National Toxicology Program
U.S. Department of Health and Human Services

PROTOCOL FOR SCOPING REVIEW OF

PARAQUAT DICHLORIDE EXPOSURE AND PARKINSON'S DISEASE

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Office of Health Assessment and Translation
Division of the National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

TABLE OF CONTENTS

LIST OF TABLES

BACKGROUND AND SIGNIFICANCE

Background

Paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium ion, hereafter referred to as paraquat) is a quaternary ammonium compound, and it is used as a broad-spectrum, fast-acting contact herbicide. Paraquat is registered for use in both agricultural and non-agricultural settings, and is used to both control weeds and as a post-harvest desiccant (harvest aid) (Bromilow 2004). It is applied as a direct spray and only kills the leaves that come in direct contact with the compound. Paraquat is inactivated upon soil contact. Importantly, paraquat is a restricted-use pesticide (i.e., it can only be purchased and used by certified applicators), and it is not registered for any homeowner or residential applications in the United States.

The primary route of exposure for paraquat is occupational exposure, including during the mixing, loading, and application of paraquat or during post-application processes (US EPA 1997). Paraquat is not registered for residential use, but residential exposure can occur for those living near farms where paraquat has been applied. However, based on normal use patterns, paraquat is not expected to be a surface water or groundwater contaminant (US EPA 1997).

High level, acute exposure to paraquat is associated with pulmonary toxicity in both humans and animal models because paraquat can accumulate in lung tissues (US EPA 1997, Dinis-Oliveira *et al.* 2008). Paraquat is categorized as having very high/high acute inhalation toxicity, moderate acute oral toxicity, and slight acute dermal toxicity (US EPA 1997). Low level, chronic exposure to paraquat is associated with various health effects, including pulmonary and central nervous system toxicity (US EPA 1987, Dinis-Oliveira *et al.* 2008). The oral reference dose for chronic oral exposure to paraquat is based on the critical effect of chronic pneumonitis (US EPA 1987).

Experimental animal studies and epidemiological studies of farmworkers suggest that paraquat exposure may affect the central nervous system (McCormack *et al.* 2002, Kamel *et al.* 2007, Prasad *et al.* 2007, Prasad *et al.* 2009, van der Mark *et al.* 2012). For example, controlled experiments in mammals have demonstrated appreciable paraquat accumulation in brain tissue (Prasad *et al.* 2007, Prasad *et al.* 2009) and neurological tissue damage consistent with the etiology of Parkinson's disease (McCormack *et al.* 2002). Moreover, meta-analyses of epidemiological data from the literature and the agricultural health study identified an elevated risk of Parkinson's disease in humans for general herbicide exposure (van der Mark *et al.* 2012) and for paraquat exposure alone (Kamel *et al.* 2007), respectively.

Significance

The association between paraquat exposure and Parkinson's disease was identified as a potential candidate for systematic review as a result of an NTP scoping activity of Parkinson's disease (NTP report under review) and was also identified by several outside groups. Given the interest and extent of the evidence, the National Toxicology Program (NTP) at NIEHS will conduct a targeted scoping review of the literature on paraquat exposure and Parkinson's disease to systematically collect and categorize the evidence to develop a systematic evidence map of the key Parkinson's related health effects, types of evidence, and gaps in research. NTP considered input from outside groups (EPA and a research group from the University Estadual Paulista, São Paulo, Brazil) to support a consistent process, promote data access and data sharing, and to avoid duplication of effort. The information contained in this scoping review will be made publicly available in an NTP Research Report, which can be used to support a full systematic review or for consideration of future research on this topic.

OBJECTIVE AND SPECIFIC AIMS

Objective

The primary objective of this evaluation is to undertake a scoping review of existing human, animal, and *in vitro* studies and identify the literature relevant to paraquat exposure and neurobehavioral and neuropathological endpoints associated with Parkinson's disease. The scoping review will systematically collect and categorize the evidence to map the key Parkinson's disease-related health effects and identify gaps in research.

Specific Aims

- Identify literature reporting the effects of paraquat exposure on neurobehavioral and neuropathological endpoints associated with Parkinson's disease in humans, animals, and *in vitro* model systems.
- Extract data on potential health effects from relevant studies (data extraction files of the included studies will be shared upon release of final report).
- Summarize/map the health effects and mechanistic data relevant to Parkinson's disease (i.e., the extent and types of health effects evidence available).

PECO Statement

A PECO statement (**P**opulation, **E**xposure(s), **C**omparator(s), and **O**utcome(s)) (Table 1) was developed to address and understand the potential effects of paraquat on neurological outcomes associated with Parkinson's disease in humans, animals, and *in vitro* model systems (Table 1). The PECO statement is used to help develop the specific research questions, search terms, and inclusion/exclusion criteria for the systematic review (Higgins and Green 2011).

Table 1. Human PECO (Population, Exposure, Comparator and Outcome) Statement

Element	Evidence
Population	**Human:** Humans, without restriction based on age, sex, or lifestage at exposure or outcome assessment. **Animal:** Experimental animals without restriction based on species (including Drosophila and other non-mammalian species), age, sex, or lifestage at exposure or outcome assessment. ***In vitro*:** Human or animal cells, tissues, or model systems with *in vitro* exposure regimens. Examples of cell lines typically used for *in vitro* Parkinson's disease mechanistic study include: SK-N-SH, SH-SY5Y, PC12, RBE, astrocytes, and dopaminergic neurons.
Exposure	Exposure to paraquat dichloride (CAS#1910-42-5) based on administered dose or concentration, biomonitoring data (e.g., urine, blood, or other specimens), environmental measures (e.g., air, water levels), or indirect measures such as job title or occupational history.

