disease (Shrestha et al., 2020), 2 studies evaluated renal disease (Lebov et al., 2015, 2016), 2 evaluated possible links to cancer development (Alexander et al., 2017, Andreotti et al, 2020), and 1 investigated dream enacting behaviors (Yuan et al., 2022). In investigating the association between paraquat exposure and Parkinson's disease, Shrestha et al. (2020) did not find an overall significant association. However, the investigators did find that there may be an interaction between head injury and paraquat exposure in the development of Parkinson's disease as the hazard ratio (HR) for those with head injuries was 3.2 (1.38, 7.45). In investigating end-stage renal disease following potential chronic paraquat exposures, Lebov et al. (2015) found an association with paraquat exposure with a significant hazard ratio of 1.99. After making further adjustments for the correlation between exposure to other pesticides included in the final model, Lebov et al. (2016) the

association between end-stage renal disease and paraquat exposure could not be made. Alexander et al. (2017) evaluated the association between paraquat exposure and Long Interspersed Nucleotide Element 1 (LINE-1) methylation, a possible indicator of cancer risk. For “ever use” of paraquat, there was a significantly negative association (beta: -0.45 (SE: 0.23)) in LINE-1 methylation. Andreotti et al. (2020) evaluated the association between paraquat exposure and renal cell carcinoma. For the 20-year lag model, there was a significant 1.95 times increase in the risk of renal cell carcinoma among median intensity-weighted lifestyle days (IWLDs) of paraquat when compared to never users (Andreotti et al., 2020). The final AHS cohort study evaluated the association between paraquat exposure and dream enacting behaviors, a characteristic feature of rapid eye movement sleep behavior disorder, which was found to be significant at an OR of 3.48 (Yuan et al., 2022).

In general, a strength of a prospective or retrospective cohort study is the ability to follow participants over time from exposure to outcome. When compared to other observational studies, this provides a clearer and more objective temporal sequence and strengthens causal inferences about the relationship between exposure and outcome. The prospective cohort design incorporated by the AHS studies can also be an important factor in avoiding information bias from recall and other factors related to exposure assessment.

However, these studies had limitations that were inherent in the study methodologies. For example, all studies were exploratory analyses in nature in that they modeled multiple comparisons of exposure to outcome. Anywhere from 16 to 57 different pesticides were investigated for potential association with a specific health outcome. This increases the chance of finding a significant result by chance alone, making any positive association less meaningful. Another weakness was the evaluation of exposure of the participants. The exposure assessment used in these AHS cohort studies relied on self-report or recall questionnaire which can introduce bias into the study, especially if the participant already has or develops a health condition. This may potentially lead to misclassification and bias.

Lebov et al., 2015

This is a prospective cohort study with the aim of evaluating the relationships between end-stage renal disease among wives of licensed pesticide applicators in Agricultural Health Study (AHS) and personal pesticide use, exposure to the husband's pesticide use, and other pesticide-associated farming and household activities. This study included the wives of private pesticide applications enrolled in the AHS the between 1993 and 1997 and were followed-up until December 2011 and excluded private pesticide applicators. Wives of pesticide applicators may be exposed to pesticides through take-home exposures, such as pesticide residues carried home on their husband's boots, clothing, and skin or by washing pesticide-contaminated clothing. Additionally, women who live on farms where pesticides are applied may experience

exposure through spray drift and water contamination; proximity of household to pesticide application areas has been positively correlated with levels of pesticides found in household dust. Because the distribution of risk factors for end-stage renal disease differs by gender, and because few spouses of applicators were male (<1%), they excluded male spouses. Of the 31,142 wives eligible for analysis, this study included 98 cases (0.3% of wives) were diagnosed with end-stage renal disease during an average of 15.4 years of follow-up. Pesticide use information was obtained from the spouse enrollment questionnaire as ever/never use of 50 specific pesticides. Among the 31,142 wives, 17,425 women applied pesticides themselves while 13,717 were not involved in pesticide application. Direct exposure was defined as the wives' personal use of 50 specific pesticides. The wives' pesticide-specific indirect exposure duration was defined as the number of years that wives could be potentially exposed, based on the estimated start date for living together, multiplied by the annual probability of the husband's use. “Ever use” of paraquat by the husbands was significantly associated with end-stage renal disease among non-applying wives (HR = 1.99, 95% CI: 1.14, 3.47), with some evidence of a positive exposure–response trend.

Strengths

The prospective design (regarding incidence of end-stage renal disease) was a good way to avoid the potential for recall bias when relying on self-reporting for assessing exposure. Using the midpoint of the decade for reported pesticide use data helps to address the possible misclassification from assuming that frequency of use was representative of the two to ten years since enrollment.

Weaknesses

- This study did not assess paraquat specifically among wives who directly applied pesticides. They did assess the general herbicide category of pesticides, and they did not find a significant association between herbicides and end-stage renal disease. This relationship needs to be included and assessed to understand the association between paraquat and end-stage renal disease.
- This analysis was exploratory as the researchers investigated 16 and 43 different chemicals, for wives who applied pesticides and wives who did not respectively, for potential associations with end-stage renal disease. This increases the chances of finding a statistically significant result, making any results found less meaningful.
- The assessment of exposure relied on self-report. This may potentially lead to misclassification and bias.
- Study enrollment was 1993–1997, with some participants likely using paraquat before this time. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.

- Cases may have already experienced the effects of earlier stages of renal disease prior to study enrollment, which could have influenced their pesticide use. If they have reduced exposure due to modified application practices, effect estimates for specific pesticide use would be biased towards the null.

DPR Conclusion

This study provided mixed results at best on the association between paraquat and end-stage renal disease. Among wives who directly applied pesticides there was no association found between herbicides and paraquat. The results do indicate an association between end-stage renal disease and indirect paraquat exposure among wives who did not apply pesticides because of the significant hazard ratio of 1.99; however, if we assume that wives with direct exposure are also experiencing this indirect exposure, then it more likely that something else may be contributing towards that hazard ratio. With the limitations inherent to the methods of the study, this finding in isolation does not merit action beyond additional research being needed.

Lebov et al., 2016

This is a prospective cohort study with the aim of evaluating associations between chronic and acute pesticide exposure and end-stage renal disease risk among private pesticide applications enrolled in the Agricultural Health Study (AHS) between 1993 and 1997 and provides a different analysis of association than the previous study (Lebov et al., 2015). Because the distribution of risk factors for end-stage renal disease differs by gender, and because few applicators were female, they excluded female applicators (N=1,562; 2.7%). Of the 55,580 male participants eligible for analysis, this study included 320 participants who were diagnosed with end-stage renal disease over an average 15.7-year follow-up period. For each pesticide, an intensity-weighted lifetime exposure metric was generated by multiplying lifetime-days of use (product of duration and frequency of use) by an intensity score that accounts for differences in exposure resulting from variation in pesticide application methods, repair of pesticide application equipment, and use of personal protective equipment. These intensity-weighted lifetime-days were used as their primary exposure metric. In intensity-weighted cumulative use analyses, positive association with end-stage renal disease risk was observed with the highest tertile of intensity-weighted use of paraquat at an hazard ratio of 2.23 (95% CI 1.18,4.21). However, after adjusting for correlation between other pesticides included in the model, end-stage renal disease risk was no longer associated with top tertile of intensity-weighted use of paraquat.

Strengths

The prospective design (regarding incidence of end-stage renal disease) was a good way to avoid the potential for recall bias when relying on self-reporting for assessing exposure. Hazard ratios with the use of person-time can account for loss to follow-up over long periods of time in a much more appropriate way than simple logistic

regressions can. Addressing the potential for a healthy worker survivor effect was another strength of this study as it reduces the likelihood of participants having chronic kidney disease prior to or at enrollment.

Weaknesses

- This analysis was exploratory as the researchers investigated 41 different chemicals for potential associations with end-stage renal disease. This increases the chances of finding a significant result, making any results found less meaningful.
- The assessment of exposure relied on self-report. This may potentially lead to misclassification and bias.
- Study enrollment was 1993–1997, with some participants likely using paraquat before this time. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.
- It is possible that 5 years after enrollment as the cutoff to satisfy concerns of participants possibly having poor renal health at enrollment is still too few years as the researchers stated that there is typically a decades-long progression of this disease from chronic stage 1 to end-stage renal disease.
- The previous study by these investigators using the same AHS cohort as the source population found a significant association between a husbands paraquat use among non-applying wives and the wives' end-stage renal disease; however, after adjusting for correlation, that association is not found to be significant among the husbands themselves. One would think that pesticide applicators with actual contact with paraquat would have a stronger association between that exposure and end-stage renal disease than non-applying wives. This inconsistency calls into question any association or causality between paraquat and end-stage renal disease.

DPR Conclusion

This study provided mixed results at best on the association between paraquat and Parkinson's disease. Initially, the study found a significant association between paraquat and Parkinson's disease at an hazard ratio of 2.23; however, after adjusting for the correlation between other pesticides included in the final model, the results do not indicate an association between end-stage renal disease and paraquat exposure.

Alexander et al., 2017

This study aimed to evaluate the association between pesticide usage and Long Interspersed Nucleotide Element 1 (LINE-1) methylation among male private pesticide applicators in the Agricultural Health Study (AHS). Global DNA methylation (DNAm) is a

commonly studied epigenetic mechanism, with lower levels of global DNAm often associated with carcinogenesis in the form of chromosomal instability and increased mutation rates. A previous study found correlations between global DNAm and DNAm levels in repetitive elements such as (LINE-1) (Kazazian & Goodier, 2002). In addition, Barchitta et al. (2014) have found associations between reduced levels of LINE-1 DNAm and risk of various malignancies. This study linked these results suggest that LINE-1 DNAm may be used as a surrogate marker of global methylation in order to investigate the relationship of the latter to cancer risk. Exposure was assessed with questionnaires at enrollment in 1993–97 and at two follow-ups between 1993 and 2010. They classified exposure as Ever exposure by asking if a pesticide applicator had ever personally mixed or applied a given pesticide (such as paraquat). They also assessed exposure with intensity-weighted lifestyle days and made cut points of amount of use based on that measure. They assessed LINE-1 methylation through EZZ DNA Methylation-Gold Kits and QCpG software at enrollment and follow-ups. For “ever use” of paraquat, there was a significant negative association (beta: -0.45 (SE: 0.23)) in LINE-1 methylation. When broken down by the lifetime number of days that they used paraquat, the highest levels of paraquat (beta: -0.88 (SE: 0.34)) had significantly lower LINE-1 DNAm levels compared with those who never used each of these pesticides.

