AWARD NUMBER: W81XWH-17-1-0536

TITLE: Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System

PRINCIPAL INVESTIGATOR: Dr. Honglei Chen, Professor

CONTRACTING ORGANIZATION: Michigan State University
                    East Lansing, MI 48824

REPORT DATE: Sept 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
                    Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
Sept 2019	Annual	1 Sep 2018 - 31 Aug 2019

4. TITLE AND SUBTITLE	
Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System	**5a. CONTRACT NUMBER**
	5b. GRANT NUMBER W81XWH-17-1-0536
	5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)	
Dr. Honglei Chen E-Mail: hchen@epi.msu.edu	**5d. PROJECT NUMBER**
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT
Michigan State University 426 Auditorium Road, RM 2 East Lansing, MI 48824-2600	

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012	
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT: In this multidisciplinary project, we proposed to examine the central hypothesis that ambient air pollutants contribute to Parkinson's disease (PD) development by initiating and/or exacerbating alpha-synuclein pathology at olfactory structures via inflammation. In the epidemiologic arm, we plan to investigate 1) the effect of long-term exposure to air pollutants on olfactory impairment (OI); 2) whether early PD pathogenesis is exacerbated by ambient air pollutants; and 3) whether lifetime use of ibuprofen modifies potential adverse effects of air pollutants on OI. The project will leverage ten years of extensive data collection on environmental exposures, medical history, and biospecimen from the well-established Sister Study of the National Institute of Environmental Health Sciences (NIEHS). Importantly, we proposed to objectively evaluate the sense of smell of approximately 3,400 Sister Study participants, using the brief smell identification test, efficiently administered by mail. We collected data between March 2018 and March 2019. Of the 4,020 eligible participants, 3,535 (87.9%) have provided some data and 3,431 (85.3%) returned the smell test kit. In May 2019, we shipped DNA samples to NIA for genotyping, expecting completion late in 2019 or early in 2020. We have also performed analyses using interim data to understand the outcomes and to develop analytic programs. We received complete dataset from our field team at NIEHS/SSS in August 2019. We are currently checking data integrity and expect to update our data analyses previously conducted based on interim dataset.

15. SUBJECT TERMS
Parkinson's Disease, Olfaction, Sense of Smell, Air Pollutants, Prodromal, Inflammation, Risk Factor

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	**b. ABSTRACT** U	**c. THIS PAGE** U	UU	11	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

1. INTRODUCTION

Olfactory impairment (OI) is an under-appreciated and under-studied health problem among older adults. More importantly, OI is an early warning for several major neurodegenerative diseases such as Parkinson's (PD) and Alzheimer's (AD) diseases. However, the causes of age-related OI and how it may contribute to neurodegenerative diseases are largely unknown. We therefore proposed a case-control study to investigate risk factors for age-related OI in order to better understand PD prodromal development. The goal of this specific project is to define the role of ambient air pollutants in OI and to explore its relevance to PD development. Specifically, we aim to 1) assess the effect of long-term exposure to air pollutants on OI; 2) investigate whether early PD pathogenesis is exacerbated by ambient air pollutants; and 3) examine whether lifetime use of non-steroidal anti-inflammatory drugs (NSAIDs), ibuprofen in particular, modifies potential adverse effects of air pollutants on OI. The project will leverage ten years of extensive data collection on environmental exposures, medical history, and biospecimen from the well-established Sister Study of the National Institute of Environmental Health Sciences (NIEHS). Specifically, we have objectively evaluated the sense of smell of more than 3,400 participants from the Sister Study, using a validated and self-administered brief smell identification test (BSIT). Participants further completed a short survey on medical history relevant to their senses of smell and taste. In addition, we will perform genotyping to quantify their genetic risk for PD. We will analyze these data together with the tremendous exposure data that the Sister Study has already collected. We expect this project will significantly improve our understanding about risk factors for OI and provide novel insights into prodromal development of PD and related neurodegenerative diseases.