Table 1. Human PECO (Population, Exposure, Comparator and Outcome) Statement	
Comparators	**Human:** A comparison population exposed to lower levels (or no exposure/exposure below detection levels) of paraquat. **Animal:** Comparable animal populations that were untreated or exposed to vehicle-only treatment in experimental animal studies. ***In vitro*:** Comparable cells or tissues exposed to vehicle-only treatment or untreated controls.
Outcomes	**Human:** ***Primary outcomes:*** Diagnosis of Parkinson's disease and/or clinical observations, neurobehavioral, or neuropathological outcomes typically associated with Parkinson's disease following *in vivo* exposure, focusing on tissue level and functional abnormalities, descriptive and/or functional assessment of the central nervous system, including the nigrostriatal (dopamine) system. Examples of relevant outcomes include tremor, bradykinesia, rigidity, postural instability, and any other movement abnormalities associated with Parkinsonism (a group of neurological disorders associated with movement abnormalities similar to those observed in Parkinson's disease patients). ***Secondary outcomes:*** Targeted molecular assays that investigate proposed cellular, biochemical, and/or molecular pathways for the etiology of Parkinson's disease following *in vivo* exposure. **Animal:** ***Primary outcomes:*** Neurobehavioral or neuropathological outcomes, focusing on whole body and tissue level abnormalities typically associated with Parkinson's disease following *in vivo* exposure. Endpoints may include motor activity and coordination, sensorimotor reflexes, effects on cognitive function, quantitative or qualitative assessment of dopaminergic neuron counts in the substantia nigra and dopaminergic neuron terminals in the striatum, and other descriptive and/or functional assessments of the central nervous system including the nigrostriatal (dopamine) system that are considered hallmarks of Parkinson's disease (e.g. detection of intracytoplasmic Lewy bodies). ***Secondary outcomes:*** Targeted molecular assays that investigate proposed cellular, biochemical, and/or molecular pathways for the etiology of Parkinson's disease following *in vivo* exposure, including measures of oxidative stress, inflammation, mitochondrial and/or proteasomal dysfunction, dopamine and metabolite levels in the nigrostriatal pathway, or other key molecular initiating events related to Parkinsonism. ***In vitro*:** *In vitro* assays investigating either cellular responses commonly attributed to Parkinson's disease (e.g. assessment of functionality, integrity, and viability for nerve cells critical to the nigrostriatal (dopamine) system). • Mechanistic assays investigating proposed pathways for the etiology of Parkinson's disease (e.g. enzyme interactions, cell signaling).

METHODS

The systematic review techniques in the protocol adhere to the framework developed by the Office of Health Assessment and Translation (OHAT) (Rooney *et al.* 2014). The OHAT systematic review framework consists of a 7-step process: the first 3 steps are relevant to produce a scoping review, whereas the last 4 are relevant to assessing study quality and synthesizing evidence. Thus, this protocol follows procedures outlined in the *OHAT Handbook for Conducting a Literature-Based Health Assessment* (NTP 2015) restricted to the first 3 steps: 1) Problem Formulation, 2) Search and Select Studies for Inclusion, and 3) Data Extraction.

Step 1. Problem Formulation

The association between paraquat exposure and Parkinson's disease was identified as a potential candidate for systematic review as a result of an NTP scoping activity of Parkinson's disease (NTP report under review) and was also identified by several outside groups. Therefore, NTP is considering input from these groups (the U.S. EPA and a research group from the University Estadual Paulista, São Paulo, Brazil) to support a consistent process, promote access and data sharing, and to avoid duplication of effort. As a result, the goals of this process are to develop a scoping review that will systematically collect and categorize the evidence to develop a systematic evidence map of the key Parkinson's related health effects, types of evidence, and gaps in research will facilitate data sharing and could support a systematic review. The lead toxicologist and epidemiologists for EPA's paraquat registration review, the systematic review team, and an expert on neurotoxicity collectively defined the outcomes of interest and the inclusion/exclusion criteria for the title/abstract screen and full text review. The current protocol was developed using the OHAT Systematic Review framework through step 3, data extraction.

Step 2. Search and Select Studies for Inclusion

Literature Search Strategy

Database search strategies were developed to identify all relevant published evidence that addresses the relationship between Parkinson's disease and paraquat exposure. The following databases will be searched (full details of the search strategies are presented in **Appendix 1**):

- EMBASE (Elsevier)
- PubMed (NLM)
- Scopus (Elsevier)
- Web of Science (Thomson Reuters)
- Toxline

All searches include general terms associated with paraquat toxicity and chemical induced toxicity in the nervous system. Further, keywords specific to Parkinson's disease were derived from review articles on proposed mechanistic pathways for the etiology of Parkinson's disease (Baltazar *et al.* 2014, Zhang *et al.* 2016) and a systematic review investigating the relationship between chemical exposure and Parkinson's disease (Choi et al. *in review*). Searches will not be limited by language restrictions or publication year.

Searching Other Resources

NTP will examine additional resources to find studies that were not identified through the electronic searches. Studies will be evaluated using the same inclusion and exclusion criteria as used for screening records retrieved from the electronic search. Relevant studies identified through these steps will be

marked as "provided from other sources" in the study selection flow diagram. This will be done by hand searching the reference lists of relevant reviews identified during the initial search.

Only peer-reviewed, published data will be addressed in the systematic review.

Screening Process

Search results from each database will be compiled in Endnote and duplications will be removed. The master reference list will be filtered and sorted with the Document Classification and Topic Extraction Resource software (DoCTER), a proprietary machine learning tool developed by ICF. Preliminary clustering results from DoCTER will be used to assess the strength of the search strategy and, if necessary, refine the search terms. Once the search strategy is finalized, the reference list will be filtered through DoCTER again to group the citations into clusters based on perceived relevance to the key questions and similarity to vetted studies. The references in each cluster will then be screened for relevance and eligibility based on the inclusion/exclusion criteria using the online literature screening program, Distiller™, a web-based, systematic review software program with structured forms and procedures[1].

