No. 21-71287

**UNITED STATES COURT OF APPEALS**
**FOR THE NINTH CIRCUIT**

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CALIFORNIA RURAL LEGAL ASSISTANCE FOUNDATION, *et al.*,
*Petitioners*,

v.

U.S. ENVIRONMENTAL PROTECTION AGENCY, *et al.*,
*Respondents*,

SYNGENTA CROP PROTECTION, LLC,
*Intervenor-Respondent*.

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ON PETITION FOR REVIEW OF THE ORDER OF THE ADMINISTRATOR OF THE UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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# BRIEF OF *AMICI CURIAE* J. TIMOTHY GREENAMYRE, MICHAEL OKUN, AND BEATE RITZ IN SUPPORT OF PETITIONERS

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## INTERESTS OF THE *AMICI CURIAE*[1]

*Amici* are medical doctors and scientists with expertise in neurology or epidemiology, particularly in the relationship between toxins—such as paraquat—and neurodegenerative diseases, such as Parkinson's disease ("Parkinson's" or "PD"). *Amici* have a strong interest in supporting Petitioners' challenge to the interim registration review decision because of the robust scientific evidence *amici* and numerous other scientists have compiled showing a strong link between paraquat exposure and the onset of Parkinson's.

*Amicus* J. Timothy Greenamyre, MD, PhD, is the Love Family Professor and Vice Chair of Neurology at the University of Pittsburgh, where he also directs the Pittsburgh Institute for Neurodegenerative Diseases and the American Parkinson Disease Association Center for Advanced Research. He is an expert on gene-environment interactions in causing Parkinson's and he has investigated biochemical mechanisms of paraquat toxicity.

*Amicus* Michael S. Okun, MD, is a neurologist and neuroscientist. He is the Director of the Norman Fixel Institute for Neurological Diseases at the University of Florida and is also the chair of the Department of Neurology at the University of

[1] Pursuant to Federal Rule of Appellate Procedure 29(a)(2), *amici* state that all parties have consented to the filing of this brief. Pursuant to Federal Rule of Appellate Procedure 29(a)(4)(E), *amici* certify that no person or entity, other than *amici* or their counsel, made a monetary contribution to the preparation or submission of this brief or authored this brief in whole or in part.

Florida. He has been the Medical Director/Advisor for the Parkinson's Foundation since 2006. He is an expert on Parkinson's and has published several hundred peer reviewed research articles on the topic including in the New England Journal of Medicine, JAMA, and Lancet.

*Amicus* Beate Ritz, MD, PhD, is a Professor of Epidemiology at the UCLA Fielding School of Public Health with co-appointments in Environmental Health Sciences and Neurology at the David Geffen School of Medicine at UCLA, and a member of the UCLA Center for Occupational and Environmental Health. She co-directed the National Institute for Environmental Health Sciences-funded Center for Gene-Environment Studies in Parkinson's Disease at UCLA, which targeted occupational and environmental toxins, and especially pesticides, in relation to Parkinson's. She was also principal investigator of the large population-based case-control study of pesticide exposures and Parkinson's in California—the Parkinson's, Environment and Genes (PEG) study.

## ARGUMENT

### I. PARKINSON'S IS A WIDESPREAD NEURODEGENERATIVE DISEASE CAUSED BY ENVIRONMENTAL AND GENETIC FACTORS

Parkinson's is a progressive, neurodegenerative disorder with no known cure. The four most common motor symptoms of Parkinson's are tremors in the hands, arms, legs, jaw, or head; muscle stiffness; slow movement; and difficulties

with balance and coordination. Non-movement symptoms can include loss of smell, gastrointestinal and sleep disorders, dementia, and depression. Symptoms are usually mild at first but worsen over time, although the rate of disease progression varies among cases. There is currently no therapy to slow, stop, or reverse the disease's progression. 2-ER-254.

Parkinson's is a significant and growing threat to public health in the United States. Approximately one million Americans currently live with Parkinson's, *id.*, making it the second-most-common neurological disorder in the country, behind Alzheimer's. In 2017, Parkinson's was the fourteenth-leading cause of death in the United States.[2] Moreover, Parkinson's now outpaces Alzheimer's as the world's fastest-growing neurological disorder.[3] Approximately 60,000 Americans receive a Parkinson's diagnosis each year, and by 2030, 1.2 million Americans are expected to be living with the disease.[4] Although early-onset Parkinson's (defined as Parkinson's in those aged 50 or younger) remains less common, its rate of

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[2] Centers for Disease Control and Prevention, *Deaths, Percent of Total Deaths, and Death Rates for the 15 Leading Causes of Death: United States and Each State, 2017*, at 1 (Dec. 31, 2018), https://www.cdc.gov/nchs/data/dvs/lcwk/lcwk5_hr_2017-508.pdf.

[3] E. Ray Dorsey & Bastiaan R. Bloem, *The Parkinson Pandemic—A Call to Action*, 75 JAMA NEUROLOGY 9, 9 (2018).

[4] *Statistics*, PARKINSON'S FOUNDATION, https://www.parkinson.org/Understanding-Parkinsons/Statistics (last visited June 1, 2022).

diagnosis doubled in the United States between 2013 and 2017.[5] Neurologists now recognize that we are experiencing a Parkinson's pandemic.[6]

The hallmark symptoms of Parkinson's result from both a loss of the neurochemical dopamine and degeneration of multiple brain circuits referred to as the basal ganglia. Dopamine is a neurotransmitter that allows neurons to communicate with each other and control movement. In Parkinson's, dopaminergic (dopamine-producing) neurons in one of the basal ganglia, called the substantia nigra, are lost or impaired.