2. KEY WORDS: Parkinson's Disease, Olfaction, Sense of Smell, Air Pollutants, Prodromal, Inflammation, Risk Factors

3. **ACCOMPLISHMENTS**

3.A. What were the major goals of this project?
By objectively assessing the sense of smell of selected participants from the NIEHS Sister study and leveraging the study's extensive environmental data collection, we aim to examine the role of ambient air pollutants in olfactory impairment (OI) and to explore its relevance to Parkinson's disease (PD). Specifically, we aim to 1) assess the effect of long-term exposure to air pollutants on OI; 2) investigate whether early PD pathogenesis is exacerbated by ambient air pollutants; and 3) examine whether lifetime use of NSAIDs, ibuprofen in particular, modifies potential adverse effects of air pollutants on OI. We initially proposed to collect data from 2,713 Sister study participants; later with a cost-share agreement established with the Parkinson's Foundation ($151,399) we were able to collect the sense of smell data from more than 3400 participants. This will allow us to comprehensively evaluate the proposed aims with enhanced statistical power and sensitivity analyses.

3.B. What was accomplished under these goals?
We started data collection in March 2018 and completed data collection on Feb. 28, 2019. Of the 4,020 Sister participants we selected, 3,535 (87.9%) responded and 3,431 (85.3%) returned the sense of smell test kit. The response rate was slightly higher than what we had ambitiously projected (85.0%). In May, a total of 3,696 DNA samples (including duplicates for quality control) were shipped to NIA for genotyping. We expect genotyping to be completed late in 2019 or early in 2020. We have also performed analyses using interim data to understand the outcomes and to develop analytic programs. Upon complete data delivery in August 2019, we are currently checking data integrity and will soon update our data analyses previously conducted based on the interim dataset. Please see below our proposed major tasks and milestones for the first two years of the project as listed in the SOW and our progress report:

Study participation
1. *"Obtain IRB approval or exemption from DOD and relevant study sites"* by month 4.
   Progress: The study involves multiple sites. We obtained standalone IRB approvals from MSU (IRB# 17-1208) on Nov 20, 2017 and the DoD (#A-20425) on Jan 10th, 2018. In addition, NIEHS/SSS (Sister study contractor of NIEHS) and University of Washington approved relevant study activities by amending their existing protocols. All field data collection has been carried out by the Sister Study team at NIEHS/SSS. After initial approval, we made multiple minor revisions that were swiftly approved by MSU IRB. All revisions did not affect the risk and benefit of study participants, and thus are not reportable to DoD IRB.

2. *"Select participants and design survey/study materials"* by month 4
   Progress: In January 2018, we selected 2,820 eligible Sister Study participants, ages 50-79 and alive, who reported a poor sense of smell at a recent survey and a random sample of 1,200 participants who did not. Study materials were ready by January 2018.

3. *"Obtain survey data from the Sister study"* by month 6
   Progress: We obtained relevant survey data from the Sister Study on June 1st, 2018.

**Participant recruitment and data collection**
1. *"Mail/receive test kit and questionnaire"* by month 18
   Progress: We completed data collection by Feb 2019. Of the 4,020 Sister participants, 3,535 (87.9%) responded and 3,431 (85.3%) returned the sense of smell test kit. The response rate was slightly higher than what we had ambitiously projected (85.0%). The

response rates were comparable between participants who self-reported a poor sense of smell and those who did not, and was little affected by age or education level.