Selection Criteria for the Evidence

Inclusion/exclusion criteria is designed to identify relevant publications that comply with each aspect of the PECO statement. The eligibility of each citation from the paraquat literature will be considered based on the criteria outlined in **Table 2**.

Primary and Secondary Outcomes

The publications selected during the paraquat literature screen will highlight studies that examine primary and/or secondary outcomes related to Parkinson's disease. Both primary and secondary outcomes will be used to evaluate the connection between pesticide exposure and the disease. Primary outcomes directly associate pesticide exposure with the manifestation of Parkinson's disease (or symptoms of neurological disruption commonly attributed to Parkinsonism). In humans, primary outcomes include abnormal neurobehavioral clinical observations, clinical diagnoses consistent with Parkinsonism, and neuropathological aberrations. Animal primary outcomes include abnormal neurobehavioral clinical observations, neuropathological aberrations, and changes in locomotor activity. Outcomes for *in vitro* studies include loss of nerve cell integrity and viability and/or altered functionality of nerve cells critical to the nigrostriatal system. Other *in vitro* outcomes are physiological changes attributed to paraquat exposure that are hypothesized to play a role in the etiology of Parkinson's disease but are not unique consequences of the disease. These include oxidative stress, proteasomal and mitochondrial dysfunction in nervous tissues, and epigenetic changes following *in vivo* exposure. Mechanistic data can come from a wide variety of studies that are not intended to identify a disease phenotype. This source of experimental data includes *in vitro* and *in vivo* laboratory studies directed at cellular, biochemical, and molecular mechanisms that explain how a chemical (in this case paraquat) produces particular adverse health effects. Secondary outcomes can be considered with the corresponding primary health effects to examine support for biological plausibility of those outcomes or may support the analysis of a causal relationship, or lack thereof, between paraquat exposure and Parkinson's disease.

[1]DistillerSR® (http://systematic-review.net/) is a proprietary project management tool for tracking studies through the screening process and storing data extracted from these studies using user-customized forms.

Title/Abstract Review

Screeners will be trained using project-specific written instructions outlined in **Table 2** with an initial pilot phase undertaken to improve clarity of the inclusion and exclusion instructions and to improve accuracy and consistency among screeners. If changes to the inclusion criteria are made based on the pilot phase, they will be documented in a protocol amendment along with the date modifications were made and the logic for the changes. Trained screeners from the evaluation design team will then conduct a title and abstract screen of each reference to determine whether a reference meets the inclusion or exclusion criteria. All references will be independently screened by two screeners. Studies that are not excluded based on the title and abstract will be screened through a full-text review. In case of screening conflicts, screeners will independently review their screening results to confirm the inclusion/exclusion decision and, if needed, discuss discrepancies with the other screeners. If a true disagreement exists between screeners, the study passes to the full-text review.

Table 2. Detailed inclusion and exclusion criteria to determine study eligibility

	Inclusion Criteria	Exclusion Criteria (or blank if none)
Participants/Population (Human Studies or Experimental Model Systems)		
human	• No restrictions on sex, age, life stage (including in utero exposure) at time of exposure or outcome assessment • No restrictions on country of residence/origin, lifestyle, race/ethnicity, or occupation	
animal	• No restrictions on sex, age, species (including Drosophila and other non-mammalian species), or life stage at exposure or outcome assessment	• Studies in non-animal organisms (e.g., plants, fungi, protists, bacteria)
In vitro	• Studies involving an in vitro exposure system and neurological measures directed at cellular, biochemical, and molecular mechanisms that may explain how exposure to paraquat leads to Parkinson's disease	
Exposure		
human	• Exposure to paraquat dichloride (CAS# 1910-42-5) based on administered dose or concentration, biomonitoring data (e.g., urine, blood, or other specimens), environmental measures (e.g., air, water levels), or indirect measures (e.g., job title)	
animal	• Exposure to paraquat dichloride based on administered dose or concentration or bio-monitoring data (e.g., urine, blood, or other specimens) • No restrictions on route of administration	
In vitro	• Exposure to paraquat dichloride based on administered dose or concentration	
Comparators		
human	• Humans exposed to lower levels (or no exposure/exposure below detection levels) of paraquat dichloride	
animal	• Study must include vehicle or untreated control group	
In vitro	• Study must include vehicle or untreated control group	

Table 2. Detailed inclusion and exclusion criteria to determine study eligibility		
	Inclusion Criteria	**Exclusion Criteria (or blank if none)**
Outcomes		
human	**Primary outcomes** ***[following in vivo exposure to paraquat dichloride]:*** • Diagnosis of Parkinson's disease and/or clinical observations, neurobehavioral, or neuropathological outcomes typically associated with Parkinson's disease following in vivo exposure. Focusing on tissue level and functional abnormalities, descriptive and/or functional assessment of the central nervous system, including the nigrostriatal (dopamine) system. Examples of relevant neurobehavioral outcomes include tremor, bradykinesia, rigidity, and postural instability. **Secondary outcomes** ***[following in vivo exposure to paraquat dichloride]:*** • Targeted molecular assays that investigate proposed cellular, biochemical, and/or molecular pathways for the etiology of Parkinson's disease following in vivo exposure.	• Studies reporting on toxicity in organs or tissues not associated with the central or peripheral nervous system
animal	**Primary outcomes** ***[following in vivo exposure to paraquat dichloride]:*** • Neurobehavioral or neuropathological outcomes, focusing on whole body and tissue level abnormalities typically associated with Parkinson's disease following *in vivo* exposure. Endpoints may include motor activity and coordination, sensorimotor reflexes, effects on cognitive function, quantitative or qualitative assessment of dopaminergic neuron counts in the substantia nigra and dopaminergic neuron terminals in the striatum, and other descriptive and/or functional assessments of the central nervous system including the nigrostriatal (dopamine) system that are considered hallmarks of Parkinson's disease (e.g. detection of intracytoplasmic Lewy bodies). **Secondary outcomes** ***[following in vivo exposure to paraquat dichloride]:*** • Targeted molecular assays that investigate proposed cellular, biochemical, and/or molecular pathways for the etiology of Parkinson's disease following in vivo exposure, including measures of oxidative stress, inflammation, mitochondrial and/or proteasomal dysfunction, dopamine and metabolite levels in the nigrostriatal pathway, or other key molecular initiating events related to Parkinsonism.	• Studies reporting on toxicity in organs or tissues not associated with the central or peripheral nervous system