The causes of dopaminergic neuron degeneration are complex, but toxic effects related to a protein called alpha-synuclein are believed to play an important role. When this protein misfolds, it tends to clump together, first into small oligomers, later into larger fibrils, and eventually into Lewy bodies, the pathological hallmark of Parkinson's.[7] The exact mechanism by which alpha-synuclein causes neurodegeneration is an active area of research, but it is clear that accumulation of abnormal forms of the protein leads to death of neurons. One of

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[5] BLUE CROSS BLUE SHIELD ASS'N, THE HEALTH OF AMERICA REPORT: PREVALENCE OF PARKINSON'S DISEASE RISING IN YOUNGER ADULTS 2 (2020), https://www.bcbs.com/sites/default/files/file-attachments/health-of-america-report/HOA-Parkinsons_0.pdf.

[6] Dorsey & Bloem, *supra* note 3, at 9.

[7] *See* Kelvin C. Luk et al., *Pathological α-Synuclein Transmission Initiates Parkinson-like Neurodegeneration in Nontransgenic Mice*, 338 SCIENCE 949 (2012).

the initiating factors in causing alpha-synuclein to misfold and aggregate is oxidative stress, such as that caused by paraquat.

While the hallmark motor symptoms of Parkinson's are accompanied by the presence of Lewy bodies in the substantia nigra and other basal ganglia, there is evidence that the disease can begin outside of the brain. Early symptoms include loss of smell and constipation, and scientists have recently found that Lewy bodies can form in the nose and intestine before appearing in the brain.[8] In addition, there is experimental evidence that misfolded alpha-synuclein can propagate from cell to cell.[9] It is therefore believed that the disease can travel to the brain either from the nose through the olfactory bulb or from the gut through the vagus nerve.[10]

Parkinson's can be caused by a combination of genetic and environmental factors. While research has identified several genetic mutations that cause alpha-

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[8] *See* Morten G. Stokholm et al., *Pathological α-synuclein in Gastrointestinal Tissues from Prodromal Parkinson Disease Patients*, 79 ANNALS OF NEUROLOGY 940 (2016); G. Webster Ross et al., *Association of Olfactory Dysfunction with Incidental Lewy Bodies*, 21 MOVEMENT DISORDERS 2062 (2006).

[9] Luk et al., *supra* note 7.

[10] *See* K. Del Tredici & H. Braak, *Review: Sporadic Parkinson's Disease: Development and Distribution of α-synuclein Pathology*,42 NEUROPATHOLOGY & APPLIED NEUROBIOLOGY 33 (2016); Staffan Holmqvist et al., *Direct Evidence of Parkinson Pathology Spread from the Gastrointestinal Tract to the Brain in Rats*, 128 ACTA NEUROPATHOLOGICA 805 (2014). The vagus nerve connects the brain to the heart, lungs, and gut, and plays an important role in regulating heart rate, breathing, and digestion. RAY DORSEY ET AL., ENDING PARKINSON'S DISEASE: A PRESCRIPTION FOR ACTION 29 (2020).

synuclein misfolding, only about 10% of Parkinson's cases are primarily caused by genetics.[11] In fact, a study comparing Parkinson's in identical and fraternal twins suggests that environmental factors play a more important role in causation than genetic factors, especially for those who are diagnosed after the age of 50.[12] Environmental risk factors associated with Parkinson's include head injury, low dietary fat intake, and exposure to a number of pesticides—including paraquat.

## II. PARAQUAT IS A COMMONLY USED, ACUTELY TOXIC HERBICIDE

Paraquat is one of the most widely used herbicides in the United States. 1-ER-012. It is "a broad-spectrum, contact herbicide that targets emerged broadleaf and grass weeds by inhibiting photosynthesis." 1-ER-011. Farmers use it both to control weeds before planting and to dry out crops for harvest. 1-ER-012. Paraquat is used on many crops, with soybeans, cotton, and corn being the most common. *Id.* Each year pesticide handlers apply over eight million pounds of paraquat to more than 15 million acres of agricultural land across the United States. *Id.* Moreover, paraquat's use is rising—in part as a substitute for glyphosate (Roundup), whose effectiveness has declined with the emergence of glyphosate-

[11] Christine Klein & Ana Westenberger, *Genetics of Parkinson's Disease*, 2 COLD SPRING HARBOR PERSPECTIVES IN MEDICINE a008888 (2012).

[12] Caroline M. Tanner et al., *Parkinson Disease in Twins: An Etiologic Study*, 281 JAMA 341 (1999).

resistant weeds. The amount of paraquat used in the United States more than doubled between 1992 and 2016.[13]

Paraquat is acutely toxic. A single exposure to paraquat can cause skin and eye irritation and respiratory harm, including lung inflammation, scarring, and compromised lung function. 3-ER-407–08. Swallowing even a teaspoonful can be fatal. 3-ER-567. Because it is so toxic and so widely available, ingesting paraquat is a common suicide method in some parts of the world. In addition, as discussed below, a growing body of research has linked paraquat exposure to Parkinson's for more than twenty years.

Based on these health effects, at least thirty-three countries have banned the use of paraquat. 2-ER-256. It is one of only two pesticides that are approved for use in the United States despite being banned or phased out in the European Union, Brazil, and China. 2-ER-099.

## III. PARAQUAT LIKELY HAS A CAUSAL RELATIONSHIP WITH PARKINSON'S

Multiple lines of convergent scientific evidence, published in peer-reviewed journals over many years, establish a link between paraquat and Parkinson's. Epidemiological studies show elevated rates of Parkinson's among people exposed

[13] *Estimated Annual Agricultural Pesticide Use: Pesticide Use Maps—Paraquat*, U.S. GEOLOGICAL SURVEY (Oct. 12, 2021), https://water.usgs.gov/nawqa/pnsp/usage/maps/show_map.php?year=2018&map=PARAQUAT&hilo=L.

to paraquat; animal studies demonstrate that paraquat can elicit the hallmark symptoms of Parkinson's in mice; and *in vitro* studies provide evidence of the mechanisms underlying these findings, demonstrating that paraquat can kill dopaminergic cells and disrupt the cellular processes that regulate the alpha-synuclein protein.

