1. Final Performance Report

BIOMOSAICS
Burlington, VT 05401

Project Title: "Antibodies to hemoglobin adducts in butadiene exposed workers"
June 20, 2001
Richard Hong, M.D., Principal Investigator
and Project Director
Mark Allegretta,Ph.D., Co-Principal Investigator
Grant Number: R43 OH03878-01

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LIST OF ABBREVIATIONS

ELISA	Enzyme Linked Immunosorbent Assay
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
OD 405	Optical Density units at wavelength 405 nanometers

ABSTRACT

The principle objective of this research was to develop an assay to detect antibodies to hemoglobin adducts in butadiene exposed workers. Demonstration of such antibodies would show the feasibility of using an immune response as an index of toxin exposure. There are many advantages of utilizing antibody detection for this purpose:

1. Antibodies could persist for an appreciable length of time after the exposure, so that detection is conceivably possible after the event.
2. Antibodies can be detected in minute quantities.
3. Antibody assays are ideal for testing of large numbers of samples requiring only minute amounts of blood.
4. Antibody assays are inexpensive.
5. Ultimately, a panel of antigens could be utilized in a microchip array allowing screening for many toxins simultaneously.

We used plasma samples from workers exposed to butadiene, and tested them for IgG, IgA and IgM antibodies to a hemogloblin adduct. The antigen, *N, N*-(2,3-dihydroxybuta- 1,4,diyl) valine was kindly provided by Dr. James Swenberg, U. No. Carolina. We developed an ELISA assay for the detection.

IgM antibodies were found in 7/19 exposed and 3/10 non-exposed workers. IgG antibodies were found in 3/19 exposed and 1/10 non-exposed workers.

During the course of this study, we had the opportunity to study serums from mice that had received daily exposure to butadiene for a two week period as part of a study conducted by Dr. Vernon Walker at the Wadsworth Institute of the New York State Public Health Department. Two of 15 exposed mice had a strong response to the hemogloblin adduct (OD 405: 0.346 and 0.374). One of 10 non exposed controls also had a positive response, but the response was low (OD 405: 0.107)

We conclude that antibody assay is a method which can detect toxin exposure and should be developed further to include a wide range of antigens which would allow mass screening of populations at risk.

SIGNIFICANT FINDINGS and USEFULNESS OF FINDINGS

Antibodies to *N, N*-(2,3-dihydroxybuta- 1,4,diyl) valine were detected in humans.

These findings, if corroborated, verify the premise of the study, i.e., that toxin exposure can be detected by this simple and inexpensive procedure which can be utilized for mass screening. In order for these findings to have greater validity, the "noise", i.e., positive results found in controls must be reduced. In efforts to maximize the sensitivity of the assay, we introduced more nonspecificity. However, the approach to maximizing signal/noise ratios is straightforward and will represent a major effort for us in the coming months. Further, only 28 of the 68 samples were completely analyzed, and the specificity may improve simply with the assay of larger numbers of samples.

Antibodies of the IgM class were found in the exposed, although the incidence was not obviously different from controls. It is anticipated that the differences can be maximized by lowering the "noise". If this can be done, the kinetics of response to exposure can be followed by the isotype responses. IgM antibodies occur early after exposure and IgG antibodies are expected after prolonged exposure.

Also, the finding of antibodies to this adduct are informative in that on can infer that the di-epoxide is a metabolic product following toxin exposure. Thus, the demonstration of a response provides information about how the toxin is handled by the host.

Antibodies to *N, N*-(2,3-dihydroxybuta- 1,4,diyl) valine were detected in mice.

This extends the observation to another animal species. If verified, animal models become available for a number of toxicologic studies.

The spleens from these animals are available and will be used to develop monoclonal proteins to the adduct. The monoclonals will be valuable reagents for further assay development and also in purification of adducts.