Strengths

The prospective design is an important factor in avoiding information bias from recall and other factors related to exposure assessment. Additionally, this study had a strong methodology in assessing LINE-1 methylation with lab testing at each follow-up.

Weaknesses

- Total use of chemicals was determined by taking a survey of frequency of use of each chemical during the past year and assuming that was representative of the two to ten years since enrollment. This leaves the study susceptible to misclassification.
- The assessment of exposure relied on self-report. This may potentially lead to misclassification and bias.
- Study enrollment was 1993–1997, with some participants likely using paraquat before this time. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.
- The analysis was exploratory in nature as they investigated 57 pesticides at one time. This increases the chances of finding a significant result, making any results found less meaningful. This design is beneficial for hypothesis generation but caution is warranted in application or interpretation of these findings more broadly.

DPR Conclusion

This was a well conducted cohort study, but remains an exploratory analysis that is not independent of the other AHS studies. The results do indicate an association between decreased LINE-1 methylation and paraquat exposure because of the significant negative association. With the limitations inherent to the methods of the study, this finding in isolation does not merit action beyond additional research being needed.

Andreotti et al., 2020

This study aimed to evaluate the association between the lifetime use of individual pesticides (such as paraquat) and the incidence of renal cell carcinoma in applicators over 20 years of follow up in the Agricultural Health Study (AHS). Exposure was assessed with questionnaires at enrollment in 1993–1997 and at follow up every 5–10 years after that. They classified exposure with intensity-weighted lifestyle days (IWLDs) and made cut points of amount of use based on that measure. Linkage to state cancer registries provided information on incident cancers diagnosed between enrollment and end of follow-up. They also incorporated unlagged, 10-year lag and 20-year lag models. For the 20-year lag model, there was a significant incidence rate ratio of 1.95 for renal cell carcinoma among the highest category of IWLDs when compared to never users of paraquat.

Strengths

The prospective design is an important factor in avoiding information bias from recall and other factors related to exposure assessment. Additionally, incorporating a time lag component into their study supported the prospective study design.

Weaknesses

- Total use of chemicals was determined by taking a survey of frequency of use of each chemical during the past year and assuming that was representative of the two to ten years since enrollment. This leaves the study susceptible to misclassification.
- The analysis was exploratory in nature as they investigated 38 pesticides at one time. This increases the chances of finding a significant result, making any results found less meaningful.
- No 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.
- Study enrollment was 1993–1997, with some participants likely using paraquat before this time. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.

DPR Conclusion

This was a well conducted cohort study but remains an exploratory analysis that is not independent of the other AHS studies. The results do indicate an association between RCC incidence and paraquat exposure; however, with the limitations inherent to the methods of the study, this finding in isolation does not merit action beyond additional research being needed.

Shrestha et al., 2020

This study aimed to evaluate the association between pesticides used and incident Parkinson's disease in applicators over 20 years of follow up in the Agricultural Health Study (AHS). Exposure was assessed with questionnaires at enrollment in 1993–97 (n=37,284 applicators) and at follow up every 5–10 years after that (n=19,068 applicators). They classified exposure with intensity-weighted lifestyle days and made cut points of amount of use based on that measure. They assessed Parkinson's disease status after 15–20 years since enrollment through self-reports and death registries. Because they had data on when Parkinson's disease was developed, they used survival analysis to estimate the hazard associated with pesticide exposure in developing Parkinson's disease. For "ever use" of paraquat, there was a 9% non-significant increase in hazard of developing Parkinson's disease among applicators. When broken down by the lifetime number of days that they used paraquat, the hazard ratio was 1.03, 1.42, and 0.74 for the first, second and third tertile of use respectively indicating no dose-response based on increasing exposure. However, results indicate a significant interaction between paraquat exposure and head injury with the development of Parkinson's disease, with a hazard ratio for those with head injuries being 3.2 (1.38–7.45).

Strengths

The analysis was very appropriate given the information on time from enrollment to development of Parkinson's disease that was available. Hazard ratios can account for loss to follow-up over long periods of time in a much more appropriate way than simple logistic regressions can. The prospective design is also an important factor in avoiding information bias from recall and other factors related to exposure assessment.

Weaknesses

- Total use of chemicals was determined by taking a survey of frequency of use of each chemical during the past year and assuming that was representative of the two to ten years since enrollment. This leaves the study susceptible to misclassification.
- The analysis was exploratory in nature due to the fact that they investigated 50 pesticides at one time. This increases the chances of finding a significant result, making any results found less meaningful.

- The assessment of exposure and Parkinson's disease status both relied on self-report. This may potentially lead to misclassification and bias.
- Study enrollment was 1993–1997, with some participants likely using paraquat before this time. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.
- Only four other chemicals were ever 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.

DPR Conclusion

This was a well conducted study. The results indicate that there may be an interaction between head injury and paraquat exposure in the development of Parkinson's disease. But additional and more focused studies are needed for any action to be taken.

Yuan et al., 2022

This is a cohort study aiming to examine high pesticide exposure events in relation to dream enacting behaviors among farmers in the Agricultural Health Study (AHS). Rapid eye movement sleep behavior disorder is a parasomnia characterized by a loss of muscle atonia during rapid eye movement sleep with the presence of dream enacting behaviors. This study included 11,248 farmers from Iowa and North Carolina that were enrolled in the AHS between 1993 and 1997 and also answered phase 4 follow-up survey sometime from 2013–2015 regarding dream enacting behaviors. 939 (8.3%) of the 11,248 eligible participants reported dream enacting behaviors at the Phase 4 survey. High pesticide exposure and pesticides involved in this exposure information was obtained from enrollment questionnaire as ever/never unusually high personal exposure. High pesticide exposure events were subsequently updated in Phases 2 (1999–2003) and Phase 3 (2005–2010). They found that paraquat involved in the highest exposed event at baseline (1993–1997) was significantly associated with reported dream enacting behaviors at an OR of 3.48 (95% CI: 1.37, 8.81).

Strengths

The cohort design of this study allowed the researchers to establish temporality. The multiple sensitivity analyses done over the course of the study to avoid selection bias were appropriately done.

Weaknesses

- This analysis was exploratory as the researchers investigated 16 specific pesticides and 8 pesticide groups at once for potential associations with dream

enacting behaviors. This increases the chances of finding a significant result, making any results found less meaningful.
- The assessment of exposure and dream enacting behaviors relied on self-report. This may potentially lead to misclassification and bias.

DPR Conclusion

This study was a hypothesis generating study. The results do indicate an association between dream enacting behaviors and paraquat exposure because of the significant odds ratio of 3.48. In addition, it is unclear what, if any, link can be made between the unique parameter chosen as the subject of this investigation and any particular health impact that could be associated with paraquat exposure. With the limitations inherent to the methods of the study, this finding in isolation does not merit action beyond additional research being needed.

B. Non-AHS Cohort Studies

There were 3 non-AHS cohort studies found during this literature review exploring paraquat and human health (Pouchieu et al., 2018; Kongtip et al., 2021; Tomenson et al., 2021). Pouchieu et al., 2018 was reviewed as part of the US EPA systematic review and not included here. Of the 2 remaining cohort studies, one evaluated the relationship between paraquat exposure and acute changes in thyroid hormones levels (Kongtip et al., 2021) and the other evaluated the association between paraquat exposure and Parkinson's disease (Tomenson et al., 2021). Briefly, Kongtip et al. (2021) was a cohort study using 78 Thai farmers (74.4% male and average age of 49.6 years) recruited from a larger longitudinal study from Khao Thong Subdistrict, Phayuha Khiri District in Nakornsawan province, in the upper central area in Thailand. This study did not find any significant change in the simple paired analysis of thyroid hormone levels from the day before to the day after spraying paraquat. However, in models of thyroid hormone change that incorporated the measured change in metabolite levels and other covariates, it was found that increased urinary elimination of paraquat (from spray application exposures) significantly reduced certain thyroid hormone levels. Tomenson et al. (2021) was a retrospective cohort study which followed-up on a cohort study of all employees who had ever worked on any of four plants at Widnes, UK where paraquat was manufactured between 1961 and 1995. This study did not find evidence of an increased risk of Parkinson's disease-related mortality among the UK workforce who manufactured paraquat when compared to the expected national Parkinson's disease mortality.

In general, a strength of cohort studies is the ability to follow participants over 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. Both studies reviewed here were able to establish temporality and used biomarker measurement of exposure, thus avoiding self-report of

paraquat exposure and possible recall bias. However, both studies were vulnerable to confounding, in that Kongtip et al. (2021) did not control for co-exposure to other pesticides while the standardized mortality ratio analysis done in Tomenson et al. (2021) did not allow for the inclusion of covariates into the study.

Kongtip et al., 2021

This study aimed to investigate the relationship of acute pesticide exposures and acute changes in thyroid hormones among Thai sugarcane farmers before and after spraying. This is a cohort study using 78 Thai farmers (74.4% male and average age of 49.6 years) recruited from a larger longitudinal study from Khao Thong Subdistrict, Phayuha Khiri District in Nakornsawan province, in the upper central area in Thailand. The field staff set an appointment with the farmers when they planned to spray chlorpyrifos, cypermethrin, paraquat or glyphosate in their fields. Data collection for this study was conducted from November 2016 to January 2019. Outcome and exposure assessment was done through measuring thyroid hormone in blood samples and analyzing urine samples for biomarkers of the sprayed pesticide, respectively. Before the spraying day, urine samples were collected at waking and brought to a nearby clinic, where blood samples were collected between 7–9 a.m. On the spray day, the field staff observed the spraying and interviewed the subject about the amount of pesticide used and the subject provided a urine sample at the end of the spraying event. The next day, the subject collected a first morning void urine and came to the clinic between 7–9 a.m. for blood collection. The detection frequency of urinary paraquat was 62.7% on the day before spraying, 94.1% directly after the spraying event and 76.5 % in the morning after spraying. This study did not find any significant change in the simple paired analysis of thyroid hormone levels from the day before to the day after spraying paraquat. However, in models of thyroid level change that incorporated the measured change in metabolite levels and covariates (gender, debt, and smoking), results indicated that increases in urinary paraquat levels from the day before to the day after spraying were significantly associated with reduced thyroid hormone levels, including reduced total triiodothyronine (T3) (p=0.036) and reduced free triiodothyronine (FT3) (=0.036).