### A. Epidemiological Studies Demonstrate an Association between Paraquat and Parkinson's

The weight of the available epidemiological evidence reveals a positive association between exposure to paraquat and Parkinson's, which, in combination with the animal and *in vitro* studies described below, suggests a causal relationship. This conclusion is supported by additional epidemiological evidence that paraquat's relationship with Parkinson's is dose-dependent. Moreover, the paraquat-Parkinson's association significantly increases when it is combined with other Parkinson's risk factors, which likely magnifies the risk of paraquat for certain subgroups of the population.

First, several epidemiological studies link paraquat exposure to an increased likelihood of developing Parkinson's. For example, papers from the Agricultural Health Study ("AHS"), a prospective study of "84,740 private pesticide applicators, recruited in 1993–1997 in Iowa and North Carolina, and their spouses," found a significant positive association between paraquat exposure and

Parkinson's.[14] In one paper (Tanner et al. 2011), a case-control study nested within the AHS classified by the Environmental Protection Agency ("EPA") as "high quality," 2-ER-280–81, the authors found that people who reported mixing or applying paraquat at least once were 2.5 times as likely to develop Parkinson's as those who had never used paraquat. 3-ER-623.[15] Numerous studies from other

[14] 3-ER-623; Freya Kamel et al., *Pesticide Exposure and Self-reported Parkinson's Disease in the Agricultural Health Study*, 165 AM. J. EPIDEMIOLOGY 364, DocID 993 (2007).

[15] EPA attempted to downplay the Tanner et al. paper by citing a more recent paper from the AHS study (Shrestha et al. 2020, 2-ER-159). Paraquat: HED Response to Comments on the Proposed Interim Decision for Registration Review and updated Occupational Handler Exposure and Risk Estimates, DocID 625, at 6–7 (June 22, 2021). While the Shrestha et al. paper found a strong positive association between paraquat use and Parkinson's among the subset of study participants with a history of head injury, the paper did not find a significant association between paraquat use alone and Parkinson's. Nonetheless, the authors did not assert that their paper undermined the findings of Tanner et al., describing differences in the two papers' methodologies that could explain the divergent results. 2-ER-160–61. Indeed, Shrestha et al. stated that, "for the herbicide paraquat, animal and earlier human studies offer persuasive evidence for a potential link with PD, despite continuing debate," and added that "[w]e cannot rule out the possibility that limited evidence of independent associations between PD and ever-use of some pesticides (including paraquat) in the current study resulted from non-differential bias attenuating [hazard ratio] estimates." 2-ER-161. EPA, in discussing the Shrestha et al. paper, failed to mention these qualifications or any of the many other limitations provided by the authors. Moreover, a more recent paper from the AHS study found a significant relationship (OR=3.48) between the experience of a high-paraquat exposure event and later development of dream-enacting behavior, which is "very common" among patients with Parkinson's. Yaqun Yuan et al., *High Pesticide Exposure Events and Dream-Enacting Behaviors Among US Farmers*, 37 MOVEMENT DISORDERS 962, 967 (2022).

regions also report odds ratios (ORs) above one—indicating that Parkinson's is more likely among the exposed group than the control group—including one study with an OR as high as 3.5.[16] In fact, a recent systematic review and meta-analysis, which synthesized the results from 13 case control studies with a total of 3,231 patients and 4,901 controls, found a statistically significant association between paraquat exposure and Parkinson's, with an overall 64% increase in risk. 3-ER-548.

Second, the evidence of a paraquat-Parkinson's link is strengthened by studies suggesting that the association is dose-dependent. For example, in the AHS nested case-control study mentioned above, whereas individuals with less than eight cumulative lifetime days' worth of paraquat use were 2.4 times more likely

[16] Camille Pouchieu et al., *Pesticide Use in Agriculture and Parkinson's Disease in the AGRICAN Cohort Study*, 47 INT'L J. EPIDEMIOLOGY 299, DocID 1020 (2018) (OR=1.43 for ever-use of paraquat among French agricultural workers); Nicole M. Gatto et al., *Well-water Consumption and Parkinson's Disease in Rural California*, 117 ENVTL. HEALTH PERSPS. 1912, DocID 978 (2009) (OR=1.10 for paraquat exposure from well water in rural California); Caroline M. Tanner et al., *Occupation and Risk of Parkinsonism: A Multicenter Case-control Study*, 66 ARCHIVES NEUROLOGY 1106, DocID 1037 (2009) (OR=2.80 for ever-use of paraquat in among North American Parkinson's patients); Amanpreet S. Dhillon et al., *Pesticide/environmental Exposures and Parkinson's Disease in East Texas*, 13 J. AGROMEDICINE 37 (2008) (OR=3.5 for ever-paraquat use in Parkinson's patients in East Texas); C. Hertzman et al., *A Case-control Study of Parkinson's Disease in a Horticultural Region of British Columbia*, 9 MOVEMENT DISORDERS 69 (1994) (OR=1.11-1.25 for paraquat exposure among Parkinson's patients in an agricultural region of Canada).

than the control group to develop Parkinson's, those who used paraquat for a longer period were *3.6 times more likely* to develop the disease.[17] Similarly, a study of Parkinson's patients in Taiwan found an OR of 6.44 for individuals who had used paraquat for at least 20 years, compared to no association for shorter term use. 3-ER-654.