4. *"Data QC, entry, and delivery"* by month 21

Progress: Data were entered and cleaned by the field team at NIEHS/SSS and were delivered to MSU in August 2019. We are currently double-checking data quality and

Figure 1 BSIT smell test score by self-reported olfaction status
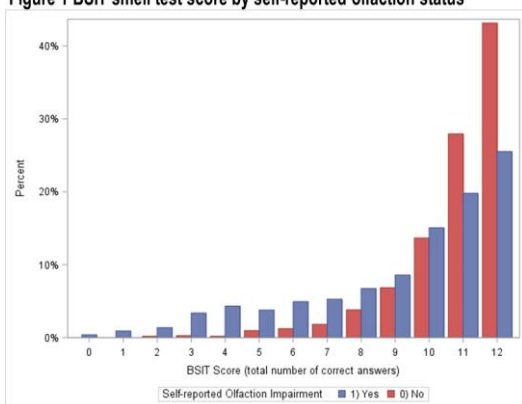
integrity. Preliminary results showed exceptional quality of data collection. Of those who returned the BSIT test-kit, 94.5% completed all 12 items, 4.3% missing on 1 or 2 items, and only 1.2% missing on 3 or more. The score ranges from 0 to 12 (Figure 1). Participants with self-reported OI overall scored lower than those who reported normal sense of smell. Initially we proposed to use a cutoff of 8 or the lowest 20% among controls. Based on distribution of these preliminary data, we may choose to use 9. This is reasonable as our study population are all women and are relatively young with an average age ~67 years, and 60% of participants are younger than age 70.

Specific Aim 5

1. *"Air pollutant assessment and data delivery to MSU"* by month 12

Progress: The aforementioned Sister Study data delivery included air pollution estimates based on participants' baseline residential addresses, the longest-lived addresses, and childhood addresses. Our collaborators at the University of Washington are currently updating air pollutant data by incorporating primary residential addresses after study enrollment.

2. *"Data analysis and manuscript preparation/submission"* (months 21-36)

Progress: Using interim dataset of 2,668 participants, we analyzed air pollutants based on enrollment address in relation to OI. We found that $PM_{2.5}$ concentration was significantly associated with higher odds of having OI (Table 1). NO_2 level on the other hand was not significantly related to OI. We will soon update this analysis with full data delivered in August 2019.

Table 1 Ambient air pollutants in relation to OI		
Exposure level	Multivariable OR and 95%CI	
	PM2.5	NO2
Quartile 1	Reference	Reference
Quartile 2	1.50 (1.06-2.13)	0.85 (0.60-1.19)
Quartile 3	1.48 (0.98-2.26)	0.76 (0.52-1.10)
Quartile 4	2.09 (1.48-3.45)	0.67 (0.44-1.01)

Specific Aim 6

1. *"DNA extracted and shipped to NIA"* by month 24

Progress: DNA extraction was completed by March 2019. In May 2019, we shipped DNA samples to NIA for genotyping, expecting completion late in 2019 or early in 2020.

3.C. What opportunities for training and professional development has the project provided?
Nothing to report – this project has no training component.

3.D. How were the results disseminated to communities of interest?
Frank Purdy, a Master student, presented the preliminary results at the "Grand Challenges in Parkinson's Disease" meeting at the Van Andel Institute in Grand Rapids, MI on Aug 21, 2019. The title for his poster is "Ambient Air Pollutants and Olfaction in A Large Cohort of Women: Preliminary Results".

3.E. What do you plan to do during the next reporting period to accomplish the goals?
As described above, we have achieved major goals proposed so far, and plan to accomplish additional goals as proposed.

4. IMPACT:

4.A. What was the impact on the development of the principal discipline(s) of the project?
The human sense of smell decreases with age, affecting 15-25% of older US adults. Although most do not even realize they have it, OI adversely affects human functioning such as detecting environmental hazards, nutrition, mood and behavior, sexuality, emotional and physical well-being, and quality of life. Further, OI independently predicts both short-term and long-term mortality in older adults.