Strengths

This study uses biomarkers of exposure and thus avoids potential recall bias that occurs when establishing exposure through self-report. This study is able to establish temporality as the thyroid hormone levels were measured following pesticide spraying. Additionally, this study had a strong methodology for assessing thyroid hormone through blood sampling and for assessing paraquat exposure through urine analysis.

Weaknesses

- This study did not adjust for coexposure to other pesticides in this analysis. The participants in this study sometimes mixed with 1–2 pesticides to save time and cost of spraying. They stated that paraquat was sprayed by itself for only 33% of the sprayings, thus 77% of the time there were other chemicals mixed or used

alongside paraquat usage. This leaves the possibility of confounding in this 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.
- The cohort of this study included farmers that have been working with and exposed to pesticides for an average of 15 years and have thus experienced chronic exposure to pesticides and may not be the ideal population for assessing outcomes after acute exposure.

DPR Conclusion

This is a well conducted cohort study with strong outcome and exposure ascertainment. In the LN models, the study found that an increase in urinary paraquat from the day before to the day after spraying significantly reduced total triiodothyronine and free triiodothyronine. With the limitations inherent to the methods of the study, this finding in isolation does not merit action beyond additional research being needed. This study was conducted in Thailand, where paraquat regulations and use restrictions may be different than those in the US.

Tomenson et al., 2021

This retrospective cohort study is a follow-up to their previous study (Tomenson & Campbell, 2011), the aim of which was to update information on the risk of Parkinson's disease and mortality from major causes of death among a United Kingdom (UK) workforce who manufactured paraquat by extending the follow-up by seven and a half years. The cohort included all employees who had ever worked on any of the four plants at Widnes, UK where paraquat was manufactured between 1961 and 1995 with vital statistics assessed in June 2009. Updated vital status information for 926 males (as of December 2017) and cause of death information was obtained from NHS Digital, the national provider of data for the UK's National Health Service. Pesticide exposure was assessed using job histories to identify those with the highest potential for exposure, 1330 static monitoring results collected at Widnes between 1979 and 1987 and through 100 personal monitors between 1983 and 1993 but there wasn't sufficient sampling information to perform a quantitative exposure assessment. A limited qualitative exposure assessment was conducted in the mid-1980s that found approximately 300 of the 729 male works included in the prior mortality investigation had high or medium exposure to paraquat. The observed number of deaths from selected causes was compared with the expected number calculated based on country (England and Wales) and local age and period-specific mortality rates. The standardized mortality ratio (SMR) was calculated as the ratio of the observed to the expected deaths. Female workers were not included in the SMR analysis because their numbers were small. Four death certificates of male workers mentioned Parkinson's disease, including two deaths that were due to Parkinson's disease. At least 6 death certificates of male employees would have been expected to have mentioned Parkinson's disease (SMR = 0.67; 95% CI 0.18–1.72). This study did not find evidence of an increased risk of Parkinson's disease-

related mortality among the UK workforce who manufactures paraquat when compared to the expected national mortality.

Strengths
This study uses biomarkers of exposure and thus avoids potential recall bias that occurs when establishing exposure through self-report. By following the workers over time, the cohort design enables them to establish temporality.

Weaknesses
- This study conducted its analysis through calculating SMR. This analysis method does not allow the inclusion of covariates into the study. The lack of covariates increases the chance of confounding and thus is a significant weakness in the design of the analysis.
- This study assessed mortality from Parkinson's disease and not risk of developing Parkinson's disease as many previous studies have done, thus if the worker did develop Parkinson's disease but died of unrelated causes then it would not be included in the study as Parkinson's disease might not be listed as the certified or underlying cause of death. This might introduce the possibility of false negatives.

DPR Conclusion
Due to the limitations inherent in the methods of the design, additional studies will be needed in this cohort. This study did not find evidence of an increased risk of Parkinson's disease-related mortality among the UK workforce who manufactures paraquat when compared to the expected national mortality.

C. Residential or Environmental Exposure Studies (by year of publication)

There were 10 studies found for this literature review that assessed residential or environmental exposure to paraquat through indirect means (e.g., geolocation, residential address, location of pesticide applications, etc.). Two of these studies were included in the US EPA systematic review (Brouwer et al., 2017; Sanders et al., 2017) and two others were reviewed in the main preliminary report as part of public comment submissions (Caballero et al., 2018; Omidakhsh et al., 2022), and therefore not included here. One study did not include paraquat specific findings (Paul et al., 2023) and was not reviewed further. Of the remaining five studies, two investigated the relationship of paraquat exposure and birth defects (Ling et al., 2018; Rappazzo et al., 2019), two evaluated the relationship between paraquat exposure and Parkinson's disease (Cheng et al., 2017; Paul et al., 2024), and one investigated chronic kidney disease (Holliday et al., 2022).

Briefly, Ling and colleagues (2018) investigated the association between paraquat exposure and preterm births and low birthweights. After adjusting for covariates,

paraquat exposure by mothers during gestation was significantly associated with preterm birth in the first and second trimester, but no association was found with low birthweight. Rappazzo et al., (2019) investigated associations between birth defects and residential exposure to seven pesticides. Hypospadias was positively associated with exposures to paraquat but associations were null or inconsistent for atrial septal defects. Cheng et al., 2017 found associations between airborne paraquat exposure and the development of Parkinson's disease, although the exposure determination was also called into question in a subsequent publication (Travis et al., 2018). Paul et al., 2024 was a continuation of Parkinson's Environment and Genes (PEG) Study, a population-based case-control study in California's Central Valley. Several publications from the PEG Study were reviewed in the 2019 US EPA systematic review (US EPA. 2019). Results showed a significant association between paraquat exposure and Parkinson's disease diagnosis across exposure locations (residential and workplace exposure), exposure schemes (ever/never, count, and average exposure per year), and "overall" versus "lagged" exposure. A case control study evaluated the association between paraquat exposure and chronic kidney disease (Holliday et al., 2022). Results showed an initial association between paraquat exposure (yes vs no) and Mesoamerican nephropathy kidney failure; however, when adjusted for covariates, the association disappeared.

For the studies that assessed potential paraquat exposure by self-report, recall, or questionnaire, there is a chance that recall bias could be introduced to the results, especially for study subjects who are already experiencing health conditions. In contrast, studies that rely on geographical methods for assigning exposure status reduce the likelihood of error due to recall bias, a particularly problematic source of bias in retrospective studies. However, using geographical location, address, or records chemical use as proxy measures of exposure is an imprecise estimation of who was exposed, to what concentrations, and for what duration. Studies that utilize proxy measures of exposure based on agricultural pesticide application data and maternal address can lead to exposure misclassification. In general, the exposure misclassification resulting from these types of geographic models of environmental exposures can be differential and result in bias away from the null (Chang et al., 2014). In the absence of supporting environmental or biological monitoring data, results from studies with these proxy measures of exposure should be interpreted with caution and results should not be viewed as definitive.

Cheng et al., 2017

The objective of this study was to quantify the contribution of airborne paraquat exposure to Parkinson's disease risk in Taiwan. The investigators estimated relative risk (RR) and proportion of Parkinson's disease cases given paraquat exposure (θ) from an epidemiological case-control study (Liou et al., 1997) in order to estimate paraquat exposure-associated population attributable fraction (PAF). In the original study, 120 cases (patients with Parkinson's disease) were recruited and matched with 240 hospital

controls by age and sex from the National Taiwan University Hospital. The cases and controls were administered a structured interview to determine demographics and pesticide exposure history. There were 31 cases and 22 controls exposed to paraquat Liou et al. (1997). The investigators used the PAF, θ, and RR parameters to construct a Hill model which expressed the dose-response relationship of paraquat exposure. This Hill-based dose-response relationship and inhibition effect on dopaminergic cell variability were used to estimate paraquat burden in the brain causing 10% inhibition effect (ID10). Paraquat-induced Parkinson's disease risk models were then conducted, and the investigators obtained population attributable risk (PAR) of Parkinson's disease and assessed population exceedance risk by multiplying PAF with annual Parkinson's disease prevalence. Results showed that the largest paraquat exposure contributions occurred in its positive trend during 2004–2011, with the paraquat contributing nearly 21 and 24% to Parkinson's disease prevalence rates among the age groups of 70–79 and ≥ 80 years, respectively, in Taiwan.

Strengths
The methods used to calculate paraquat exposure contribution to Parkinson's disease rate were statistically sound and unique from the other studies exploring paraquat and Parkinson's disease. The Liou et al. (1997) study, which was used to calculate PAF, matched controls to the cases by age and sex, thus avoiding selection bias and confounding.

Weaknesses
- The attribution of paraquat exposure to Parkinson's disease prevalence rate from 2004–2011 used data from the Liou et al. (1997) case-control study. There is upwards of a 14 year gap where pesticide use behaviors and practices have likely changed thus possibly introducing significant bias into the study.
- No updated exposure data was available for this study, as it relied on data originally published in 1997. The authors assumed that there was a uniform airborne concentration of paraquat available to the entire study population, an averaging methodology that is not realistic with point/area source emissions or flux following pesticide applications, as detailed in a subsequent publication (Travis et al., 2018). As such, the exposure estimates were overestimated, thus skewing any potential association.

DPR Conclusion
The results of this case-control study indicate that paraquat exposure contributed 21% and 24% to the Parkinson's disease prevalence rates among individuals aged 70–79 and ≥ 80, respectively. With the limitations inherent in this study, namely the lack of exposure determination for the current study participants and the methodology used to estimate airborne exposures, caution is warranted in applying the findings more broadly. Air monitoring studies conducted by California Air Resources Board on behalf of DPR, no samples detected paraquat above the limit of

quantitation in several communities in a county of high use during the month of expected peak use (Baker et al., 1996), calling into question the exposure estimation used by Cheng et al. (2017). Additionally, this study was conducted in Taiwan which may have different pesticide regulations and requirements than in the US.