Table 2. Detailed inclusion and exclusion criteria to determine study eligibility		
	Inclusion Criteria	**Exclusion Criteria (or blank if none)**
In vitro	***Following <u>in vitro exposure</u> to paraquat dichloride:*** • *In vitro* assays investigating either cellular responses commonly attributed to Parkinson's disease (e.g. assessment of functionality, integrity, and viability for nerve cells critical to the nigrostriatal (dopamine) system) or generic cellular responses commonly attributed to paraquat exposure but are not unique to Parkinson's disease (e.g. measures of oxidative stress and mitochondria dysfunction in nerve cells, epigenetic changes). • Mechanistic assays investigating proposed pathways for the etiology of Parkinson's disease (e.g. enzyme interactions, cell signaling)	• Studies reporting on toxicity unrelated to the central or peripheral nervous system
Publications (e.g., language restrictions, use of conference abstracts, etc.)		
human, animal, *In vitro*	• Study must contain original data • Studies published in a language other than English will be collected and categorized by health effect or mechanism to the extent they can be categorized without full translation as extensive translation and level of effort are beyond the goals of this scoping review.	• Articles with no original data (e.g., editorial or review*) • Studies published in abstract form only (grant awards, conference abstracts) • Retracted articles • Non-English language articles that cannot be categorized based on English abstract
*Relevant reviews are used as background and for reference scanning.		

Full-Text Review

After completion of the title/abstract screen, full-text articles will be retrieved[2] for those studies that either clearly meet the inclusion criteria or where eligibility to meet the inclusion criteria is unclear. Full-text review will be independently conducted by two screeners that participated in the title/abstract screening. True disagreements will be resolved by discussion through consultation with other members of the evaluation design team and technical advisors.

Reason for exclusion at the full-text-review stage will be annotated and reported in a study selection flow diagram in the final report (using reporting practices outlined in Moher et al. 2009). Although more than one reason may apply, for simplicity, only one of the following reasons for exclusion will be documented: (1) lacks a comparator (e.g., a control or baseline group); (2) conducted with a non-animal model (e.g., plants, fungi, protists, or bacteria); (3) lacks neurobehavioral or neuropathological health outcome information; (4) conducted in wildlife; (5) lacks paraquat exposure; (6) mixture study lacking paraquat-only exposure; (7) is a conference abstract, grant application/registration, thesis/dissertation, or otherwise not a peer-reviewed scientific publication; or (8) study only available non-English language.

[2]OHAT will initially attempt to retrieve a full-text copy of the study using an automated program, such as QUOSA, when possible, and NIH library services (NIH subscriptions and interlibrary loans). For publications not available through NIH, OHAT will search the Internet and/or may attempt to contact the corresponding author. Studies not retrieved through these mechanisms are excluded and notated as "not available."

(A list of included and excluded studies including those excluded during the title and abstract screen will be itemized in the appendix of the paraquat draft risk assessment for reference.

Multiple Publications of Same Data
Multiple publications with overlapping data for the same study (e.g., publications reporting subgroups, additional outcomes or exposures outside the scope of an evaluation, or longer follow-up) are identified by examining author affiliations, study designs, cohort name, enrollment criteria, and enrollment dates. If necessary, study authors will be contacted to clarify any uncertainty about the independence of two or more articles. NTP will include all publications on the study, select one study to use as the primary, and consider the others as secondary publications, with annotation as being related to the primary record during data extraction. The primary study will generally be the publication with the longest follow-up, or for studies with equivalent follow-up periods, the study with the largest number of cases or the most recent publication date. NTP will include relevant data from all publications of the study, although if the same outcome is reported in more than one report, NTP will include a single instance of the data.

The list of included and excluded studies will be provided in the scoping report. The results of the literature search will be presented in a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) diagram (Moher *et al.* 2009).

Step 3. Data Extraction and Content Management

Data will be extracted from individual studies by members of the evaluation team. Data extraction and warehousing will be carried out using Health Assessment Workspace Collaborative (HAWC), a free and open-source, web-based software application[3] as well as Microsoft Excel. Data extraction elements to be collected from epidemiological studies are listed in **Appendix 2** and from animal studies in **Appendix 3**. Some animal studies (e.g., those using alternative toxicological models like drosophila and zebrafish) as well as in vitro and mechanistic studies will be included as supporting information, and will not be fully extracted into HAWC. Instead, basic information (e.g., species, cell type, exposure duration and route, dose, etc.) will be extracted from these studies into Microsoft Excel to facilitate data visualization and summary. These Excel tables will be appended to the final report. Data flagged for extraction will include, but not be limited to: information on author affiliations and funding, characteristics of the model organism, the exposure methodology, the exposure conditions, the route of administration, and the comparators, and quantitative and qualitative data on health effects. The data extraction results for included studies will be presented in the technical report and the data extraction results will be available for download from HAWC in Excel format when the project is completed. Data extraction will be performed by one member of the evaluation team or contract support and checked by a second member for completeness and accuracy. Any discrepancies in data extraction will be resolved by discussion or consultation with a third member of the evaluation team. Study authors will be contacted if integral data is missing or incomplete. A note indicating if contact was made with the study author(s) will be appended to the appropriate datasets. Inferences and estimations for missing/incomplete data may be necessary if study authors cannot be reached and will be properly annotated in the extracted dataset.

