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LLNL-TR-682042

Mechanism for Clastogenic Activity of Naphthalene. Quarterly Technical Progress Report

B. A. Buchholz

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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
Principal Investigator Organization and Address:	Lawrence Livermore National Laboratory P.O. Box 808, L-397 Livermore, CA 94551
Principal Investigator Phone and Email:	925-422-1739 buchholz2@llnl.gov
Report Date:	Jan 20, 2016
Report Period:	September 1, 2015-December 31, 2015

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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 and mouse samples exposures completed. Monkey samples need to be exposed in next quarter.
Task 3: Perform In Vitro Study for Goal 2. Mouse and rat ex vivo exposures completed. Monkey samples need to be completed in the next quarter.
Task 4: Sample Preparation and Analysis. Mouse and Rat Goal 2 samples completed. Monkey samples remain to be done for Goal 2. Rat samples completed for Goal 1. Mouse and Monkey samples for Goal 1 need to be completed.
Task 5: Data Interpretation and Reporting. Poster will be presented at 2016 Society of Toxicology Meeting. Outline for paper on adduct formation complete and similar to poster for SOT meeting.

What was accomplished under these goals?

The major activity of the past quarter was digest of rat DNA with naphthalene and running it on UPLC-MS-AMS for Goal 1. Adducts were not stable and broke apart from DNA somewhere in the process of analyses.

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?

The conditions for DNA digest and UPLC-MA-AMS analysis will be adjusted in an effort to prevent adduct destruction. We will switch to mouse airway DNA which had much higher adduct levels (approx. 100x) than rat nose DNA. We will perform monkey exposures as animals become available.

2. Products:

Abstract was submitted to Society of Toxicology for March 2016 meeting.

3. Participants & Other Collaborating Organizations

Bruce Buchholz and Laura Van Winkle have worked 1 person month on the project.
Name: Bruce Buchholz
Project Role: PI
Nearest person month worked: 1
Contribution to Project: Analyzed ex vivo mouse and rat exposure samples. Completed quarterly and annual reports.
Name: Laura Van Winkle
Project Role: Co-investigator
Nearest person month worked: 1
Contribution to Project: Performed tissue micro-dissections. Drafting poster and paper.

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 metabolites 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. The new system spits UPLC eluent to simultaneously measure ^{14}C 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 ^{14}C analysis of fractions. Very few monkeys were culled from the colony in the past quarter and none were available for this project. We hope to get four monkey samples in the next quarter.

5. Special Reporting Requirements:

Quad Charts: Quad chart attached.