Third, epidemiological studies suggest that the Parkinson's risk from paraquat exposure is even higher for people with other risk factors—including genetic predisposition, prior head injury, low fat intake, and exposure to other pesticides. Several studies demonstrate that individuals who are genetically predisposed to Parkinson's have a much greater risk of developing the disease after paraquat exposure than the general population. For example, one study found that people with a mutation in the GSTT1 gene were *11 times as likely* to develop Parkinson's if they used paraquat, whereas paraquat users who did not have the mutation were only 1.5 times as likely. 2-ER-281; 3-ER-617. Several other studies have shown significantly higher Parkinson's risk for people with other genetic mutations after paraquat exposure.[18]

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[17] Caroline M. Tanner et al., *Rotenone, Paraquat, and Parkinson's Disease*, 119 ENVTL. HEALTH PERSPS. 866, DocID 1036 (2011) (supplemental materials, table 3, https://ehp.niehs.nih.gov/action/downloadSupplement?doi=10.1289%2Fehp.1002839&file=1002839art_suppl.pdf).

[18] Nicole M. Gatto et al., *α-Synuclein Gene May Interact with Environmental Factors in Increasing Risk of Parkinson's Disease*, 35 NEUROEPIDEMIOLOGY 191,

Non-genetic risk factors are also associated with an increased risk of developing Parkinson's after paraquat exposure. Traumatic brain injury increases the Parkinson's OR for paraquat exposure from 1.36 to 3.01.[19] Similarly, low dietary fat intake increases the risk of paraquat exposure, with low omega-6 intake increasing the OR for paraquat exposure from 1.2 to 4.0, and low linoleic acid intake increasing the OR from 1.2 to 3.8.[20]

Exposure to other pesticides also increases the Parkinson's risk of paraquat.[21] The Taiwan study mentioned above found that participants who used

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193, DocID 978 (2010) (OR=1.45 and 1.35 for individuals with two variants of the alpha-synuclein gene and high paraquat exposure, compared to OR=1.22 and 0.77 for individuals with those variants and low or no paraquat exposure); Laurie H. Sanders et al., *Base Excision Repair Variants and Pesticide Exposure Increase Parkinson's Disease Risk*, 158 TOXICOLOGICAL SCI. 188, DocID 1030 (2017) (OR=2.38 for paraquat-exposed individuals with two or more alleles of DNA repair genes associated with Parkinson's risk, compared to OR=1.13 for exposed individuals with no more than one such risk allele); Beate Ritz et al., *Dopamine Transporter Genetic Variants and Pesticides in Parkinson's Disease*, 117 ENVTL. HEALTH PERSPS. 964, DocID 1027 (2009) (OR=4.53 for individuals with above-median paraquat and maneb exposure and at least two risk alleles, compared to OR=2.99 for above-median exposure and one risk allele).

[19] Pei-Chen Lee et al., *Traumatic Brain Injury, Paraquat Exposure, and their Relationship to Parkinson's Disease*, 79 NEUROLOGY 2061, DocID 996 (2012).

[20] Freya Kamel et al., *Dietary fat intake, Pesticide Use, and Parkinson's Disease*, 20 PARKINSONISM & RELATED DISORDERS 82, DocID 992 (2014).

[21] Anthony Wang et al., *Parkinson's Disease Risk from Ambient Exposure to Pesticides*, 26 EUROPEAN J. EPIDEMIOLOGY 547, DocID 1043 (2011) (OR=1.26 for individuals with ambient paraquat-only exposure, and OR=1.50 for individuals exposed to a combination of paraquat, maneb, and ziram).

paraquat and other pesticides occupationally were twice as likely to develop Parkinson's as those who had only been exposed to pesticides other than paraquat. 3-ER-653. Thus, while the use of other pesticides alone or alongside paraquat may increase the risk of Parkinson's, 2-ER-159, paraquat is independently associated with Parkinson's and may further increase the overall risk with co-exposures. Therefore, the paraquat-Parkinson's association cannot be attributed to a confounding effect with other pesticides.

### B. Paraquat Exposure Can Cause Parkinson's-like Symptoms in Animal Studies

Animal studies bolster the evidence for a link between paraquat and Parkinson's through several lines of evidence. They show that paraquat can accumulate in tissues, including the brain. In addition, mice exposed to paraquat exhibit the hallmark pathological signs and symptoms of Parkinson's. Finally, paraquat exposure reduces dopamine levels in animals, providing further evidence of the loss of dopaminergic neurons—a key step in the development of Parkinson's in humans.

Animal studies on paraquat exposure reveal that body tissues, including the brain, sequester paraquat.[22] Mice exposed to paraquat through injection, inhalation,

[22] R.E. Murray & J.E. Gibson, *Paraquat Disposition in Rats, Guinea Pigs, and Monkeys*, 27 TOXICOLOGY & APPLIED PHARMACOLOGY 283, DocID 1007 (1974); R.J. Dinis-Oliveria et al., *Paraquat Poisonings: Mechanisms of Lung Toxicity,*

and oral administration have shown paraquat accumulation in their brain tissue[23]—including in the striatum and midbrain portions of the brain, regions that house the substantia nigra and are affected in Parkinson's.[24] EPA recognized that "the observation of paraquat in the midbrain is particularly important for connecting environmentally relevant exposure to PD because it demonstrates that ingested paraquat is able to cross the blood-brain-barrier (BBB) and distribute to a region of the brain that is involved in the pathology of PD." 2-ER-321.[25]

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*Clinical Features, and Treatment*, 38 CRITICAL REVS. TOXICOLOGY 13, DocID 967 (2008).