FINANCIAL STATUS REPORT

(Long Form)

(Follow instructions on the back)

1. Federal Agency and Organizational Element to Which Report is Submitted	2. Federal Grant or Other Identifying Number Assigned By Federal Agency	OMB Approval No.	Page of
CDC/NIOSH	R43 OH03878-01	0348-0039	1 of 1 pages

3. Recipient Organization (Name and complete address, including ZIP code)
BioMosaics, Inc. P.O. Box 281, Charlotte, VT 05445

4. Employer Identification Number	5. Recipient Account Number or Identifying Number	6. Final Report	7. Basis
13-4036306	80B7G	☒ Yes ☐ No	☒ Cash ☐ Accrual

8. Funding/Grant Period *(See instructions)* From: (Month, Day, Year)	To: (Month, Day, Year)	9. Period Covered by this Report From: (Month, Day, Year)	To: (Month, Day, Year)
9/30/99	3/31/01	9/30/99	3/31/01

10. Transactions:	I Previously Reported	II This Period	III Cumulative
a. Total outlays			63,600
b. Refunds, rebates, etc.			0.00
c. Program income used in accordance with the deduction alternative			0.00
d. Net outlays *(Line a, less the sum of lines b and c)*	0.00	0.00	63,600
Recipient's share of net outlays, consisting of:			
e. Third party (in-kind) contributions			0.00
f. Other Federal awards authorized to be used to match this award			0.00
g. Program income used in accordance with the matching or cost sharing alternative			0.00
h. All other recipient outlays not shown on lines e, f or g			0.00
i. Total recipient share of net outlays *(Sum of lines e, f, g and h)*	0.00	0.00	0.00
j. Federal share of net outlays *(line d less line i)*	0.00	0.00	63,600
k. Total unliquidated obligations			0.00
l. Recipient's share of unliquidated obligations			0.0
m. Federal share of unliquidated obligations			0.0
n. Total Federal share *(sum of lines j and m)*			63,600
o. Total Federal funds authorized for this funding period			63,600
p. Unobligated balance of Federal funds *(Line o minus line n)*			0.0
Program income, consisting of:			
q. Disbursed program income shown on lines c and/or g above			
r. Disbursed program income using the addition alternative			
s. Undisbursed program income			
t. Total program income realized *(Sum of lines q, r and s)*			0.00

11. Indirect Expense	a. Type of Rate *(Place "X" in appropriate box)* ☒ Provisional ☐ Predetermined ☐ Final ☐ Fixed	b. Rate	c. Base	d. Total Amount	e. Federal Share
		0.19566	50,250	9,832	9,832

12. *Remarks: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation.*

13. Certification: **I certify to the best of my knowledge and belief that this report is correct and complete and that all outlays and unliquidated obligations are for the purposes set forth in the award documents.**

Typed or Printed Name and Title	Telephone (Area code, number and extension)
Mark Allegretta, President	802-425-5545
Signature of Authorized Certifying Official	**Date Report Submitted**
Mark Allegretta	6/29/01

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269-104

Standard Form 269 (Rev. 7-97)

Prescribed by OMB Circulars A-102 and A-110

200-498 P.O. 139 (Face)

3. Equipment Inventory:

Item	Serial or ID #	Cost
EL800 Elisa Reader (Bio-Tek)	150256	$ 62127.50
Microplate washer	104137	322.50

Notes: All items were acquired on 07-07-2000
All items purchased entirely (100%) with Federal funds
All items are in excellent condition

4. Final Invention Statement

No inventions were conceived during the course of this award.

Memorandum

Date: December 11, 2001

From: Michael J. Galvin, Jr., Ph.D., Lead Program Activity
Office of Extramural Programs, NIOSH, D30

Subject: Final Report Submitted for Entry into NTIS for Grant 1 R43 OH003878-01.

To: William D. Bennett
Data Systems Team, Information Resources Branch, EID, NIOSH, P03/C18

The attached final report has been received from the principal investigator on the subject NIOSH grant. If this document is forwarded to the National Technical Information Service, please let us know when a document number is known so that we can inform anyone who inquires about this final report.

Any publications that are included with this report are highlighted on the list below.

Attachment
cc: Sherri Diana, EID, P03/C13

List of Publications

Title:	Hemoglobin Adduct Antibodies: Butadiene Exposed Workers
Investigator:	Richard Hong, M.D.
Affiliation:	BioMosaics
City & State:	Burlington, VT
Telephone:	(802) 656-8335
Award Number:	1 R43 OH003878-01
Start & End Date:	9/30/1999–3/31/2001
Total Project Cost:	$63,600
Program Area:	NORA
Key Words:	

Abstract:
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Publications
No publications to date.