Ling et al., 2018

The objective of this study was to examine whether prenatal exposure to agricultural pesticides contributes to the risk of preterm birth or term low birth weight. To do so, the investigators combined two sets of birth records randomly selected from all California births between 1998 and 2010. These birth records were originally matched controls for an autism study (1:10 matched by sex and year of birth) and a cancer study (1:20 matched by year of birth). The investigators restricted the study population to those residing at birth within 2 km of fields on which agricultural use pesticides were applied. Included were 24,693 preterm births, and 220,297 term births, 4412 term low birthweight births, and 194,732 term normal birthweight infants. Pesticide exposure estimates during each month of pregnancy were calculated using California's Pesticide Use Report (PUR) data source, land use maps, and geocoded birth addresses. Monthly exposure estimates (pounds per acre) were calculated by adding the poundage of pesticide applied in a 2 km buffer surrounding each address and weighting the total poundage by the proportion of acreage treated within the buffer. For each trimester, prenatal pesticide exposure to mothers was classified as "ever" or "never exposed" (1 vs. 0) for selected individual chemicals within each chemical class. For each chemical class, the investigators generated a total count that was categorized into three levels (exposed to 2 or more pesticides, exposed to 1 pesticide, and no exposure). Then unconditional logistic regression analyses was conducted, adjusting for matching the controls by infant sex, year of birth, and the source of control subjects (autism vs. cancer study) to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of associations between pesticide exposure and preterm birth or term low birthweight. After adjusting for covariates, the investigators found that paraquat exposure to mothers during gestation was significantly associated with preterm birth in the first trimester (OR: 1.07, 95% CI: 1.03, 1.11) and in the second trimester (OR: 1.06, 95% CI: 1.02, 1.10).

Strengths

Using geographical methods for exposure assessment reduces the likelihood of error due to recall bias, a particularly problematic source of bias in retrospective studies.

Weaknesses

- Despite the complexity of the recruitment process, there were important differences in demographic factors between the cases and the controls. On average, the cases (preterm and born term with a low birthweight) had younger mothers, infants born preterm were more likely to have Hispanic or Black mothers, and term low birthweight infants were more likely to have Black or Asian mothers when compared to the controls (term births and term births with normal

birthweight). 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 avoided this issue entirely. As designed, the recruiting methods may have introduced selection bias and confounding.

- This analysis was exploratory as the researchers investigated 17 different chemicals for potential associations with preterm and term low birthweight. This increases the chances of finding a significant result, making any results found less meaningful.
- The geographical exposure assessment is a proxy measure of exposure and is imprecise in who was exposed and how much exposure there was. While this avoids the downsides of recall bias, it is too imprecise of a measure of exposure to avoid vulnerability to information bias.

DPR Conclusion

This study was a hypothesis generating study. The results do indicate an association between preterm births in both the first and second trimesters and paraquat exposure because of the significant odds ratios of 1.07 and 1.06, respectively. However, with the limitations inherent to the methods of the study, this finding in isolation does not merit action beyond additional research being needed.

Rappazzo et al., 2019

In this cohort study, the association between pesticide exposure and birth defects was examined. Data on birth defects from live births in North Carolina was gathered for the years 2003 through 2005. The specific birth defects evaluated were atrial septal defect (ASD, secundum atrial septal defects, separate from patent foramen ovale), patent ductus arteriosus (PDA) (>2500 gram birth weight), hypoplastic left heart syndrome (HLHS), tracheal esophageal fistula (TEF), hypertrophic pyloric stenosis (HPS), Hirschsprung's disease, hypospadias, upper and lower limb deficiencies, and choanal atresia. Exposure to seven pesticides for which there were over 100,000 application instances including paraquat for the years 2003–2005 was estimated through a calculation based on crop maps from 2002 and 2008–2010. The number of pounds of each pesticide applied within 500 meters of mothers' residences from one month before conception to the 3rd month of pregnancy was estimated based on crop type. Level of exposure was categorized based on percentile within the study population: <10th percentile, 10-<50th percentile, 50-<90th percentile, and >=90th percentile versus unexposed. A total of 51,227 participants (25,066 female, 26,161 male) were not exposed to paraquat at all and 253,679 participants (123,394 female, 130,285 male) were exposed at some level. Paraquat exposure at the 90th percentile was significantly associated with ASD, PDA, HPS, and hypospadias compared to participants who were not exposed to paraquat with odds ratios ranging from 1.32 (CI=1.06, 1.64) to 1.75 (CI=1.29, 2.39). Additionally, paraquat exposure at the median was significantly associated with lower limb defects compared to participants who were not exposed to

paraquat with an odds ratio of 4.65 (CI=1.09, 19.84). Odds ratios were also elevated for paraquat exposure above the median compared to unexposed for HLHS, TEF, choanal atresia, Hirschsprung's, and upper limb defects, although they were not significant.

Strengths
Using geographical methods for exposure assessment reduces the likelihood of error due to recall bias, a particularly problematic source of bias in retrospective studies. The methodology for determining birth defect endpoints was strong, utilizing an established registry to access data for many participants with high reliability. Finally, the timing of the exposure assessment was appropriate for establishing that exposure occurred before health endpoints.

Weaknesses

- The geographical exposure assessment is a proxy measure of exposure and is imprecise with respect to who was exposed and how much exposure there was. This approach avoids recall bias of the subjects. However, it is too imprecise a measure of exposure to avoid information bias.
- Co-exposure to two pesticides correlated with paraquat applications were examined in a sensitivity analysis, but the potential confounding of other combinations of pesticides was not rigorously evaluated.
- The analysis was exploratory in nature due to the fact that seven pesticides and ten health endpoints were examined at one time. This increases the chances of finding a significant result, making any results found less meaningful. This design is beneficial for hypothesis generation, but one should use caution in inferring causality.

DPR Conclusion
All of the birth defect endpoints were more likely to occur in participants in the highest category of paraquat exposure compared to the unexposed participants although some of the associations were not significant. The significant observation of skeletal defects in this study is consistent with registrant submitted developmental toxicity studies showed increased fetal skeletal variations, although maternal toxicity was noted in several studies. However, the consistency of effects noted in both animal and human studies, coupled with the significance of the finding point to the possibility of an association of certain development effects with paraquat exposure that warrant further review.

Holliday et al., 2022
The objective of this case-control study was to investigate potential etiologies for chronic kidney disease of unclear etiology, also known as Mesoamerican nephropathy. Investigators recruited Latin American immigrant patients from the Harris Health System outpatient dialysis unit between 2015 and 2019. Cases were determined to have Mesoamerican nephropathy kidney failure if they had no known cause of primary or

secondary kidney disease (n=52). Controls were extracted from one of two cohorts: Cohort 1 consisted of patients without a known cause of primary or secondary kidney disease (n=63); Cohort 2 were case-matched participants who had no knowledge of having kidney disease and were referred by the cases. Controls from Cohort 2 were of similar age, sex and place of origin and not related to cases. Cases and controls were administered a questionnaire through which self-reported pesticide exposure frequency and duration was obtained (as well as demographics). The investigators estimated the odds ratio (OR) of Mesoamerican nephropathy kidney failure for each agrochemical exposure variable using logistic regression models adjusted for non-agrochemical covariates that were significantly associated with Mesoamerican nephropathy kidney failure in age- and sex-adjusted models. Sixteen healthy controls from Cohort 2 were matched to 16 cases. The Wilcoxon signed rank test was used to compare continuous parameters and McNemar test was used to compare dichotomous parameters. When adjusted for age and sex, paraquat exposure (yes vs no) was significantly associated with Mesoamerican nephropathy kidney failure (OR 2.25; 95% CI: 1.51, 99.36). However, when adjusted for additional covariates (rodent/bird, drinking water, and working with cotton/corn), the significance disappeared.

Strengths

This study benefitted from not investigating multiple pesticides at once. Although the sample size was small, Cohort 2 controls were case-matched by age, sex and place of origin thus reducing the likelihood of selection bias and confounding with this cohort which can often be a limitation in case-control studies.

Weaknesses

- Despite the complexity of the recruitment process, there were important differences in demographic factors between the cases and the controls from Cohort 1. On average, the controls were younger, almost exclusively men, and were more likely to have worked in agriculture while in their home country of origin when compared to the cases. 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 avoided this issue entirely. As designed, the recruiting methods may have introduced selection bias and confounding.
- The assessment of exposure relied on self-report. This may potentially lead to misclassification and bias
- There were no other chemicals 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.

DPR Conclusion

This study provided mixed results at best on the association between paraquat and Mesoamerican nephropathy kidney failure. Although the investigators did find a

positive association between paraquat and Mesoamerican nephropathy kidney failure with an OR of 12.25, in the subsequent three models that further adjusted for confounding, an association was no longer found. In addition, the participants in the study were likely exposed in their home counties in Latin American where paraquat use restrictions and regulations are likely different than in the US.

Paul et al., 2024

The objective of this case-control study was to examine the risk of Parkinson's disease from ambient paraquat exposure. Parkinson's Environment and Genes (PEG) study is a population-based case-control study that examines the connection between Parkinson's disease and exposure to pesticides in three agricultural counties in California's Central Valley. PEG participants with Parkinson's disease and control subjects were recruited between 2000 and 2015 in Kern, Fresno, and Tulare counties in California. Parkinson's disease status for each patient was confirmed by a movement disorder specialist from UCLA. A total of 829 Parkinson's disease patients (519 men, 301 women, 9 unknown) and 824 controls (380 men, 437 women, 7 unknown) were recruited. Exposure to paraquat was assessed through a geographical analysis identifying which participants lived within 500 meters of an application of paraquat using the PUR database and a detailed residential and work history for the years 1974–2015 obtained from interviews with the subjects. Three different exposure schemes were assessed: 1) a participant with any paraquat applied within 500 meters of their work or residence at any time was considered exposed; 2) a count of the number of years with at least one application of paraquat within 500 meters of the work or residence for each participant; and 3) the average pounds of paraquat applied within 500 meters of the work or residence for each participant per year. Overall exposure from 1974 through year of diagnosis or interview and lagged 10 – 20-year exposure to Parkinson's disease diagnosis or interview were examined. Moderately strong and significant associations between paraquat exposure and Parkinson's disease diagnosis were found across exposure locations (residential and workplace exposure), exposure scheme (ever/never, year count, and average exposure per year), and overall versus lagged exposure. Significant odds ratios for this association ranged from 1.19 (CI=1.03, 1.38) to 2.15 (CI=1.46, 3.19).