[23] Kavita Prasad et al., *Prolonged Toxicokinetics and Toxicodynamics of Paraquat in Mouse Brain*, 115 ENVTL. HEALTH PERSPS. 1448, DocID 1022 (2007); Charles B. Breckenridge et al., *Pharmacokinetic, Neurochemical, Stereological and Neuropathological Studies on the Potential Effects of Paraquat in the Substantia Nigra Pars Compacta and Striatum of Male C57BL/6J Mice*, 37 NEUROTOXICOLOGY 1, DocID 941 (2013); Richard J. Smeyne et al., *Assessment of the Effects of MPTP and Paraquat on Dopaminergic neurons and Microglia in the Substantia Nigra Pars Compacta of C57BL/6 Mice*, 11 PLOS ONE e0164094, DocID 1035 (2016); Timothy Anderson et al., *Paraquat Inhalation, a Translationally Relevant Route of Exposure: Disposition to the Brain and Male-Specific Olfactory Impairment in Mice*, 180 TOXICOLOGICAL SCI. 175 (2021).

[24] Prasad et al., *supra* note 23; Alison McCormack & Donato Di Monte, *Effects of l-dopa and Other Amino Acids against Paraquat-induced Nigrostriatal Degeneration*, 85 J. NEUROCHEMISTRY 82 (2003); Keiko Shimizu et al., *Paraquat Leads to Dopaminergic Neural Vulnerability in Organotypic Midbrain Culture*, 46 NEUROSCIENCE RSCH. 523, DocID 1033 (2003); Lina Yin et al., *Genetic-based, Differential Susceptibility to Paraquat Neurotoxicity in Mice*, 33 NEUROTOXICITY & TERATOLOGY 415 (2011).

[25] More recent research has confirmed that inhaled paraquat can also reach the midbrain. Anderson et al., *supra* note 23.

Short-term paraquat exposure through a variety of routes elicits hallmark Parkinson's behavior in mice, including tremors and motor impairment.[26] Male mice exposed to a daily oral dose exhibit trembling and decreased ambulatory activity.[27] 3-ER-628. Reduced motor coordination has been observed in mice,[28] and one maze test study observed dose-dependent motor impairment in mice.[29] In fact, injecting mice with paraquat so reliably elicits Parkinson's-like symptoms that paraquat has been used to create an animal model for study of the disease.[30]

In addition to these behavioral effects, paraquat exposure can elicit neurochemical changes. Following several weeks of an oral paraquat dose, male mice have decreased levels in the substantia nigra of dopamine, dopamine

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[26] Because only humans can be diagnosed with Parkinson's, animal studies instead investigate the typical, or "hallmark," symptoms of the disease. 2-ER-321.

[27] Female mice also exhibit signs of motor impairment at a similar dose level. Nicolas Naudet et al., *Oral Exposure to Paraquat Triggers Earlier Expression of Phosphorylated α-synuclein in the Enteric Nervous System of A53T Mutant Human α-synculein Transgenic Mice*, 76 J. NEUROPATHOLOGY & EXPERIMENTAL NEUROLOGY 1046, DocID 1009 (2017).

[28] R.M. Satpute et al., *Effect of Resveratrol and Tetracycline on the Subacute Paraquat Toxicity in Mice*, 36 HUMAN & EXPERIMENTAL TOXICOLOGY 1303, DocID 1031 (2017).

[29] Dan Lou et al., *Does Age Matter? Comparison of Neurobehavioral Effects of Paraquat Exposure on Postnatal and Adult C57BL/6 Mice*, 26 TOXICOLOGY MECHANISMS & METHODS 667, DocID 489 (2016).

[30] Kim Tieu, *A Guide to Neurotoxic Animal Models of Parkinson's Disease*, 1 COLD SPRING HARBOR PERSP. MEDICINE a009316, a009321 (2011).

metabolites, and tyrosine hydroxylase, an enzyme involved in dopamine synthesis and used to identify dopaminergic neurons. 3-ER-628. These results are "consistent with dopaminergic neuron degeneration." 2-ER-323. Furthermore, a dose-dependent decrease in dopamine has been observed in the midbrain of male mice.[31] Female mice exposed to paraquat through drinking water also exhibit signs of neuroinflammation.[32]

### C. *In Vitro* Studies Suggest a Plausible Mechanism for How Paraquat Can Cause Parkinson's

*In vitro* studies performed at the cellular and subcellular level complement the evidence provided by animal studies, further supporting the likelihood that paraquat has a causative relationship with Parkinson's.

First, *in vitro* studies suggest that paraquat decreases the viability of dopaminergic neurons. For instance, reduced cell viability following paraquat exposure has been observed in human neural progenitor cell lines ("hNPCs")—a type of human stem cell that is a precursor to several types of neurons of the central nervous system, including dopaminergic neurons.[33] Reduced viability has

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[31] A. Endo et al., *Effects of a Paraquat-containing Herbicide, Gramoxon®, on the Central Monoamines and Acetylcholine in Mice*, 13 RSCH. COMMC'NS PSYCHOLOGY PSYCHIATRY BEHAVIOR 261, DocID 969 (1988).

[32] Naudet et al., *supra* note 27.

[33] *See, e.g.*, S. McCarthy et al., *Paraquat Induces Oxidative Stress and Neuronal Cell Death; Neuroprotection by Water-soluble Coenzyme $Q_{10}$*, 201 TOXICOLOGY &

also been observed in developed rodent dopaminergic neurons following several days of paraquat exposure at low doses.[34] Rat neurons also exhibited a significant dose-dependent decrease in dopamine uptake.[35]

Furthermore, intracellular studies suggest mechanisms for how paraquat exposure causes dopaminergic neuron death. Paraquat elicits intracellular markers of apoptosis—the process of programmed cell death—in hNPCs.[36] Paraquat also causes oxidative stress in cells. In fact, similar to how animal studies use paraquat to elicit hallmark Parkinson's symptoms and create an animal model for the study of Parkinson's, *in vitro* studies use paraquat to elicit oxidative stress in cells and create a positive control for the study of oxidative stress. 2-ER-343.