Most importantly, converging evidence suggests OI is one of the earliest and most important prodromal symptoms for PD. OI research may therefore represent an unprecedented opportunity to understand the early stages of PD development. Late-onset PD takes years, if not decades, to develop, and by the time of diagnosis, is generally too advanced to decelerate, stop, or reverse. Research on OI may help in the war against PD in two ways: 1) characterize at-risk populations which may eventually facilitate early diagnosis and treatment, and 2) elucidate disease etiology. Current research, including ours, has focused on how OI predicts the risk of PD. We, however, also see OI research as an excellent opportunity to open the etiological "black-box" of the disease. A major challenge in such research is the current lack of understanding of the decades of PD prodromal development, during which many factors may come into play to initiate pathology or modify progression. By using OI as an easily-measured and noninvasive intermediate marker of PD, we expect to bring new insights into this "black-box" by identifying factors that contribute to OI and factors that modify its progression to PD, fundamentally improving understanding of the poorly understood etiology of PD.

4.B. What was the impact on other disciplines?
OI or hyposmia is also an early marker for several other neurodegenerative diseases such as Alzheimer's disease. Therefore, this project may eventually help understand a common pathway that leads to neurodegeneration.

4.C. What was the impact on technology transfer?
Nothing to report

4.D. What was the impact on society beyond science and technology?
Nothing to report during this period, but eventually data from this project will raise public awareness of the importance of sense of smell in aging, especially brain aging.

5. CHANGES/PROBLEMS:

5.A. Changes in approach and reasons for change
There was no substantial change to the project or its direction. As explained in the Accomplishments, we developed a cost-share with Parkinson Foundation ($151,399) approved

on August 8, 2019. With this additional support, we were able to extend data collection to all age-eligible Sister participants, regardless of their smoking, moving, or disease status. Our initial proposal to limit to nonsmokers and non-movers was mainly constrained by the budget allocation of the project ($1.5 million per study team). This addition does not affect scientific goals, study protocol, or risks and benefits of study participants, and brings substantial benefits to this project and beyond. At the end, we recruited 3,431 participants with smell test data as compared to 2,713 initially planned, we will be able to more comprehensively examine the proposed specific aims with larger statistical power and possibilities of sensitivity analyses.

5.B. Actual or anticipated problems or delays and actions or plans to resolve them
Nothing to report.

5.C. Changes that had a significant impact on expenditures
As explained above, we have developed a cost-share agreement to add $151,399 to expand data collection from the originally proposed 2,713 to 3,431 participants. The agreement was approved by DoD in August 2019.

5.D. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Nothing to report. Human subject study protocol was approved as detailed in 3.B.a.

6. PRODUCTS:

6.A. Publications, conference papers, and presentations
Nothing to report.

Journal publications:
No publications yet directly from this project, but I acknowledged DoD support in the following publications.

1 Liu B, Luo ZH, Pinto JM, Shiroma EJ, Tranah GJ, Wirdefeldt K, Fang F, Harris TB, **Chen H**: Relationship between poor olfaction and mortality among community-dwelling older adults: A cohort study. _Ann Intern Med_ Apr 30 2019. doi: 10.7326/M18-0775. PMID: 31035288.
2 Heinzel S, Berg D, Gasser T, **Chen H**, Yao C, Postuma RB; MDS Task Force on the Definition of Parkinson's Disease. Update of the MDS research criteria for prodromal Parkinson's disease. _Mov Disord_. 2019 Aug 14. doi: 10.1002/mds.27802. Review. PMID: 31412427.
3 **Chen H,** Ritz B. (Invited): The Search for Environmental Causes of Parkinson's Disease: Moving Forward. _J Parkinsons Dis._ 2018;8(s1):S9-S17. doi: 10.3233/JPD-181493. PMID: 30584168; PubMed Central PMCID: PMC6311360.

Books or other non-periodical, one-time publications: Nothing to report
Other publications, conference papers, and presentations:
A poster presentation titled "Ambient Air Pollutants and Olfaction in A Large Cohort of Women: Preliminary Results" at the "Grand Challenges in Parkinson's Disease" meeting at the Van Andel Institute in Grand Rapids, MI on Aug 21, 2019.