[3]Health Assessment Workspace Collaborative (HAWC): A Modular Web-based Interface to Facilitate Development of Human Health Assessments of Chemicals. https://hawcproject.org/portal/.

Step 4. Study Results and Summaries

The results of all included human, animal, and *in vitro* studies will be summarized by outcome and chemical in text and tables to develop a systematic evidence map of the evidence by health effect and types of evidence, and identify data gaps in available research. In addition, visualizations using the extracted data in HAWC will also be generated to summarize the data.

SCOPING REVIEW: OUTLINE

The NTP Scoping Report on paraquat exposure and neurobehavioral and neuropathological endpoints associated with Parkinson's disease will include the following information:

Introduction

This section will provide a brief background on the topic.

Methodology

This section will provide a brief overview of the methodologies used in the review process, including:

- the research question
- the search strategy used to identify and retrieve studies
- the process for selecting the included studies
- the methods of data extraction

Results

This section will include the results from the scoping efforts. Results will be presented in tables or figures as appropriate using HAWC, Tableau, and Microsoft Excel. The results from the included studies will be discussed by outcome. This will include a description of:

- the number of studies identified that reported the outcome
- the full list of excluded studies, with reasons for exclusion documented for studies excluded at the full text review stage
- the results and summaries for each included study (including files in downloadable format)
- the visualization of result summaries for included studies (generated using HAWC, Tableau, or other formats as appropriate)

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ABOUT THE PROTOCOL

Contributors

Evaluation teams are composed of federal staff and contractor staff. Contractor staff members are screened for potential conflicts of interest. Federal staff members should do a self-evaluation. Technical advisors were screened for conflict of interest prior to their service and did not report any conflicts of interest. Epidemiologists and toxicologists on OHAT evaluation teams should have at least three years' experience and/or training in reviewing studies, including summarizing studies and critical review (e.g., assessing study quality and interpreting findings). Team members should have at least a master's degree or equivalent experience in epidemiology, toxicology, environmental health sciences, or a related field.

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Technical advisors are outside experts retained on an as-needed basis to provide individual advice to the NTP for a specific topic. The technical advisors were selected for their experience with Parkinson's disease, paraquat, and environmental health.

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Sources of Support

National Institute of Environmental Health Sciences/Division of the National Toxicology Program

Protocol History and Revisions

Date	Activity or revision
May 9, 2017	Draft Protocol reviewed: sent to peer reviewers for comment/review
January 10, 2018	Protocol posted publicly at (https://ntp.niehs.nih.gov/go/parkinson)
May 21, 2018	• Updated animal primary and secondary outcomes in the PECO statement and the inclusion criteria to reflect adjustments made to study screening. While screening studies, the evaluation team realized that the assessment of dopaminergic neurons and other hallmarks of Parkinson's disease would be more appropriate as a primary outcome, rather than a secondary outcome. Thus, after evaluation team discussions, hallmarks of Parkinson's disease, including assessment of dopaminergic neurons and detection of Lewy bodies, were moved from secondary to primary outcomes in the PECO (Table 1) and study inclusion criteria (Table 2). All previously screened references were re-screened according to the revised criteria. • Revised language in "Searching Other Resources" to reflect that only reference lists of review articles were searched to find studies that were not identified through the electronic searches. A robust dataset and relatively large number of studies was included after searching electronic databases and completing both the title-abstract and full text screening (n=428 records). References from 194 relevant review articles were then screened (n = 116 records after duplicate removal) and only 2 additional studies were included as studies with secondary outcomes. Thus, the evaluation team considered the included studies sufficient to support a scoping review and representative evidence map, and the decision was made to limit searching of other resources to reference lists of relevant review articles rather than also searching the reference lists of all included studies and non-research records.
May 24, 2018	Revised protocol posted publicly at (https://ntp.niehs.nih.gov/go/parkinson)

APPENDICES

Appendix 1. Literature Search Strategy

The strategy for this search is broad for the consideration of neurobehavioral and neuropathological endpoints associated with Parkinson's disease and comprehensive for paraquat dichloride as an exposure or treatment in order to ensure inclusion of relevant papers.

Database	Search Terms
EMBASE	(paraquat OR 1910-42-5 OR gramoxone OR methyl-viologen OR paragreen-A) AND (alpha-synuclein OR apoptosis OR astrocyte OR astrocytes OR ataxia OR autophagy OR axon OR axonal OR axons OR bradykinesia OR brain OR central-nervous OR dendrite OR dendrites OR dentritic OR dj-1 OR dopamine OR dopaminergic OR gait OR ganglia OR glial OR gliosis OR glutamate OR glutamates OR Glutamic Acids OR glutathione OR Lewy bodies OR lewy body OR locomotion OR locomotor-activity OR lrrk2 OR Mesencephalon OR Mesencephalons OR microglia OR microglial OR microglials OR midbrain OR mitochondria OR Mitochondrial OR Mitochondrion OR motor-activity OR mpp OR mptp OR NADPH-oxidase OR nerve OR nerves OR nervous OR neural OR neurobehavior OR neurobehavioral OR neurobehaviour OR neurobehavioural OR neuroblastoma OR neurodegeneration OR neurodegenerative OR neuroglia OR neurological OR neuromotor OR neuron OR neuronal OR neuronopathy OR neurons OR neuropathies OR neuropathology OR neuropathy OR neurotoxic OR neurotoxicity OR neurotransmitter OR neurotransmitters OR nigral OR nigrostriatal OR nitric-oxide OR nitrosative-stress OR oxidative-stress OR paralysis-agitans OR parkin OR parkinson OR parkinsons OR parkinsonian OR parkinsonism OR pink1 OR reactive-oxygen-species OR rigidity OR snpc OR striatal OR striatum OR substantia-nigra OR synapse OR synapses OR synaptic OR synuclein OR synucleins OR tau OR tauopathies OR tauopathology OR tauopathy OR Thioredoxin-Disulfide OR thioredoxin-reductase OR tremor OR tremors OR Tyrosine 3-Monooxygenase OR tyrosine-hydroxylase OR ubiquitin)