Strengths

Using geographical methods for exposure assessment reduces the likelihood of error due to recall bias (although recall was relied upon for residential and occupational history which was used to assess exposure), a particularly problematic source of bias in retrospective studies. Unlike many studies utilizing this design, this hypothesis was specific to a single combination of pesticides and one endpoint. Finally, using lagged exposure assessment compared to time of Parkinson's disease diagnosis was a strength, enhancing the establishment of a temporal relationship.

Weaknesses

- Despite the complexity of the recruitment process, there were important differences in demographic factors between the subjects and the controls. On average, the controls were younger, more likely to be female, and more likely to be non-white. While these factors were controlled for statistically using the logistic regression models, there is a potential for error with this approach. Matching control subjects based on these demographic factors would have reduced selection bias and confounding.
- Using geographical location as a proxy measure of exposure is an imprecise method for determining if exposures actually occurred. This approach avoids recall bias of the subjects. However, it is too imprecise a measure of exposure to avoid information bias.
- Co-exposure to four pesticides (chlorpyrifos, glyphosate, isopropylamine salt and diazinon) was controlled for statistically in the measurement of these associations. Parkinson's disease is a complex disease with multi-factorial origins. However, no other co-exposures were examined. With the many other potential pesticide exposures associated with living or working near agricultural application of pesticides, confounding cannot be ruled out.
- Work histories were obtained back to 1974, Study enrollment was 1993–1997, with some participants likely using paraquat before this time. Federal regulatory and label-based restrictions for paraquat use have undergone significant changes since this time. Current handler, reentry worker and bystander protections are different than what study participants likely experienced, such that paraquat during the study period does not reflect current paraquat use.

DPR Conclusions

Findings from this study showed that while there were moderate to strong associations of increased risk of developing Parkinson's disease with proxy measures of residential paraquat exposure, almost all risk estimates were attenuated when examining residential exposures as opposed to workplace exposures. In addition, the authors note that these workplace exposures showed stronger associations in multi-pesticide adjusted models, thus emphasizing the multifactorial origin of Parkinson's disease. This latter finding was also emphasized in another publication by the same research group which found increased motor or cognitive decline in Parkinson's patients associated with residential or workplace proximity to the use of numerous pesticides other than paraquat, including 2-methyl-4-chlorophenoxyacetic acid (MCPA) dimethylamine salt, copper sulfate pentahydrate, S,S,S-tributyl phosphorotrithioate, sodium cacodylate, and methamidophos (Li et al., 2023).

D. Occupational Exposure Studies

Two occupational exposure studies were reviewed that investigated the association between occupational exposure to paraquat and certain health outcomes. Ferri et al.

(2017) considered the association with risk of lymphoma and Diaz-Criollo et al. (2019) evaluated the relationship to adverse respiratory outcomes. One study did not specifically evaluate associations between occupational exposures to paraquat and health outcomes, and was not reviewed further (Salazar-Flores et al., 2020). Briefly, Ferri et al. (2017) found positive association between paraquat and risk for all Lymphoma with an OR of 2.8. However, these findings were complicated by an inverse dose response relationship. Diaz-Criollo et al. (2019) found that chronic paraquat exposure was significantly associated with self-reported asthma. Different pesticide mixtures, combined with paraquat, were also found to be associated with obstructive pattern in spirometry, flu, thoracic pain, and allergic rhinitis.

Ferri et al., 2017

The objective of this case-control study was to explore the occupational risk of the major B-cell lymphoma subtypes in Southern Italy using a case–control study design. To do so, the investigators recruited 158 lymphoma cases and 76 controls in the provinces of Bari and Taranto (Apulia, Southern Italy) from 2009 to 2014. Controls were recruited from the assisted regional register and were selected based on having the same sex, same age class, and same province of residence as the cases; however, due to the small number of controls, no matching analysis was performed. The participation rates were 50% for the population controls, 80% for hospitalized controls, and 75% for cases. Questionnaires were administered to cases and controls to assess demographic characteristics and specific job activity. The participant's work histories and the carcinogen exposure (CAREX) job-exposure matrix were used to determine occupational exposure to pesticides and carcinogens. A score was assigned to each risk factor from the report frequencies contained in the CAREX tables for each job sector [0 = no exposure (no reports); 1 = low exposure (<75% of report frequency); 2 = medium-high exposure (>75% of frequency of reports)]. A score was assigned to each frequency of exposure in CAREX for that sector: [0 = no exposure (no reports), 1 = low exposure (<75% of report frequency), and 2 = medium-high exposure (>75% of frequency of reports)]. This score was multiplied by the duration of each job activity (in years) to calculate the CEI which was categorized as CEI = 0 [no exposure (cumulative indicator = 0)], CEI = 1 [low exposure (cumulative indicator < = 30)], and CEI = 2 [medium-high exposure (cumulative indicator >30)]. The analysis was done using two-sample test of proportions and unconditional logistic regression. Investigators found that participants with low levels of paraquat showed an increased risk for all lymphomas [Odds ratio (OR)= 2.8 (1.0-8.2)].

Strengths

Using work histories and CAREX for exposure assessment reduces the likelihood of error due to recall bias, a particularly problematic source of bias in retrospective studies. Though not individually matched, the controls seem to be representative of the cases thus reducing the likelihood of selection bias and confounding which can often be a limitation in case-control studies.

Weaknesses

- This analysis was exploratory as the researchers investigated 22 different chemical products for potential associations with the development of B-cell lymphoma. This increases the chances of finding a significant result, making any results found less meaningful.
- The work history exposure assessment method is a proxy measure of exposure and is imprecise in how much exposure there was thus does not avoid vulnerability to information bias. While this reduced the likelihood of recall bias as participants are not reporting pesticide exposure, participants are still self-reporting work history which is used to pair with CAREX to determine exposure.

DPR Conclusion

Although the results point to a positive association between paraquat and risk of B-cell lymphoma with an OR of 2.8, the investigators found an inverse dose response relationship. This is contrary to the basic tenants of toxicology, where increasing dose generally results in increasingly severe outcomes, and limits the applicability of the results. In addition, this study was conducted outside the US where pesticide use regulations and restrictions may vary.

Diaz-Criollo et al., 2019

The objective of this study was to explore the association between chronic exposure to pesticide mixtures, including paraquat, and respiratory outcomes among Colombian farmers. To do so, the investigators conducted a cross-sectional study with a volunteer sample of 217 farmworkers from the Colombian municipalities of Carmen de Viboral, Granada, and La Unión. Trained interviewers administered a survey which obtained demographics, occupation (including exposure time at the office and outside of work), clinical symptoms (respiratory symptoms), and toxicological history (smoking and alcohol use). Pulmonary functioning of the participants was evaluated through spirometry using Sibilmed Micro spirometer. The forced vital capacity (FVC) and forced expiratory volume in one second (FEV) were used to determine the presence of obstruction through their ratio (FEV/FCV). Ratios less than 80% were considered obstructed. Participants self-reported respiratory outcomes as well such as the flu, asthma, allergic rhinitis, etc. Participants self-reported that they had been “periodically exposed to paraquat over the past two years and having applied or handled it one week to two days before collection of a urine sample.” Participants also self-reported exposure to other pesticides. Urine samples were collected and used to determine paraquat levels through higher performance liquid chromatography with solid-phase extraction. This values was multiplied by the reported number of months of paraquat exposure at work due to the expectation that respiratory effects were chronic. One-way analysis of variance (ANOVA) or Kruskal-Wallis tests were used to compare the variables among the municipalities. Poisson regression models with robust variance were used to adjust prevalence. The Kernel density estimator through urine analysis found a peak of individuals with no exposure (n=147) and another peak at around 30

ng/ml (starting at 18 and ending around 40 ng/ml). Chronic paraquat exposure was found to be significantly associated with self-reported asthma (prevalence ratio (PR): 1.06; 95% CI: 1.00, 1.13). Different pesticide mixtures combined with paraquat were also found to be associated with obstructive pattern in spirometry, flu, thoracic pain, and allergic rhinitis.

Strengths
One of the outcomes, obstructive pattern in spirometry, was assessed using a spirometer rather than self-report which avoids the possibility of recall bias. Additionally, having paraquat levels detected in urine samples contribute toward the chronic paraquat exposure strengthens the exposure more than relying solely on work history as other studies have.

Weaknesses

- Researchers investigated chronic paraquat exposure for potential associations with 8 different outcomes. This increases the chance of finding a significant result, making any results less meaningful.

DPR Conclusion
The investigators found a positive association between paraquat and asthma with a PR of 1.06, which is consistent with findings of acute inhalation toxicity following high levels of exposure (see Appendix C). However, the 95th%-ile confidence interval was 1.00–1.13, indicating the general weakness of the finding. In addition, this study was conducted outside the US where pesticide use regulations and restrictions may vary.

V. Discussion

A. Parkinson's Disease-Related Studies

There were 5 observational studies found during this literature review that evaluated the relationship between paraquat and Parkinson's disease (Cheng et al., 2017;Shrestha et al., 2020; Tomenson et al., 2021; Yuan et al., 2023; Paul et al. 2024). Findings from Yuan et al., 2023 were difficult to associate with the development of Parkinson's disease from the measured outcome (dream-enacting behaviors). Tomenson et al. (2021) did not find evidence of an increased risk of Parkinson's disease-related mortality among the UK workforce who manufactures paraquat when compared to the expected national mortality. And, while Cheng et al., 2017 found a significant association between ambient exposure to paraquat and the development of Parkinson's disease, issues with the way investigators ascribed paraquat for the Taiwanese population call into question the veracity and applicability of the findings.