Second, and of critical importance to understanding paraquat's relationship with Parkinson's, *in vitro* studies suggest that paraquat causes dysfunction in the cellular processes responsible for regulating the alpha-synuclein protein. As

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APPLIED PHARMACOLOGY 21, DocID 1005 (2004); Xiuli Chang et al., *Paraquat Inhibits Cell Viability via Enhanced Oxidative Stress and Apoptosis in Human Neural Progenitor Cells*, 206 CHEMICO-BIOLOGICAL INTERACTIONS 248, DocID 946 (2013).

[34] *See, e.g.*, Xue-Fei Wu et al., *The Role of Microglia in Paraquat-induced Dopaminergic Neurotoxicity*, 7 ANTIOXIDANTS & REDOX SIGNALING 654, DocID 1047 (2005); Heather Klintworth et al., *Activation of c-Jun N-terminal Protein Kinase is a Common Mechanism Underlying Paraquat- and Rotenone-induced Dopaminergic Cell Apoptosis*, 97 TOXICOLOGICAL SCI. 149, DocID 995 (2007).

[35] Wu et al., *supra* note 34.

[36] McCarthy et al., *supra* note 33.

discussed above, misfolding and accumulation of the alpha-synuclein protein, which manifests as Lewy bodies, is a marker of Parkinson's. In hNPCs, paraquat has been shown to increase alpha-synuclein protein levels just 48 hours after exposure.[37] Paraquat concentration is also correlated with an acceleration in the rate of alpha-synuclein fibril formation—a precursor to Lewy body formation.[38] Taken together, these studies suggest that paraquat may lead to the aggregation of alpha-synuclein protein in cells.

Typically, the cellular processes of autophagy and the ubiquitin-proteasome system ("UPS") break down misfolded and damaged proteins in cells.[39] These processes are also specifically involved in regulating the alpha-synuclein protein.[40]

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[37] Francesca F. Caputi et al., *Proteasome Subunit and Opioid Receptor Gene Expression Down-regulation Induced by Paraquat and Maneb in Human Neuroblastoma SH-SY5Y Cells*, 40 ENVTL. TOXICOLOGY & PHARMACOLOGY 895, DocID 943 (2015).

[38] Amy B. Manning-Bog et al., *The Herbicide Paraquat Causes Up-regulation and Aggregation of α-synuclein in Mice*, 277 J. BIOLOGICAL CHEMISTRY 1641, DocID 1004 (2002); 3-ER-550.

[39] Juliana Navarro-Yepes et al., *Inhibition of Protein Ubiquitination by Paraquat and 1-methyl-4-phenylpyridnium Impairs Ubiquitin-dependent Protein Degradation Pathways*, 53 MOLECULAR NEUROBIOLOGY 5229, DocID 1010 (2016).

[40] 2-ER-344 (citing Diogo M. Branco et al., *Cross-talk Between Mitochondria and Proteasome in Parkinson's Disease Pathogenesis*, 2 FRONTIERS AGING NEUROSCIENCE 17 (2010)).

Paraquat has been shown to disrupt both processes in human cells,[41] suggesting another mechanism for how paraquat might contribute to the accumulation of misfolded alpha-synuclein. In human cells, a duration- and concentration-dependent reduction in UPS activity has been observed within just six hours of exposure to paraquat.[42]

In summary, *in vitro* studies demonstrate that paraquat both causes the aggregation and misfolding of alpha-synuclein (via oxidative stress) and disables the cellular systems that normally detoxify such misfolded proteins. Both alpha-synuclein aggregation and misfolding are central to the causation of neurodegeneration in Parkinson's. These studies therefore provide evidence for a causal mechanism that complements the evidence from the epidemiological and animal studies described above.

## IV. EPA MADE SIGNIFICANT ERRORS IN ITS REVIEW OF THE EVIDENCE LINKING PARAQUAT AND PARKINSON'S

EPA discounted many important epidemiological and animal studies for reasons that were not sufficiently grounded in science. Had EPA used accepted

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[41] Navarro-Yepes et al., *supra* note 39; Wonsuk Yang & Evelyn Tiffany-Castiglioni, *Paraquat-induced Apoptosis in Human Neuroblastoma SH-SY5Y Cells: Involvement of p53 and Mitochondria*, 71 J. TOXICOLOGY & ENVTL. HEALTH 289, DocID 1050 (2008).

[42] Qunxing Ding & Jeffrey N. Keller, *Proteasome Inhibition in Oxidative Stress Neurotoxicity: Implications for Heat Shock Proteins*, 77 J. NEUROCHEMISTRY 1010, DocID 966 (2001).

scientific methods to adequately account for potential bias or confounding factors, which are omnipresent in these sorts of studies to one degree or another, EPA would have recognized the compelling coherence along the three lines of evidence—human epidemiology, animal models, and *in vitro* studies. Properly weighed together, these multiple lines of evidence point towards a causal relationship between paraquat exposure and Parkinson's.

### A. EPA Incorrectly Discounted Epidemiological Studies Demonstrating an Association between Paraquat and Parkinson's

In its Systematic Review, EPA considered 26 epidemiological studies. 2-ER-279. Of the studies reporting an odds ratio, all but two reported an odds ratio above one for at least one category of analysis or participants.[43] 2-ER-304. Nevertheless, EPA found that these studies provided only "limited, but insufficient" evidence of a "clear associative or causal relationship" between occupational paraquat exposure and Parkinson's, and "insufficient" evidence of "clear associative or causal relationship" between non-occupational paraquat exposure and Parkinson's. 2-ER-299, 303. EPA's conclusions are contrary to the weight of the epidemiological evidence.