Database	Search Terms
PubMed	(paraquat[tiab] OR paraquat[mh] OR gramoxone[tiab] OR methyl-viologen[tiab] OR paragreen-A[tiab]) AND (alpha-synuclein[tiab] OR alpha-synuclein[mh] OR apoptosis[tiab] OR apoptosis[mh] OR astrocyte[tiab] OR astrocytes[tiab] OR astrocytes[mh] OR ataxia[tiab] OR autophagy[tiab] OR autophagy[mh] OR axon[tiab] OR axonal[tiab] OR axons[tiab] OR axons[mh] OR bradykinesia[tiab] OR brain[tiab] OR central-nervous[tiab] OR dendrite[tiab] OR dendrites[tiab] OR dentritic[tiab] OR dj-1[tiab] OR dopamine[mh] OR dopamine[tiab] OR Dopamine Plasma Membrane Transport Proteins[mh] OR dopaminergic[tiab] OR gait[tiab] OR gait[mh] OR ganglia[tiab] OR glial[tiab] OR gliosis[tiab] OR gliosis[mh] OR glutamate[tiab] OR glutamates[mh] OR glutamates[tiab] OR Glutamic Acids[tiab] OR glutathione[tiab] OR glutathione[mh] OR Lewy bodies[tiab] OR lewy body[tiab] OR locomotion[mh] OR locomotion[tiab] OR locomotor-activity[tiab] OR lrrk2[tiab] OR Mesencephalon[tiab] OR Mesencephalons[tiab] OR microglia[tiab] OR microglial[tiab] OR microglials[tiab] OR midbrain[tiab] OR mitochondria[tiab] OR mitochondria[mh] OR Mitochondrial[tiab] OR Mitochondrion[tiab] OR motor-activity[tiab] OR motor-activity[mh] OR mpp[tiab] OR mptp[tiab] OR NADPH-oxidase[mh] OR NADPH-oxidase[tiab] OR nerve[tiab] OR nerves[tiab] OR nervous[tiab] OR nervous-system[mh] OR nervous-system-diseases[mh] OR nervous-system-physiological-processes[mh] OR neural[tiab] OR neurobehavior[tiab] OR neurobehavioral[tiab] OR neurobehaviour[tiab] OR neurobehavioural[tiab] OR neuroblastoma[tiab] OR neuroblastoma[mh] OR neurodegeneration[tiab] OR neurodegenerative[tiab] OR neuroglia[tiab] OR neurological[tiab] OR neuromotor[tiab] OR neuron[tiab] OR neuronal[tiab] OR neuronopathy[tiab] OR neurons[tiab] OR neuropathies[tiab] OR neuropathology[tiab] OR neuropathy[tiab] OR neurotoxic[tiab] OR neurotoxicity[tiab] OR neurotransmitter[tiab] OR neurotransmitter agents[mh] OR neurotransmitter agents[Pharmacological Action] OR neurotransmitters[tiab] OR nigral[tiab] OR nigrostriatal[tiab] OR nitric-oxide[tiab] OR nitric-oxide[mh] OR nitric-oxide-synthase[mh] OR nitrosative-stress[tiab] OR oxidative-stress[tiab] OR paralysis-agitans[tiab] OR parkin[tiab] OR parkin protein[supplementary concept] OR parkinson[tiab] OR parkinsons[tiab] OR parkinson's[tiab] OR parkinsonian[tiab] OR parkinsonism[tiab] OR pink1[tiab] OR reactive-oxygen-species[tiab] OR reactive-oxygen-species[mh] OR rigidity[tiab] OR snpc[tiab] OR striatal[tiab] OR striatum[tiab] OR substantia-nigra[tiab] OR synapse[tiab] OR synapses[tiab] OR synaptic[tiab] OR synuclein[tiab] OR synucleins[tiab] OR synucleins[mh] OR tau[tiab] OR tau proteins[mh] OR tauopathies[tiab] OR tauopathology[tiab] OR tauopathy[tiab] OR Thioredoxin-Disulfide[tiab] OR Thioredoxin-Disulfide Reductase[mh] OR thioredoxin-reductase[tiab] OR tremor[tiab] OR tremors[tiab] OR Tyrosine 3-Monooxygenase[mh] OR Tyrosine 3-Monooxygenase[tiab] OR tyrosine-hydroxylase[tiab] OR ubiquitin[tiab] OR ubiquitin[mh])