The strongest findings for an association between paraquat exposure and the development of Parkinson's disease came from Shrestha et al., 2020 and Paul et al.,

2024. Shrestha et al. (2020) found a non-significant increase in risk of development Parkinson's disease among pesticide applicators in the Agricultural Health Study. But when broken down by the lifetime number of days, the hazard ratio was 1.03, 1.42, and 0.74 for the first, second, and third tertile of use respectively indicating no dose-response based on increasing exposure. The importance of this study, however, was the 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 (1.38–7.45).

Paul et al., 2024 was part of the PEG study. Investigators assessed exposure to paraquat was assessed through a geographical analysis identifying which participants lived within 500 meters of an application of paraquat using the PUR database and a detailed residential and work history for the years 1974–2015 obtained from interviews with the subjects. Moderately strong and significant associations between paraquat exposure and Parkinson's disease diagnosis were found across exposure locations (residential and workplace exposure), exposure scheme (ever/never, count, and average exposure per year), and "overall" versus "lagged" exposure. Significant odds ratios for this association ranged from 1.19 (CI=1.03, 1.38) to 2.15 (CI=1.46, 3.19). Paul and colleagues used a combination of work history and geolocational methods for determining exposure status. Doing so subjected the study to a unique combination of introducing recall bias for residential and occupational history and reducing the likelihood of the same bias by relying on geographic pesticide application data. One strength of using both methods to determine potential exposure is that a more comprehensive pattern of residential movement/migration for study subjects could be developed. However, caution is warranted in making the assumption that pesticide application records are directly related to actual exposure of individuals. In addition, as described in the Regulatory and Registration History of Paraquat section in the main document, there have been significant changes in restrictions and personal protective equipment requirements for paraquat use over the years. Exposures that may have occurred in California during the work/residential history questionnaire timeline (1974–2015) would likely not occur with the numerous restrictions and protections currently in place at both the state and federal level.

B. Non-Parkinson's Disease Related Studies

Ten observation studies were reviewed that evaluated the association between paraquat exposure and various human health outcomes including thyroid hormone effects, respiratory effects, renal disease, birth defects, and cancer.

Kongtip et al., (2021) studied acute pesticide exposures and acute changes in thyroid hormones among Thai sugarcane farmers before and after spray applications of pesticides including paraquat. The strength of the study was that it used biomarkers of exposure and effect, thus reducing potential bias. Results showed urinary paraquat

levels were significantly associated with reduced total triiodothyronine (T3) (p=0.036) and reduced free triiodothyronine (FT3) (=0.036). However, the study failed to adjust for co-exposures, as the applicators reported that they mixed and/or applied multiple pesticides together over 77% of the time. Diaz-Criollo and colleagues (2019) investigated associations between chronic exposure to pesticide mixtures and respiratory outcomes among Colombian farmers. Urine samples were used to determine the level of paraquat exposure in conjunction with reported use. Most participants had no internal paraquat exposure (n=147/217) even if they reported using the herbicide. Chronic exposure was found to be associated with self-reported asthma (prevalence ratio 1.06; 95% CI: 1.00, 1.13). It's important to note that both of these studies were conducted outside of the US where they may be different occupational protection requirements for the use of paraquat or other pesticide.

Four studies investigated the link between paraquat exposure and renal disease or renal cell carcinoma. Lebov et al. (2015) initially reported an association between private pesticide applicator exposure to paraquat prior to 1993 and the incidence of end-stage renal disease in the applicators' spouses who did not apply the herbicide themselves. This highlighted the potential role of take-home exposures among the study subjects in the Agricultural Health Study. However, the same association was not found for spouses who reported applying paraquat themselves (and therefore higher exposure than spouses who were not pesticide applicators). In a follow up study that adjusted for correlation between other pesticide use, no association could be made between end-stage renal disease and paraquat, even at the highest reported uses(Lebov et al., 2016). Holliday and colleagues (2022) investigated chronic renal disease in a population of Latin American immigrants. An association was initially identified with paraquat exposure. But when adjusted for additional covariates, the findings were no longer significant.

The strongest findings of renal disease were from a prospective AHS study by Andreotti et al. (2020). They investigated the association between lifetime use of individual pesticides and the incidence of renal cell carcinoma. The authors used a lagged analysis in which they estimated cumulative exposure for each year of follow-up until cancer diagnosis or other events, and then discounted exposure during the most proximal years. Results indicated an exposure-response association with paraquat and the development of renal cell carcinoma (relative risk = 1.95; 95% CI: 1.03, 3.70). AHS Study participants (private pesticide applicators from Iowa and North Carolina) were recruited between 1993–97, and likely worked applying pesticides for many years prior to enrollment. As described in the main document, there have been significant changes in restrictions and personal protective equipment requirements for paraquat use over the years. Exposures that may have occurred at of AHS recruitment would not occur with the current federal restrictions and protections in place.

Two additional population-based studies with cancer outcomes were reviewed. Alexander et al. (2017) evaluated the association between paraquat exposure and Long Interspersed Nucleotide Element 1 (LINE-1) methylation, a possible epigenetic indicator of cancer risk. For “ever users” of paraquat among male private pesticide applicators in AHS, there was a significant association (beta: -0.45 (SE: 0.23)) in reduction of LINE-1 methylation. Caution is warranted in interpreting these findings in that the authors investigated 57 different pesticides at one time without statistical controls, thus increasing the probability of finding a significant result by chance alone. Ferri et al. (2017) conducted a case-control study to explore the relationship between occupational risk and B-cell lymphoma subtypes. The results pointed to a positive association between paraquat exposure and risk of B-cell lymphoma with an OR of 2.8. However, this was accompanied by an inverse dose-response relationship, meaning those with higher paraquat exposure showed lower risk of developing this cancer.

Finally, two studies investigated the possible link between paraquat exposure and birth defects. Ling et al. (2018) examined whether prenatal exposure to agricultural pesticides contributes to the risk of preterm birth or term low birth weight. After adjusting for covariates, the investigators found that paraquat exposure in mothers during gestation was significantly associated with preterm birth in the first and second trimester. Caution is warranted in interpreting these results as there were multiple differences between the cases and controls and multiple potential exposures were investigated at once and not controlled for statistically. Rappazzo et al. (2019) conducted a cohort study in North Carolina in which data on birth defects from live births were gathered from 2003–2005. Paraquat was significantly associated with several congenital heart defects and lower limb defects. Both studies utilized proxy measures of exposure based on agricultural pesticide application data and maternal address, which can lead to exposure misclassification. In general, the exposure misclassification resulting from these types of geographic models of environmental exposures can be differential and result in bias away from the null (Chang et al., 2014). In the absence of supporting environmental or biological monitoring data, results from studies with these proxy measures of exposure should be interpreted with caution and results should not be viewed as definitive.

VI. Conclusion

The strength and applicability of findings from such studies could increase by designing single exposure (paraquat) - single outcome studies. If co-exposures exist, statistical approaches should be used to avoid compounding. Likewise, data collection and analysis should be designed to avoid recall and information bias, or have methods in place to address and resolve any impact from such bias.

Overall, after exploring the literature on studies investigating paraquat and human health outcomes, this review found that the association with kidney disease-related outcomes are equivocal and although there are numerous limitations in their studies (imprecise geographical exposure assessment, confounding). The association between paraquat and thyroid outcomes and between paraquat and birth defects warrant additional evaluation. With regards to the association between paraquat and Parkinson's disease, the strongest findings came from Shrestha et al., 2020 which found a significant association between occupational paraquat exposure and head injury with the development of Parkinson's disease. This is consistent with the multivariate origins of disease onset and development.

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Appendix C.

Summary of Human Clinical Case Reports and Case Series of Paraquat Poisoning

2015 – 2024

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I. Purpose

The purpose of this literature review was to summarize human clinical case reports and case series of acute paraquat exposure or poisoning to provide additional context for the paraquat injury and illness reports described in the main preliminary report. The studies summarized herein were published from 2015 through July 2024. The result can be considered with the weight of evidence of paraquat associated health effects as DPR considers mitigation or other control measures surrounding the registration and use of this paraquat in California.

II. Human Clinical Case Reports and Case Series

A search of the PubMed data source (https://pubmed.ncbi.nlm.nih.gov/) resulted in 33 case reports and case series of the clinical signs, symptomology and treatment of paraquat poisoning cases published in the open literature since 2015. The case reports largely involve patients that ingested paraquat as a suicide attempt resulting in serious hospitalization or mortality. Pesticide ingestion is a leading method for suicide worldwide (Chang et al., 2021). There is evidence that bans on production or import of paraquat results in a predictive reduction in the use of this herbicide in cases of self-harm or suicide because of increased difficulty in obtaining the chemical (Chang et al., 2021). Fifteen articles investigated acute symptomology and sequelae resulting from paraquat poisoning (Delirrad et al., 2015; Kanchan et al., 2015; Asl and Dadashzadeh, 2016; Ntshalintshali et al., 2017; Fléchel et al., 2018; Isha et al., 2018; Lin et al., 2018; Chen et al., 2019b; Liu et al., 2019; Sharma et al., 2019; Panda et al., 2021; Qiu and Deng, 2021; Eizadi et al., 2022; Liu et al., 2022; Sarkar & Santra, 2022). The most pertinent findings are summarized below.

