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LLNL-TR-672405

Quarterly Technical Progress Report June 2015

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June 8, 2015

Disclaimer

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Quarterly Technical Progress Report

Award Number:	10567486
Log Number:	LC130820
Project Title:	Mechanism for Clastogenic Activity of Naphthalene
Principal Investigator Name:	Bruce Buchholz
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Report Date:	June 10, 2015
Report Period:	March 1, 2015-May 31, 2015

LLNL-TR-672405

This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

1. Accomplishments:

The project has two main goals: 1) Identify the types of adducts naphthalene (NA) forms with DNA and 2) determine whether adduct formation correlates with site selective tumor formation in defined subcompartments of the respiratory tract (respiratory and olfactory nasal epithelium and airways of mice, rats and rhesus monkeys). Five tasks are associated with the completion of the goals.
Task 1: Contracting and Animal Use Approvals. IACUC and ACURO approvals are complete, The subcontract with UC Davis (UCD) was executed in December 2014.
Task 2: Perform In Vitro Study for Goal 1. Rat samples exposed and in freezer while adduct standards are being made. Mouse samples need to be exposed in next quarter.
Task 3: Perform In Vitro Study for Goal 2. Mouse ex vivo samples completed. Rat and monkey samples need to be completed in the next quarter.
Task 4: Sample Preparation and Analysis. Mouse Goal 2 samples completed. Other samples remain to be done.
Task 5: Data Interpretation and Reporting. Need rat data to write paper on adduct formation.

What was accomplished under these goals?

The major activity of the past quarter was pursuit of naphthalene metabolite DNA adduct standards that identify which adducts are formed for Goal 1.

Describe the Regulatory Protocol and Activity Status (if applicable).

(a) ***Human Use Regulatory Protocols***

TOTAL PROTOCOLS: *No human subjects research will be performed to complete the Statement of Work.*

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

No human cadaver research will be performed to complete the Statement of Work.

(c) Animal Use Regulatory Protocols

TOTAL PROTOCOL(S): 1 animal use protocol is required to complete the Statement of Work.

PROTOCOL (1 of 1 total):

Protocol [ACURO Assigned Number]: LC130820

Title: Quantitation of DNA Adducts from Naphthalene

Target required for statistical significance: 36 mice, 18 rats

Target approved for statistical significance: 36 mice, 18 rats

SUBMITTED TO AND APPROVED BY:

Submitted to USAMRMC 14-AUG-2014

Approved by USAMRMC (ACURO) 15-OCT-2014 by Bryan K. Ketzenberger, DVM, DACLAM

IACUC 18172 (UC Davis) submitted by Protocol PI Alan Buckpitt was approved 15-MAY-2014, renewed 15-May-2015.

STATUS:

No technical or logistical issues.

What do you plan to do during the next reporting period to accomplish the goals and objectives?

We plan to complete the rat ex vivo exposures required for Goals 1 and 2. Samples will be analyzed by AMS.

We anticipate most of the HPLC separation work for Goal 1 will be done in the next quarter.

2. Products:

Nothing to report.

3. Participants & Other Collaborating Organizations

No Personnel have worked 1 person month on the project.
Name: Bruce Buchholz
Project Role: PI
Nearest person month worked: 0
Contribution to Project: Analyzed ex vivo mouse exposure samples. Completed quarterly reports.

4. Changes/Problems:

a. Actual Problems or delays and actions to resolve them

Initial rat ex vivo samples had elevated controls making them unsuitable for use for Goal 2. Samples are being stored at -80C for use in Goal 1. Lab used in DNA separation had high level 14C use and another lab has been identified and certified for processing.

We were unable to schedule a block of several consecutive days to complete the remaining rat microdissections during the spring quarter at UC Davis and will complete them now that the term is finishing in early June.

It has been more difficult than expected to acquire the naphthalene metaobolites and generate DNA adducts for use as internal standards for Goal 1. We have secured the metabolites and are working on producing stable adducted bases. If not all the target adducted bases can be made and retain stability, we will use what we have for analyses.

b. Anticipated Problems/Issues

We want to use a new hybrid interface UPLC-AMS-MS/MS system in the lab for the metabolite-adduct analyses but it is not yet working reliably. The new system spits UPLC eluent to simultaneously measure 14C by AMS (quantitation) and traditional small molecule mass to provide identification. The mass identification would be better than identification by co-elution of standards and 14C analysis of fractions.

5. Special Reporting Requirements:

Quad Charts: Quad chart attached.

Mechanism for Clastogenic Activity of Naphthalene

LC130820
MIPR:10567486

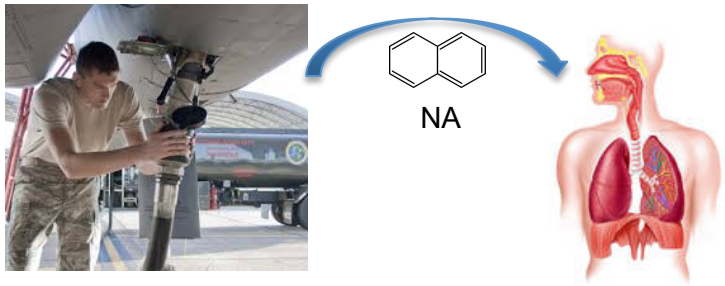
PI: Bruce Buchholz **Org:** Lawrence Livermore National Laboratory **Award Amount:** $165K

Study/Product Aim(s)

- Identify the types of adducts naphthalene (NA) forms with DNA
- Determine whether adduct formation correlates with site selective tumor formation in defined subcompartments of the respiratory tract

Approach

The location of lung and nasal tumors in rodents exposed to NA are highly specific to defined regions within the respiratory tract. We will utilize microdissection methods to isolate live tissue from these target areas. This approach combined with accelerator mass spectrometry (AMS) will determine whether DNA adducts are formed in the target tissue following incubation with ^{14}C-NA.



Determine if naphthalene (NA) in jet fuel and cigarette smoke forms DNA adducts that can lead to cancer in respiratory tissues

Accomplishment: Remaining in vitro scheduled for start of next quarter. Acquisition NA metabolites and generation of adducted DNA bases for HPLC underway.

Timeline and Cost

Activities CY	14	15
Animal Protocol & Contract Complete		
In Vitro Studies for Aim 1 Complete		
In Vitro Studies for Aim 2 Complete		
Sample Analyses Complete		
Data Analyses and Reporting		
Estimated Budget ($K)	**$15**	**$150**

Updated: (3-June-2015)

Goals/Milestones

Q1 Goals – Approval of Animal Protocol and Subcontract Executed
☑ Animal protocols approved
☑ Subcontract with UCD executed
Q2 Goal – Begin Experimental Work
☑ Begin in vitro studies and sample analyses for aims 1& 2
Q3 Goal – Complete Experimental Work
☐ Finish all in vitro studies and sample analyses
Q4 Goals – Complete Data Analyses and Reporting
☐ Complete and submit peer-reviewed publication
☐ Complete and submit final report

Comments/Challenges/Issues/Concerns

- In vitro exposures scheduled to be complete in next month.
- Delay in securing metabolite adducted bases has HPLC work for Goal 1 behind schedule.

Budget Expenditure to Date

Projected Expenditure: $90K
Actual Expenditure: $45K