6.B. Website(s) or other Internet site(s)
Nothing to report

6.C Technologies or techniques
Nothing to report

6.D Inventions, patent applications, and/or licenses
Nothing to report

6.E. Other Products
As reported earlier in 3.B. We have collected the sense of smell data from 3,431 participants of the NIEHS Sister Study, creating one of the largest databases on the sense of smell among middle to older age women.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

7.A What individuals have worked on the project?

Name:	Honglei Chen
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0003-3446-7779
Nearest person month worked:	2
Contribution to Project:	Oversaw all activities of the study, including study design, material development and purchase, IRB approvals, filed data collection, and DNA extractions, and data management and analysis.
Funding Support:	

Name:	Frank Purdy
Project Role:	Graduate Student function as project manager
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	6
Contribution to Project:	Helped Dr. Chen manage various aspects of study activities, received and managed Sister Study data, conducted preliminary data analyses.
Funding Support:	

Name:	Aiwen Yang
Project Role:	Data analyst
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	9

Contribution to Project:	Under Dr. Chen's supervision, conducted preliminary data analyses.
Funding Support:	

7.B. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Title: Prodromal symptoms in the Sister Study
    Time Commitments: Chen H, PI, in-kind (0% effort)
    Supporting Agency: Parkinson's Foundation - PF-IMP-1825
    Address: 1359 Broadway Suite 1509; New York, NY 10018
    Performance Period: 06/01/2018 – 05/31/2019
    Level of funding: $151,399 total
    Project Goals: Supplemental funding to expand data collection in the above-referenced DoD study to a larger sample which enables more comprehensive analyses of risk factors for olfactory impairment and their relevance to Parkinson's development as detailed in 3.A. & 5.A.

Title: Determinants of depression in Parkinson's disease
    Time Commitments: Chen H, co-I, in-kind (0% effort)
    Supporting Agency: Michigan State University
    Address: Office of Research, A209 East Fee Hall, 965 Wilson Road, East Lansing, Michigan 48824-1316
    Performance Period: 07/01/2018 – 06/30/2021
    Level of funding: $299,975 total
    Project Goals: To determine role of appendectomy in depression among Parkinson's patients
    Specific Aims: To evaluate appendectomy in relation to depression in Parkinson's disease using data from the Swedish Patient Registry. No scientific or budgetary overlap with this project.

Title: R01ES029227-01A1 Pesticides, Olfaction, and Neurodegeneration Among US Farmers
    Time Commitment: Chen H, PI, 1.35 AY months & 1 Sum month
    Address: Office of Research, A209 East Fee Hall, 965 Wilson Road, East Lansing, Michigan 48824-1316
    Performance Period: 02/01/19 – 01/31/24
    Level of funding: $4,999,267 total
    Project Goals: To examine the connections among pesticides, olfactory impairment, and prodromal neurodegeneration.
    Specific Aims: To investigate roles of pesticides in olfactory impairment among farmers and their relevance to prodromal development of neurodegenerative diseases such as dementia and Parkinson's. No scientific or budgetary overlap with this project.

7.C. What other organizations were involved as partners?

    Organization Name: The Social & Scientific Systems, Inc.
    Location of Organization: Durham, North Carolina
    Partner's contribution to the project: collaboration

    Organization Name: National Institute of Environmental Health Sciences
    Location of Organization: Durham, North Carolina
    Partner's contribution to the project: collaboration

Organization Name: ReproCell, Inc. (previously called Bioserve)
Location of Organization: Beltsville, MD
Partner's contribution to the project: collaboration

Organization Name: University of Washington
Location of Organization: Seattle, WA
Partner's contribution to the project: collaboration

Organization Name: Chicago University
Location of Organization: Chicago, IL
Partner's contribution to the project: collaboration

Organization Name: National Institute on Aging
Location of Organization: Bethesda, MD
Partner's contribution to the project: collabroation

Organization Name: Parkinson's Foundation
Location of Organization: New York, NY
Partner's contribution to the project: Supplemental financial support as explained above

8. SPECIAL REPORTING REQUIREMENTS

8.A. COLLABORATIVE AWARDS: Other co-PI will submit their own reports
8.B. QUAD CHARTS: attached.