[43] As explained above, an odds ratio greater than one indicates that the effect (here the development of Parkinson's) is higher in the treated group (here, people exposed to paraquat) than in the control group.

Of the 26 studies EPA considered, EPA gave less weight to 22 of them because it ranked them as either "moderate" or "low" quality. EPA's criteria included the degree to which a study could verify paraquat exposure and study outcomes, control for confounders, recruit an adequate sample size, and avoid risk of bias. 2-ER-367. While these criteria are useful in evaluating epidemiologic evidence, they need to be applied carefully and not used to dismiss valuable information regarding causality.

The National Research Council cautions against employing a blunt approach to evaluating a study's limitations, emphasizing that:

> [A]ll studies have "flaws" in the sense of limitations that add uncertainty about the proper interpretation of the results. Some flaws are inevitable given the limits of technology, resources, the ability and willingness of persons to participate in a study, and ethical constraints. In evaluating epidemiologic evidence, the key questions, then, are the extent to which a study's limitations compromise its findings and permit inferences about causation.[44]

Similarly, the National Academies of Sciences, Engineering, and Medicine have also cautioned that:

> [T]he possibility that a single unsatisfactory rating could completely nullify the use of a particular study from synthesis is problematic as it may lead to a biased review…. In fact, combining multiple small,

[44] NATIONAL RESEARCH COUNCIL, REFERENCE MANUAL ON SCIENTIFIC EVIDENCE 553 (3d ed. 2011).

> low-powered but similar studies in a synthesis is one of the benefits of systematic review.[45]

EPA violated these principles in its treatment of studies based on their potential bias. (In this context, "bias" refers to any source of systematic error, not the researchers' subjective intentions.[46]) EPA's approach had two key flaws: first, it failed to assess the likelihood that bias did in fact occur, and second, it made no attempt to estimate the magnitude of its effect on the reported results.

EPA identified the potential for recall and selection bias in several studies' reliance on self-reporting to assess a participant's paraquat exposure. 2-ER-298. While this method can introduce the risk of bias into a study, EPA should have employed a more nuanced approach to assessing this risk.

EPA also discounted studies that eliminated this risk of bias by using Geographic Information System mapping to assess paraquat exposure, asserting that this method did not identify exposure to specific pesticides. 2-ER-303. However, isolating paraquat-only exposure is impossible to achieve in any human study that does not intentionally treat subjects with toxins—which would be

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[45] NATIONAL ACADEMIES OF SCIENCES, ENGINEERING, AND MEDICINE, THE USE OF SYSTEMATIC REVIEW IN EPA'S TOXIC SUBSTANCES CONTROL ACT RISK EVALUATIONS 6 (2021).

[46] HARVEY CHECKOWAY ET AL., RESEARCH METHODS IN OCCUPATIONAL EPIDEMIOLOGY 89 (2d ed. 2004).

ethically impermissible. Throughout their lifetimes, people are exposed to a multitude of chemical agents both in combination and sequentially.

Moreover, all exposure assessment methods have downsides, whether because they introduce a risk of recall bias or reporting error or a risk of exposure misclassification (errors in exposure measurements).[47] In fact, most exposure assessment errors (those classified as "non-differential," i.e., the same in cases and controls) are more likely to result in null findings (i.e., the failure to find an effect) because any true signal is likely to get drowned out in the noise of the data. Discounting studies due to some degree of exposure misclassification is not recommended because no exposure assessment method can fully eliminate the risk of such errors. For example, a systematic review protocol on the paraquat-Parkinson's relationship recommended using four categories to assess bias risk (definitely low, probably low, probably high, or definitely high) in combination with sensitivity analyses.[48]

Second, EPA erred by giving less weight to "secondary" papers. EPA identified "primary" articles as those that examined only the association between

[47] *See id.* at 10 ("[E]ven the most well designed and conducted epidemiologic study is limited by potential bias and uncontrolled confounding.").

[48] Carolina Vaccari et al., *Paraquat and Parkinson's Disease: A Systematic Review Protocol According to the OHAT Approach for Hazard Identification*, 6 SYSTEMATIC REVS. 98, at 4–5 (2017).

paraquat and Parkinson's, and "secondary" articles as those that examined potential effect modification by environmental, dietary, and behavioral factors on the same study population examined in a primary article. EPA asserted that secondary articles did not "provide additional, independent information" on the association between paraquat and Parkinson's. 2-ER-279.

However, these "secondary" articles both reveal that paraquat's risk is elevated for certain subpopulations and point to a causal interpretation because the interaction observed is between certain risk genes and exposures. As a result, these findings should reframe the analysis of earlier "primary" studies, as they suggest that the association found in the "primary" studies masks a much stronger association occurring for certain study subgroups. Indeed, they mean that even studies with null findings might mask significant impacts among susceptible subpopulations that are not visible when analyzing the data of the whole population. Other recent systematic reviews and meta-analyses did not discount the findings of secondary studies in the way EPA did.[49]

[49] Wimonchat Tangamornsuksan et al., *Paraquat Exposure and Parkinson's Disease: A Systematic Review and Meta-analysis*, 74 ARCHIVES ENVTL. & OCCUPATIONAL HEALTH 225, 226, DocID 1055 (2019); Carolina Vaccari et al., *Paraquat and Parkinson's Disease: A Systematic Review and Meta-analysis of Observational Studies*, 22 J. TOXICOLOGY ENVTL. HEALTH, PART B: CRITICAL REVS. 172, 178, DocID 1054 (2019); Minako T. Allen & Leonard S. Levy, *Parkinson's Disease and Pesticide Exposure—A New Assessment*, 43 CRITICAL REVS. TOXICOLOGY 515, 517 (2013).