Database	Search Terms
Web of Science	All terms searched in Title, Abstract, or Keywords LIMITS: Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, CCR-EXPANDED, IC Timespan=All years (paraquat OR 1,1'-Dimethyl-4,4'-bipyridinium-dichloride OR 1910-42-5 OR gramoxone OR methyl-viologen OR paragreen-A) AND (alpha-synuclein OR apoptosis OR astrocyte OR astrocytes OR ataxia OR autophagy OR axon OR axonal OR axons OR bradykinesia OR brain OR central-nervous OR dendrite OR dendrites OR dentritic OR dj-1 OR dopamine OR dopaminergic OR gait OR ganglia OR glial OR gliosis OR glutamate OR glutamates OR Glutamic Acids OR glutathione OR Lewy bodies OR lewy body OR locomotion OR locomotor-activity OR lrrk2 OR Mesencephalon OR Mesencephalons OR microglia OR microglial OR microglials OR midbrain OR mitochondria OR Mitochondrial OR Mitochondrion OR motor-activity OR mpp OR mptp OR NADPH-oxidase OR nerve OR nerves OR nervous OR neural OR neurobehavior OR neurobehavioral OR neurobehaviour OR neurobehavioural OR neuroblastoma OR neurodegeneration OR neurodegenerative OR neuroglia OR neurological OR neuromotor OR neuron OR neuronal OR neuronopathy OR neurons OR neuropathies OR neuropathology OR neuropathy OR neurotoxic OR neurotoxicity OR neurotransmitter OR neurotransmitters OR nigral OR nigrostriatal OR nitric-oxide OR nitrosative-stress OR oxidative-stress OR paralysis-agitans OR parkin OR parkinson OR parkinsons OR parkinson's OR parkinsonian OR parkinsonism OR pink1 OR reactive-oxygen-species OR rigidity OR snpc OR striatal OR striatum OR substantia-nigra OR synapse OR synapses OR synaptic OR synuclein OR synucleins OR tau OR tauopathies OR tauopathology OR tauopathy OR Thioredoxin-Disulfide OR thioredoxin-reductase OR tremor OR tremors OR Tyrosine 3-Monooxygenase OR tyrosine-hydroxylase OR ubiquitin)
SCOPUS	All terms searched in Title, Abstract, or Keywords LIMITS: Indexes=SCI-EXPANDED, SSCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, CCR-EXPANDED, IC Timespan=All years (paraquat OR 1,1'-Dimethyl-4,4'-bipyridinium-dichloride OR 1910-42-5 OR gramoxone OR methyl-viologen OR paragreen-A) AND (alpha-synuclein OR apoptosis OR astrocyte OR astrocytes OR ataxia OR autophagy OR axon OR axonal OR axons OR bradykinesia OR brain OR central-nervous OR dendrite OR dendrites OR dentritic OR dj-1 OR dopamine OR dopaminergic OR gait OR ganglia OR glial OR gliosis OR glutamate OR glutamates OR Glutamic Acids OR glutathione OR Lewy bodies OR lewy body OR locomotion OR locomotor-activity OR lrrk2 OR Mesencephalon OR Mesencephalons OR microglia OR microglial OR microglials OR midbrain OR mitochondria OR Mitochondrial OR Mitochondrion OR motor-activity OR mpp OR mptp OR NADPH-oxidase OR nerve OR nerves OR nervous OR neural OR neurobehavior OR neurobehavioral OR neurobehaviour OR neurobehavioural OR neuroblastoma OR neurodegeneration OR neurodegenerative OR neuroglia OR neurological OR neuromotor OR neuron OR neuronal OR neuronopathy OR neurons OR neuropathies OR neuropathology OR neuropathy OR neurotoxic OR neurotoxicity OR neurotransmitter OR neurotransmitters OR nigral OR nigrostriatal OR nitric-oxide OR nitrosative-stress OR oxidative-stress OR paralysis-agitans OR parkin OR parkinson OR parkinsons OR parkinson's OR parkinsonian OR parkinsonism OR pink1 OR reactive-oxygen-species OR rigidity OR snpc OR striatal OR striatum OR substantia-nigra OR synapse OR synapses OR synaptic OR synuclein OR synucleins OR tau OR tauopathies OR tauopathology OR tauopathy OR Thioredoxin-Disulfide OR thioredoxin-reductase OR tremor OR tremors OR Tyrosine 3-Monooxygenase OR tyrosine-hydroxylase OR ubiquitin)

Database	Search Terms
Toxline	All terms searched in Title, Abstract, or Keywords LIMITS: Exclude PubMed Records Do NOT add chemical synonyms and CASRNs to search Search exact words (paraquat OR 1,1'-Dimethyl-4,4'-bipyridinium-dichloride OR 1910-42-5 OR gramoxone OR methyl-viologen OR paragreen-A) AND (alpha-synuclein OR apoptosis OR astrocyte OR astrocytes OR ataxia OR autophagy OR axon OR axonal OR axons OR bradykinesia OR brain OR central-nervous OR dendrite OR dendrites OR dentritic OR dj-1 OR dopamine OR dopaminergic OR gait OR ganglia OR glial OR gliosis OR glutamate OR glutamates OR Glutamic Acids OR glutathione OR Lewy bodies OR lewy body OR locomotion OR locomotor-activity OR lrrk2 OR Mesencephalon OR Mesencephalons OR microglia OR microglial OR microglials OR midbrain OR mitochondria OR Mitochondrial OR Mitochondrion OR motor-activity OR mpp OR mptp OR NADPH-oxidase OR nerve OR nerves OR nervous OR neural OR neurobehavior OR neurobehavioral OR neurobehaviour OR neurobehavioural OR neuroblastoma OR neurodegeneration OR neurodegenerative OR neuroglia OR neurological OR neuromotor OR neuron OR neuronal OR neuronopathy OR neurons OR neuropathies OR neuropathology OR neuropathy OR neurotoxic OR neurotoxicity OR neurotransmitter OR neurotransmitters OR nigral OR nigrostriatal OR nitric-oxide OR nitrosative-stress OR oxidative-stress OR paralysis-agitans OR parkin OR parkinson OR parkinsons OR parkinson's OR parkinsonian OR parkinsonism OR pink1 OR reactive-oxygen-species OR rigidity OR snpc OR striatal OR striatum OR substantia-nigra OR synapse OR synapses OR synaptic OR synuclein OR synucleins OR tau OR tauopathies OR tauopathology OR tauopathy OR Thioredoxin-Disulfide OR thioredoxin-reductase OR tremor OR tremors OR Tyrosine 3-Monooxygenase OR tyrosine-hydroxylase OR ubiquitin)