9. APPENDICES: Nothing to report

Airborne Pollutants as Triggers of Parkinson's Disease via the Olfactory System   PD160021P2
W81XWH-17-1-0536

PI: Honglei Chen   **Org:** Michigan State University   **Award Amount:** 1,500,000

Study/Product Aim(s)

The overall aim is to investigate roles of air pollutants in olfactory impairment (OI) and to examine their relevance to Parkinson's disease (PD).
• Examine the effect of air pollutants on OI.
• Examine whether air pollutants exacerbate early PD pathogenesis.
• Examine whether NSAIDs use modifies the potential adverse effects of air pollutants on OI.

Approach

This is a nested case-control study within the well-established NIEHS Sister Study. We selected 2,820 participants who self-reported a poor sense of smell and 1,200 who did not. We tested their sense of smell using the Brief Smell Identification Test (BSIT) by mail. Of these, 3,535 (87.9%) participants returned any study materials and 3,431 (85.3%) returned the sense of smell test kit. We will analyze air pollutant exposures and covariates from the Sister study in relation to the sense of smell assessed in the current study.
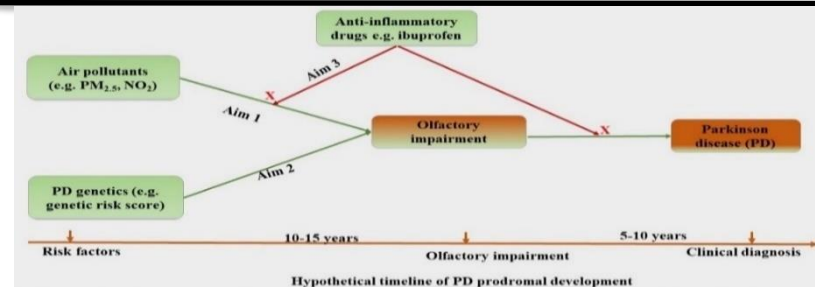

Figure. Aims: Air pollutants and PD genetic risk factors may contribute to olfactory impairment individually and synergistically (aims 1-2) and anti-inflammatory therapies may reduce the risk (aim 3).

Accomplishments: 1) Data collection, entry and cleaning completed; data delivered to MSU on August 23 2019. A total of 3,535 (87.9%) participants have provided data and 3,431 (85.3%) returned the sense of smell test kit. 2) DNA samples were shipped to NIA for genotyping. 3) developed cost-share with Parkinson Foundation (PF). 4) MSU team is double-checking data quality and conducting analyses to: a) compare self-reported vs. tested sense of smell, and b) examine air pollutants in relation to the sense of smell.

Timeline and Cost

Activities CY	17-18	18-19	19-20	20-21
Text (Major aim/study/milestone)	Planning			
Text (Major aim/study/milestone)		Data collection		
Text (Major aim/study/milestone)			Data analysis	
Text (Major aim/study/milestone)			Manuscript writing	
Estimated Budget ($K)	$407129	$684822	$193866	$214182

Updated: Completed participant recruitment, field data collection, and DNA extraction; genotyping and data analysis in progress.

Goals/Milestones
CY17 Goal – Study preparation
☑ Develop study materials, obtained IRB approval, select participant
CY18Goals – Participant recruitment and data collection
☑ Recruit participants, test the sense of smell using BSIT
☑ Data cleaning and entry
CY19 Goal – Data analyses and DNA extraction
☑ DNA extraction and send to NIA for genotyping
☑ Data delivery to PI and analysis
CY20 Goal – All data ready and data analyses
☑ NIA genotyping, completing data generation
☐ Data analyses and manuscript preparation
Comments/Challenges/Issues/Concerns
• Nothing to report
Budget Expenditure to Date
Projected Expenditure: $ 1,091,951 (DoD only) $151,399   (PF)
Actual Expenditure: $ 931,407 (DoD only) $145,914.88 (PF)