A. Acute Renal Failure and Other Organ Effects

Kanchan et al. (2015) conducted a postmortem and hospital registry-based retrospective study with the aim of identifying common clinical presentation and end-organ complications of paraquat poisoning in South India. Among the 14 cases (7 females and 7 males), the underlying cause of death included acute renal failure, adult respiratory distress syndrome, multiorgan failure, and acute liver failure. Half of the victims died within 2 days of paraquat ingestion. Fléchel et al. (2018) conducted a retrospective analysis on 26 patients admitted for intentional or accidental ingestion of paraquat at the Western French Guyana hospital. Among the sixteen females and ten males, renal function was impaired for all patients who eventually died versus only two of the six survivors ($p = 0.001$). Some factors associated with death included older age ($p = 0.003$), a higher ingested paraquat dose ($p = 0.04$), impairment of renal function on admission ($p = 0.009$), and hypokalemia ($p = 0.003$). Sarkar and Santra (2022) conducted a cross-sectional study at a tertiary care hospital in West Bengal over a period of a year and found that, among the 32 patients who had ingested paraquat, 59.4% had symptoms of vomiting, 21.9% had throat discomfort, 28.1% had oral ulcers,

56.2% had abdominal pain, 71.9% had decreased urination, and 43.8% had respiratory distress. Moreover, patients (n = 10) who had ingested >30 mL had 100% case fatality and the patients (n = 15) who ingested 16–30 mL had a case fatality rate of 53.3%. Several investigated the treatment of acute lung injury following paraquat exposure (Ntshalintshali et al., 2017; Isha et al., 2018;Wu et al., 2018; Panda et al., 2021). The causative factors of acute paraquat exposure and spontaneous pneumothorax and/or pneumomediastinum and their association with patient mortality were also investigated (Zhou et al., 2015; Sahoo et al., 2020). One acute case required life-saving lung transplant (Tang et al., 2015).

B. Dermal Poisoning

Eizadi et al. (2022) reported a rare fatal case from dermal exposure to paraquat. The patient, 45-year-old man, was admitted 6 days after first contact and presented with dysphagia, respiratory distress and grade two, and third skin burns focusing on the upper body after accidental exposure to paraquat.

C. Factors that Increase Susceptibility to Mortality

Several studies investigated pre-existing conditions or markers that made victims of paraquat poisoning more likely to die from their exposure. Kim et al. (2016) studied the effect multidrug resistance protein 1 (MDR1) gene polymorphisms might have on mortality in paraquat intoxicated patients; however, no significant relationship was found. Chen et al. (2019a) conducted a retrospective study on a sample of 92 patients, 47 females and 45 males, and found that baseline serum high-mobility group box 1 (HMGB1) level was an independent prognostic marker of 30-day mortality and that 30-day mortality was increased in patients with higher baseline serum HMGB1 levels. Oghabian et al. (2019) conducted a cross-sectional study on 126 paraquat poisoned patients who were referred to Afzalipour Hospital in Iran from 2006-2015. Among 126 patients, the highest mortality rate was in patients with respiratory distress, followed by oral ulceration and excess salivation and that ~ 2250 mg predicted death with 86.2% specificity and 75.7% sensitivity. Additionally, the dose of poison, blood sugar level, and aspartate transaminase levels were significantly associated with mortality.

D. Clinical Interventions

Additional studies investigated methods to increase survival rates and reducing mortality from paraquat-induced outcomes (Xu et al., 2015; Zhao et al., 2018). Gao et al. (2015) found that, among 1185 paraquat exposed patients admitted to the first affiliated hospital of Zhengzhou University, Zhengzhou, Henan, China, the mean survival duration was significantly longer in patients treated with hemoperfusion in combination with continuous venovenous hemofiltration than patients treated solely with hemoperfusion. In a follow up study, Chen et al. (2020) found that, among 487 paraquat poisoning cases, patients who received strengthened hemoperfusion in combination

with continuous venovenous hemofiltration had significantly reduced mortality rates when compared to others who were administered conventional therapy. Strengthened hemoperfusion combined with continuous venovenous hemofiltration was an independent factor reducing mortality from paraquat poisoning ($p<0.001$).
Khazraei et al. (2019) studied 44 patients from 3 hospital in Shiraz, Iran from 2010-2015 and found that all 44 patients that undergone mechanical ventilation due to paraquat-induced pulmonary injury were not able to wean off from the ventilator and all passed away. However, in a survival analysis conducted by Ren et al. (2021), the survival rate of patients with paraquat-induced pulmonary fibrosis treated with pirfenidone was significantly higher than that in the group not treated by pirfenidone ($p<0.05$).
While activated charcoal was found not to increase patient survivability (Sun et al. 2018), Yi et al. (2019) found that edaravone significantly protected the liver ($P=.021$), cardiovascular ($P=.031$), and renal ($P=.028$) organs of patients from paraquat associated injury 7 days after the poisoning, but that the drug had no significant protection or improvement on respiratory and digestive tract damage.

III. Conclusion

The summary of data presented above largely comes from intentional exposure to paraquat as an instrument of self-harm. This type of paraquat exposure is outside of legal use of paraquat as regulated by both the US Environmental Protection Agency (US EPA) and the Department of Pesticide Regulation (DPR). However, it is important to note that some symptoms reported from these clinical cases, most notably renal effects and lung toxicity, are consistent with animal toxicity studies and injury and illness reports summarized in the main preliminary report.

Fortunately, there is an apparent decrease in the number of reported illnesses and injuries associated with exposure to paraquat alone or in combination with other pesticides (DPR, 2019). Data for the most recent year (2019) shows only 4 cases, all reporting exposure to paraquat in combination with other active ingredients. This decrease in reported illness and injury cases parallels a downward trend in legal paraquat use in California over the same period (see Figure 1, main document).

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Appendix D.

Review of Paraquat Publications Submitted as Part of Public Comment

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I. Purpose

In November 2022, as part of the annual pesticide registration renewal cycle, the Department of Pesticide Regulation (DPR) received public comments that were accompanied by approximately 80 publications, documents, or webpage references. In November 2023, additional public comments were received by DPR which included an additional 25 references or articles. From the submitted documents, DPR's Human Health Assessment Branch (HHA) identified the scientific articles whose primary investigations were on paraquat (human, animal or in vitro). HHA identified human health and toxicology data that were original peer-reviewed research published in the open literature and conducted a more thorough evaluation of these findings, as summarized in the main document. Inclusion criteria and the evaluation process followed DPR's Guidance for Toxicology Study and Data Acceptability in Registration Review and Risk Assessment (DPR, 2023) and/or the Framework for Incorporating Human Epidemiologic & Incident Data in Risk Assessments for Pesticides from the US Environmental Protection Agency (US EPA, 2016). Submitted publications that that did not make a clear reference to or association of findings to paraquat were reviewed but not included in the preliminary report. Editorial, commentary, or published news articles were reviewed but not included.

Below is a summary of the documents received.

A. November 2022 Public Comment Submissions

Approximately 80 scientific citations or other articles were received by DPR as part of public comment in November 2022. Using the inclusion criteria above, the following scientific articles underwent further evaluation of human health related findings. They are categorized by the type of study (e.g., population-based, animal/in vitro, or reviews/other).

Population-based studies or meta analyses (in chronological order):

There were ten (10) population-based studies or meta analyses submitted to DPR for the department's consideration of human health effects of paraquat that met HHA's inclusion criteria for scientific quality. Each received a complete evaluation in the main preliminary report, including:

Kamel et al., 2006
Costello et al., 2009
Ritz et al., 2009
Gatto et al., 2010
Tanner et al., 2011
Lee et al., 2012
Caballero et al., 2018
Tangamornsuksan et al., 2019
Vaccari et al., 2019
Omidakhsh et al., 2022

Of these studies, US EPA evaluated the findings from Kamel et al., 2007, Costello et al., 2009, Ritz et al., 2009, Gatto et al., 2010, Tanner et al., 2011, and Lee et al., 2012 in the agency's systematic review of the association of paraquat and Parkinson's disease (US EPA, 2019c).

Animal/in vitro studies (in chronological order):

There were four (4) animal or in vitro studies submitted to DPR for the department's consideration of human health effects of paraquat that met HHA's inclusion criteria. Each received a complete evaluation in the preliminary report including:

McCormack et al., 2003
Peng et al., 2004
Prasad et al., 2007
Anselmi et al., 2018

Reviews or other articles (in chronological order):

There were five (5) scientific reviews or other articles submitted to DPR for the department's consideration of human health effects of paraquat that met HHA's inclusion criteria. All five articles were reviewed, and of these, three were included as background information in the preliminary report.

McDowell and Chesselet, 2012 – Study was used for background information for animal models of Parkinsonism

Nandipati and Litvan, 2016 – Study provided review of primary studies whose findings were either evaluated by US EPA or that were separately submitted to DPR, and therefore was reviewed but not included in the preliminary report

Billingsley et al., 2018 – Study was reviewed and determined to not be specific to paraquat (investigated genetic causation of Parkinson's disease)

Chen and Ritz, 2018 – Study was used for background information on the multivariate causes of Parkinson's disease

Ball et al., 2019 – Study was used for background information on the multivariate causes of Parkinson's disease

B. November 2023 Public Comment Submissions

An additional 25 scientific citations or other articles were received by DPR as part of public comment in November 2023. Using the inclusion criteria above, 15 articles underwent further review of human health related findings. They are categorized by the type of study (e.g., population-based, animal/in vitro, or reviews/other).

Population-based studies or meta analyses (in chronological order):

There were six (6) population-based studies submitted to DPR for the department's consideration of human health effects of paraquat that met HHA's inclusion criteria. All six articles were reviewed. Of these, four articles underwent further evaluation of human health related findings and two were determined to be not relevant to paraquat, as follows:

Baldereschi et al., 2000 – Study reviewed and determined to not be specific to paraquat

Karunarathne et al., 2000 – Study reviewed and determined to not be specific to paraquat

Tanner et al., 2009 – Study was used as methodological background for a study by the same research group which received a complete evaluation in the preliminary report (Tanner et al., 2011; see above)

Goldner et al., 2010 – Received a complete evaluation in the preliminary report

Fléchel et al., 2018 – Received a complete evaluation in Appendix C of this document as part of the review of case reports and case studies on paraquat used for self-harm

Chang et al., 2021 – Received a complete evaluation in Appendix C of this document as part of the review of case reports and case studies on paraquat used for self-harm

US EPA did not review any the above articles in its 2019 systematic review on Parkinson's disease and paraquat (US EPA, 2019c).