Appendix 2. Data Extraction Elements for Human Studies

HUMAN	
Funding	Funding source(s)
	Reporting of COI by authors and/or translators (*reporting bias)
Subjects	Study population name/description
	Dates of study and sampling timeframe
	Geography (country, region, state, etc.)
	Demographics (sex, race/ethnicity, age or lifestage and exposure and outcome assessment)
	Number of subjects (target, enrolled, n per group in analysis, and participation/follow-up rates) (*missing data bias)
	Inclusion/exclusion criteria/recruitment strategy (*selection bias)
	Description of reference group (*selection bias)
Methods	Study design (e.g., prospective or retrospective cohort, nested case-control study, cross-sectional, population-based case-control study, intervention, case report, etc.)
	Length of follow-up (*information bias)
	Health outcome category, e.g., neurodevelopment
	Health outcome, e.g., memory (*reporting bias)
	Diagnostic or methods used to measure health outcome (*information bias)
	Confounders or modifying factors and how considered in analysis (e.g., included in final model, considered for inclusion but determined not needed (*confounding bias)
	Substance name and CAS number
	Exposure assessment (e.g., blood, urine, hair, air, drinking water, job classification, residence, administered treatment in controlled study, etc.) (*information bias)
	Methodological details for exposure assessment (e.g., HPLC-MS/MS, limit of detection) (*information bias)
	Statistical methods (*information bias)
Results	Exposure levels (e.g., mean, median, measures of variance as presented in paper, such as SD, SEM, 75th/90th/95th percentile, minimum/maximum); range of exposure levels, number of exposed cases
	Statistical findings (e.g., adjusted β, standardized mean difference, adjusted odds ratio, standardized mortality ratio, relative risk, etc.) or description of qualitative results. When possible, OHAT will convert measures of effect to a common metric with associated 95% confidence intervals (CI). Most often, measures of effect for continuous data are expressed as mean difference, standardized mean difference, and percent control response. Categorical data are typically expressed as odds ratio, relative risk (RR, also called risk ratio), or β values, depending on what metric is most commonly reported in the included studies and on OHAT's ability to obtain information for effect conversions from the study or through author query.
	If not presented in the study, statistical power can be assessed during data extraction using an approach that can detect a 10% to 20% change from response by control or referent group for continuous data, or a relative risk or odds ratio of 1.5 to 2 for categorical data, using the prevalence of exposure or prevalence of outcome in the control or referent group to determine sample size. For categorical data where the sample sizes of exposed and control or referent groups differ, the sample size of the exposed group will be used to determine the relative power category. Recommended sample sizes to achieve 80% power for a given effect size, i.e., 10% or 20% change from control, will be compared to sample sizes used in the study to categorize statistical power as "appears to be adequately powered" (sample size for 80% power met), somewhat underpowered (sample size is 75% to < 100% of number required for 80% power), "underpowered" (sample size is 50% to < 75% of number required for 80% power), or "severely underpowered" (sample size is < 50% of number required for 80% power).

HUMAN	
	Observations on dose response (e.g., trend analysis, description of whether dose-response shape appears to be monotonic, non-monotonic)
Other	Documentation of author queries, use of digital rulers to estimate data values from figures, exposure unit, and statistical result conversions, etc.

Appendix 3. Data Extraction Elements for Animal Studies

ANIMAL	
Funding	Funding source(s)
	Reporting of COI by authors and/or translators (*reporting bias)
Animal Model	Sex
	Species
	Strain
Treatment	Chemical name and CAS number
	Source of chemical
	Purity of chemical (*information bias)
	Dose levels or concentration (as presented and converted to mg/kg bw/d when possible)
	Other dose-related details, such as whether administered dose level was verified by measurement, information on internal dosimetry (*information bias)
	Vehicle used for exposed animals
	Route of administration (e.g., oral, inhalation, dermal, injection)
	Age or lifestage at start of dosing and at health outcome assessment
	Duration and frequency of dosing (e.g., hours, days, weeks when administration was ended, days per week)
Methods	Study design (e.g., single treatment, acute, subchronic (e.g., 90 days in a rodent), chronic, multigenerational, developmental, other)
	Guideline compliance (i.e., use of EPA, OECD, NTP or another guideline for study design, conducted under GLP guideline conditions, non-GLP but consistent with guideline study, non-guideline peer-reviewed publication)
	Number of animals per group (and dams per group in developmental studies) (*missing data bias)
	Randomization procedure, allocation concealment, blinding during outcome assessment (*selection bias)
	Method to control for litter effects in developmental studies (*information bias)
	Use of negative controls and whether controls were untreated, vehicle-treated, or both
	Endpoint health category (e.g., reproductive)
	Endpoint (e.g., infertility)
	Diagnostic or method to measure endpoint (*information bias)
	Statistical methods (*information bias)
Results	Measures of effect at each dose or concentration level (e.g., mean, median, frequency, measures of precision or variance) or description of qualitative results. When possible, OHAT will convert measures of effect to a common metric with associated 95% confidence intervals (CI). Most often, measures of effect for continuous data will be expressed as percent control response, mean difference, or standardized mean difference. Categorical data will be expressed as relative risk (RR, also called risk ratio).
	No observed effect level (NOEL), lowest observed effect level (LOEL), benchmark dose (BMD) analysis, statistical significance of other dose levels, or other estimates of effect presented in paper. **Note:** The NOEL and LOEL are highly influenced by study design, give no quantitative information about the relationship between dose and response, and can be subject to author's interpretation (e.g., a statistically significant effect might not be considered biologically important). Also, a NOEL does not necessarily mean zero response. Ideally, the response rate or effect size at specific dose levels is used as the primary measure to characterize the response.

ANIMAL	
	If not presented in the study, statistical power can be assessed during data extraction using an approach that assesses the ability to detect a 10% to 20% change from control group's response for continuous data, or a relative risk or odds ratio of 1.5–2 for categorical data, using the outcome frequency in the control group to determine sample size. Recommended sample sizes to achieve 80% power for a given effect size, i.e., 10% or 20% change from control, will be compared to sample sizes used in the study to categorize statistical power. Studies will be considered adequately powered when sample size for 80% power is met.
	Observations on dose response (e.g., trend analysis, description of whether dose-response shape appears to be monotonic, nonmonotonic)
	Data on internal concentration, toxicokinetics, or toxicodynamics (when reported)
Other	Documentation of author queries, use of digital rulers to estimate data values from figures, exposure unit, statistical result conversions, etc.