In addition, EPA erred by lowering its confidence in several studies for failing to isolate paraquat-only exposure in the study population. While an inability to isolate paraquat exposure does introduce the risk of confounding effects, epidemiological researchers are inevitably constrained by the impossibility of recruiting participants who have only been exposed to one chemical agent in their lifetime. This limitation creates a trade-off, as a low sample size also reduces confidence in a study's findings.[50] But more importantly, such bias can be and has been addressed in epidemiological research by adjusting for potential confounders or performing formal bias analyses to estimate the potential size of the bias introduced. Rather than discounting the findings of studies that failed to isolate paraquat-only exposure, EPA should have conducted sensitivity analyses that extract the valuable information each study contains and integrated these into the systematic review.[51] As mentioned above, a recently-published comprehensive systematic review synthesized the results of the epidemiological studies through a meta-analysis and found a statistically significant association between paraquat exposure and Parkinson's. 3-ER-548.

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[50] *See, e.g.*, 2-ER-294–95 (classifying several studies as "low quality" due to the small number of paraquat exposure cases identified in the study population).

[51] Indeed, as described above, a study in Taiwan has shown that people exposed to paraquat and other pesticides were twice as likely to develop Parkinson's as those who had only been exposed to pesticides other than paraquat. *See* pp. 12–13, *supra*.

### B. EPA Incorrectly Excluded or Gave Lower Weight to Hundreds of Animal Studies

EPA identified 217 animal studies relevant to evaluating paraquat's association with Parkinson's. 2-ER-306. However, EPA ultimately considered only eleven of them in its weight of the evidence assessment. 2-ER-316. The majority of studies were eliminated from EPA's consideration because they used injection as the route of paraquat exposure, rather than oral, dermal, or intranasal exposure. 2-ER-306. Additionally, for the eleven studies EPA did consider, EPA found it a "major weakness for the overall database" that most studies included only male mice. 2-ER-309. Both of these are significant errors in evaluating the animal evidence.[52]

First, EPA erred by wholly excluding injection studies. EPA reasoned that "given the divergence in toxicokinetic behavior from anticipated routes of exposure, toxicity data reported for injection studies is of limited use to assessing human risk from pesticidal uses of paraquat." 2-ER-306. However, EPA is incorrect that there is a meaningful divergence in toxicokinetic behavior from injection exposure compared to other routes. EPA overlooks that for studying the paraquat-Parkinson's link, the mechanism of paraquat exposure is largely

[52] These errors are all the more concerning, given that even taking into account only the eleven studies that EPA included in its analysis, EPA should have concluded that there was a risk of concern if it had applied the appropriate uncertainty factors. *See* Pet. Opening Br. at 30–34.

irrelevant provided that paraquat enters the bloodstream. This is because, as EPA recognized, paraquat can enter the brain by crossing the blood-brain-barrier, 2-ER-321–22, meaning that paraquat exposure in the bloodstream can lead to exposure in the brain. Paraquat can be absorbed into the bloodstream through injection, ingestion, inhalation, and dermal exposure.[53] Furthermore, once in the brain, paraquat selectively enters dopaminergic neurons via a specific uptake mechanism.[54] And, once in neurons, there is no known mechanism by which paraquat can exit the cell or be metabolized.[55] In essence, once paraquat enters a neuron, it is there forever, constantly causing oxidative stress until the cell dies.

Moreover, it is also likely that paraquat does not even need to cross the blood-brain-barrier to affect dopaminergic neurons in the substantia nigra. This is because alpha-synuclein pathology can begin in the olfactory system or the gut and pass transneuronally from olfactory neurons or through the vagus nerve, respectively, to the substantia nigra.[56] Taken together, this evidence indicates that the method of paraquat exposure does not affect resulting toxicokinetic behavior in

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[53] Edward A. Lock & Martin F. Wilks, *Paraquat*, *in* HAYES' HANDBOOK OF PESTICIDE TOXICOLOGY 1771 (R. Krieger ed., 3d ed. 2010).

[54] Phillip M. Rappold et al., *Paraquat Neurotoxicity is Mediated by the Dopamine Transporter and Organic Cation Transporter-3*, 108 PROC. NAT'L ACAD. SCI. 20766 (2011).

[55] Lock & Wilks, *supra* note 53.

[56] *See* p. 5, *supra*.

such a way as to justify wholly excluding injection studies from the review of the evidence. Contrary to EPA, in a recent paper recommending systematic review protocols tailored to paraquat-Parkinson's studies, the authors chose to impose "no restrictions regarding dose or exposure route" for animal studies.[57]

Second, EPA erred in discounting the overall weight of the animal evidence because the majority of studies it considered used male mice. EPA ignores that men appear more susceptible to Parkinson's than women,[58] so a divergence in the responses of male and female mice to paraquat would not be surprising. However, the findings observed in male mice are supported by existing studies that involving female mice. For instance, a study conducted only on female mice revealed that they experienced neuroinflammation (a well-known component of Parkinson's pathology) after exposure to paraquat in drinking water.[59]

## CONCLUSION

For the foregoing reasons, *amici* respectfully request the Court remand the Registration Decision.

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[57] Vaccari et al., *supra* note 48, at 3.

[58] Silvia Cerri et al., *Parkinson's Disease in Women and Men: What's the Difference?*, 9 J. PARKINSON'S DISEASE 501 (2019).

[59] Naudet et al., *supra* note 27.

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**CERTIFICATE OF SERVICE**

I hereby certify that I electronically filed the foregoing brief with the Clerk of the Court for the United States Court of Appeals for the Ninth Circuit by using the appellate CM/ECF system on June 1, 2022. I certify that all participants in the case are registered CM/ECF users and that service will be accomplished by the appellate CM/ECF system.

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**UNITED STATES COURT OF APPEALS**
**FOR THE NINTH CIRCUIT**

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