Animal/In vitro studies (in chronological order):

There were six (6) animal or in vitro studies submitted to DPR for the department's consideration of human health effects of paraquat that met HHA's inclusion criteria. Of these, five received a complete evaluation in the preliminary report, as follows:

McCormack et al., 2005
Rudyk et al., 2015
Duan et al., 2023
Nuber and Selkoe, 2023
Zuo et al., 2023

Richardson et al., 2015 – Study was reviewed and determined to not be relevant to paraquat (investigated health effects of the pesticide deltamethrin)

Reviews or other articles (in chronological order):

There were three (3) scientific reviews or other articles submitted to DPR for the department's consideration of human health effects of paraquat that met HHA's inclusion criteria. All three articles were reviewed, and none was determined to be relevant to the preliminary report, as follows:

Lee and McEwen, 2001 – Study reviewed the neurotrophic and neuroprotective actions of estrogens

Kaasinen et al., 2015 – This was a clinical study on aging in Parkinson's disease patients

Georgiev et al., 2017 – This study reviewed the clinical data on gender differences in Parkinson's disease

TOTAL SUBMITTED STUDIES COMPREHENSIVELY EVALUATED: 26

All totaled, HHA included 26 documents submitted in a more detailed evaluation of the scientific findings of paraquat impacts to human health either in the main preliminary report or in one of the appendices. Of these, seventeen (17) articles were submitted in 2022 and nine (9) in 2023. Table D.1 summarizes the review of the documents submitted to DPR as part of public comment and their inclusion in the preliminary report.

Table D.1. Publications, documents, or webpage references received by DPR as part of public comment on the registration renewal of paraquat and their review status for the Preliminary Report on Human Health Effects of Paraquat

Citation (Alphabetical by Author)	**Review Notes**	**Warranted further review (Y/N)**
2022 Submissions		
Aminci Brief for Timothy Greenamyre et al. 2021 No. 21-71287	No. 21-71287 US Court of Appeals for the Ninth Circuit, California Rural Legal Assistance Foundation et al., Petitioners v. US Environmental Protection Agency	N
Anderson, 2016	For ecotox review	N
Anselmi et al., 2018	Study using neurotoxin-based animal models to investigate pathogenesis of Parkinson's disease	Y
Badroo et al., 2020	For ecotox review	N
Ball et al., 2019	Review of Parkinson's disease research	Y
Berger et al., 2018	For ecotox review	N
Billingsley et al., 2018	Genetic causation of Parkinson's disease	N
Caballero et al., 2018	Population based study	Y
CalEnviroScreen 4.0 Indicator Maps	CalEnviroScreen site with indicator maps	N
Canevari et al., 2017	For ecotox review	N
CDFW	5-Year Status Review: Greater Sandhill Crane; For ecotox review	N
Centers for Disease Control	CDC website, Facts About Paraquat	N

Table D.1. Publications, documents, or webpage references received by DPR as part of public comment on the registration renewal of paraquat and their review status for the Preliminary Report on Human Health Effects of Paraquat

Citation (Alphabetical by Author)	**Review Notes**	**Warranted further review (Y/N)**
Chaffin, 2022	Legal Examiner (blog), article on US EPA risk-benefit analysis of paraquat use	N
Chen and Ritz, 2018	Review of current Parkinson's research	Y
Code of Federal Regulations	70 Fed. Reg. 49,380, Designation of Critical Habitat for the California tiger salamander; For ecotox review	N
Cornell Lab of Ornithology	Swainson's hawk - Range Map; For ecotox review	N
Cornell Lab of Ornithology	Tricolored Blackbird - Range Map; For ecotox review	N
Costello et al., 2009	Population based study	Y
Cox, 2022	News article, The Bakersfield Californian	N
Donley, 2019	Review of US, EU, China, Brazil pesticide bans	N
Dorsey et al., 2020	No complete reference provided	N
DPR	Pest Management web page	N
DPR	PUR data - pounds used, 2018	Y
DPR	PUR data - total pounds, applications, acres treated, 2018	Y
DPR	PUR Report, June 2020	Y
Feigin et al., 2019	Analysis of the global burden of neurological diseases from 1990–2016	N
Fitzgerald, 2021	EarthJustice press release	N
Gatto et al., 2010	Population based study	Y
Gillam and Uteuova, 2022	News article, The Guardian	N
Hakim, 2016	News article, NY Times article	N
Huang et al., 2019	For ecotox review	N
Kamel et al., 2006	Population based study	Y
Katz et al., 2022	Petitioners Opening Brief: California Rural Legal Assistance Foundation et al. v. US Environmental Protection Agency	N
Kruse et al., 2021	Economic evaluation of late-stage PD in Germany	N
Lanini, 2016	For ecotox review	N
Lee et al., 2012	Population based study	Y
McCormack and Di Monte, 2003	Study using neurotoxin-based animal models to investigate pathogenesis of Parkinson's disease	Y
McDowell and Chesselet, 2012	Study using neurotoxin-based animal models to investigate pathogenesis of Parkinson's disease	Y
Motion for Voluntary Remand Without Vacatur	CRLA et al. v. EPA, No. 21-71287	N
Nandipati and Litvan, 2016	Review of primary studies whose findings were either evaluated by US EPA or as part of this document	N
National Institute of Neurological Disorders and Stroke, 2022	NINDS webpage for patient information	N
NOAA Fisheries	California Central Valley Steelhead; For ecotox review	N
NOAA Fisheries	Species Directory: Coho Salmon (Protected); For ecotox review	N

Table D.1. Publications, documents, or webpage references received by DPR as part of public comment on the registration renewal of paraquat and their review status for the Preliminary Report on Human Health Effects of Paraquat

Citation (Alphabetical by Author)	**Review Notes**	**Warranted further review (Y/N)**
Omidakhsh et al., 2022	Population based study	Y
Opening Brief, CRLA et al. v. EPA	CRLA et al. v. EPA, No. 21-71287	N
Peng et al., 2004	Study using neurotoxin-based animal models to investigate pathogenesis of Parkinson's disease	Y
Pesticide Action Network, 2022	Consolidated list of banned pesticides	N
Prada, 2015	News article, Reuters	N
Prasad et al., 2007	Study using neurotoxin-based animal models to investigate pathogenesis of Parkinson's disease	Y
Ritz et al., 2009	Population based study	Y
Sagar, 1987	Historical context of paraquat use	N
Tangamornsuksan et al., 2019	Epidemiological meta-analysis	Y
Tanner et al., 2011	Population based study	Y
The Lewin Group, Inc., 2019	Economic impact of PD in the USA in 2017	N
UC IPM	For ecotox review	N
US EPA	Paraquat Dichloride: Draft Human Health Risk Assessment, 2019	Y
US EPA	Federal Record announcement of availability of the Interim Decision for Paraquat Dichloride, 2021	N
US EPA	US EPA, Paraquat Dichloride: Interim Registration Review Decision, 2021	Y
US EPA	Active ingredient webpage, Paraquat Dichloride, 2022	N
US EPA	Website, Paraquat Dichloride: One Sip Can Kill	N
US EPA	Paraquat: Preliminary Ecological Risk Assessment for Registration Review, 2019; For ecotox review	N
US EPA	Risks of Paraquat Use to Federally Threatened California Red-legged Frog; For ecotox review	N
US FWS	San Joaquin kit fox 5-Year Review, 2022; For ecotox review	N
US FWS	Specie Profile for Giant kangaroo rat; For ecotox review	N
US FWS	Species Profile for California red-legged frog (Rana draytonii); For ecotox review	N
US FWS	Species Profile for California Tiger Salamander; For ecotox review	N
US FWS	Species Profile for Chinook salmon; For ecotox review	N
US FWS	Species Profile for Fresno kangaroo rat; For ecotox review	N
US FWS	Species Profile for Valley elderberry longhorn beetle; For ecotox review	N
US FWS	Species Profile for Vernal pool fairy shrimp; For ecotox review	N
US FWS	Species Profile for Yellow-billed Cuckoo; For ecotox review	N
USGS	Estimated Annual Agricultural Pesticide Use Maps- Paraquat; For ecotox review	N
Vaccari et al., 2019	Epidemiological meta-analysis	Y

Table D.1. Publications, documents, or webpage references received by DPR as part of public comment on the registration renewal of paraquat and their review status for the Preliminary Report on Human Health Effects of Paraquat

Citation (Alphabetical by Author)	**Review Notes**	**Warranted further review (Y/N)**
Yang et al., 2020	Current and projected economic burden of Parkinson's disease in the US	N
2023 Submissions		
American Association of Neurological Surgeons	Patient information webpage on Parkinson's disease	N
Baldereschi et al., 2000	Population based study; not paraquat specific	N
CAMS-care	Suicide treatment and help in California (website)	N
Chang et al., 2021	Paraquat case reports	Y
Cleveland Clinic, 2023	Patient information webpage on the substantia nigra	N
Duan et al., 2023	Study using neurotoxin-based animal models to investigate pathogenesis of Parkinson’s disease	Y
Fléchel et al., 2018	Paraquat case reports	Y
Georgiev et al., 2017	Reviewed clinical data on gender differences in Parkinson’s disease	N
Goldner et al., 2010	Population based study	Y
Kaasinen et al., 2015	Clinical study on aging in Parkinson’s disease patients	N
Karunarathne et al., 2020	Not paraquat relevant	N
Kingsley, 2022	Opinion piece, The Sacramento Bee	N
Lee and McEwen, 2001	Neurotrophic and neuroprotective actions of estrogens	N
Mayo Clinic	Patient website on Parkinson's disease diagnosis	N
McCormack et al., 2005	Study using neurotoxin-based animal models to investigate pathogenesis of Parkinson’s disease	Y
Moffitt Cancer Center, 2023	Patient information webpage on thyroid cancer	N
Nuber and Selkoe, 2023	Study using neurotoxin-based animal models to investigate pathogenesis of Parkinson’s disease	Y
Richardson et al., 2015	Deltamethrin study	N
Rudyk et al., 2015	Study using neurotoxin-based animal models to investigate pathogenesis of Parkinson’s disease	Y
Rutgers University, 2015	Rutgers University press release on study of ADHD and deltamethrin	N
Sanesco Health, 2016	Sanesco Health, Dopamine pathways (blog)	N
Stuart et al., 2023	Agricultural production article	N
Tanner et al., 2009	Population based study	Y
Tsalenchuk et al., 2023	Epigenetic evaluation of environmental exposures and Parkinson's disease; not paraquat specific	N
Zuo et al., 2023	Study using neurotoxin-based animal models to investigate pathogenesis of Parkinson’s disease	Y