

SRI International



LP1845 LIQUID GUN PROPELLANT  
DERMAL TOXICITY STUDIES:

①

EVALUATION OF THE POTENTIAL OF LP1846  
LIQUID GUN PROPELLANT TO INDUCE  
UNSCHEDULED DNA SYNTHESIS IN THE IN  
VITRO HEPATOCYTE DNA REPAIR ASSAY

Final Report

Date: March 14, 1990

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<p>At the request of the U.S. Army, SRI International<sup>4</sup> assessed the ability of the test material, LP1846 Liquid Gun Propellant (LP1846), to induce unscheduled DNA synthesis (UDS) in primary cultures of male Fischer-344 (F-344) rat hepatocytes. This study was conducted in compliance with the Good Laboratory Practice Standards.</p> <p>Laboratory work was initiated on August 23, 1989, and compound LP1846 was tested at concentrations of 0.5, 1, 5, 10, 50, 100, 250, 500, 1000, 2500, and 5000 ug/ml. No increase in UDS above that of the media control level was observed after treatment of the hepatocytes with compound LP1846. Therefore, on the basis of our criterion for a positive response, LP1846 was negative in the <u>in vitro</u> rat hepatocyte DNA repair assay.</p>			
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FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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*JB* Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of these organizations.

*JD* In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

\_\_\_\_\_ For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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*James Baker* 3/14/90  
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**QUALITY ASSURANCE UNIT  
Final Report and  
Conflict of Interest Statement**

The Quality Assurance Unit of SRI International assures that the Evaluation of the Potential of LP1846 Liquid Gun Propellant to Induce Unscheduled DNA Synthesis in the In Vitro Hepatocyte DNA Repair Assay conducted for the U.S. Army Medical Research and Development Command was performed in compliance with Good Laboratory Practices as set forth by Toxic Substances Control Act, Environmental Protection Agency (40 CFR, Part 792).

An inspection was performed on 24 August 1989 during the washdown phase that includes removal of the test or control article and media and rinsing and fixation of cells prior to autoradiographic procedures. The Study Director was informed of any findings following inspection and SRI Management was informed on 24 August 1989.

Audit of the raw data generated during the study and Draft Final Report Verification were completed 23 October 1989. The Study Director and SRI Management were notified of results on 24 October 1989. The Final Report was reviewed on 12 March 1990 and reaudit was deemed unnecessary. The Final Report accurately describes the methods and Standard Operating Procedures (SOPs) utilized during the study and does reflect the raw data that was generated during the conduct of the study. Any deviations from the protocol or SOPs were made with proper documentation.

This statement certifies that the personnel listed below participated in the inspection and audit of this study. These personnel have not been involved in the generation or evaluation of the data. Participation by the individuals listed below poses no conflict of interest.

Jill E. Kovach

I verify that the above is true to the best of my knowledge.

Jill E. Kovach

Quality Assurance Unit

12 March 1990


Date

**COMPLIANCE STATEMENT**

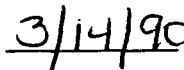
To the best of our knowledge, the in vitro hepatocyte DNA repair assay with LP1846 Liquid Gun Propellant (SRI Study No. 7662-A01-89) was conducted in general conformance with applicable Environmental Protection Agency (EPA) Good Laboratory Practice standards with the following exceptions:

1. Test substance characterization and stability data were not developed by SRI International.
2. Assays to verify concentration, stability, and homogeneity of the test substance in the carrier vehicle were not performed.

These deviations should not affect the results or conclusions of this study.



James P. Bakke  
Study Director



Date

## SUMMARY

At the request of the U.S. Army, SRI International assessed the ability of the test material, LP1846 Liquid Gun Propellant (LP1846), to induce unscheduled DNA synthesis (UDS) in primary cultures of male Fischer-344 (F-344) rat hepatocytes. This study was initiated on August 18, 1989, and conducted in compliance with the Good Laboratory Practice standards promulgated by the United States Environmental Protection Agency (EPA), 40 CFR Part 792. This study shall be completed with submission of the Final Report.

Laboratory work was initiated on August 23, 1989, and tabulation of laboratory data was completed on October 5, 1989. The compound LP1846 was tested at concentrations of 0.5, 1, 5, 10, 50, 100, 250, 500, 1000, 2500, and 5000  $\mu\text{g/ml}$ . The positive control was 2-acetylaminofluorene (2-AAF) at a concentration of 3.0  $\mu\text{g/ml}$ . No increase in UDS above that of the media control level was observed after treatment of the hepatocytes with compound LP1846. Therefore, on the basis of our criterion for a positive response, LP1846 was negative in the in vitro rat hepatocyte DNA repair assay.

CONTENTS

FOREWORD..... 1  
QUALITY ASSURANCE STATEMENT..... 2  
COMPLIANCE STATEMENT..... 3  
SUMMARY..... 4  
INTRODUCTION..... 6  
MATERIALS..... 7  
ANIMAL HUSBANDRY..... 8  
EXPERIMENTAL DESIGN..... 10  
RESULTS AND CONCLUSION..... 16  
REFERENCES..... 18  
DISTRIBUTION LIST..... 19

## INTRODUCTION AND BACKGROUND

### Study Objective

At the request of the U.S. Army, SRI International assessed the ability of the test material, LP1846 Liquid Gun Propellant (LP1846), to induce unscheduled DNA synthesis (UDS) in primary cultures of male Fischer-344 (F-344) rat hepatocytes.

The measurement of DNA repair as unscheduled DNA synthesis (UDS) following chemically induced DNA damage has been shown to be a valuable tool in assessing the genotoxic activity of chemicals. Several in vitro assays for the measurement of UDS have been described (Martin et al., 1978; San and Stich, 1975; Trosko and Yager, 1974); however, these assays often do not provide a means to metabolize chemicals to an active form. A substantial advance was made by the development of an assay that employs in vitro cultures of primary rat hepatocytes (Williams, 1977). This assay offers many advantages over other in vitro UDS systems: (1) the low level of DNA replication in the adult rat liver ( $\leq 0.1\%$ ) precludes the need for inhibitors of DNA replication; (2) the same cells serve as both activator and target; and (3) a more accurate profile of metabolism is obtained than with microsomal preparations. This assay has been shown to detect a wide variety of genotoxic agents, including polycyclic aromatic hydrocarbons, nitrosamines, mycotoxins, and aromatic amines.

### Study Purpose

The purpose of this study was to obtain data of suitable quality and integrity relating to the test article's genotoxic activity for submission pursuant to Section 4 of the Toxic Substances Control Act. This study, therefore, was conducted in compliance with the Good Laboratory Practice standards of The Environmental Protection Agency (40 CFR Part 792).

## MATERIALS

### Test Article

- Name: LP1846 Liquid Gun Propellant (LP1846).
- Lot no.: 1846-03.
- Purity: (not reported to SRI by Sponsor).
- Physical state at room temperature: Liquid.
- Physical description: Water white to very pale straw colored.
- Stability in test system: Sponsor assumes responsibility.
- Storage conditions: Stored at room temperature.
- Date received: July 3, 1989.

### Positive Control

- Name: 2-Acetylaminofluorene (2-AAF, 3.0  $\mu\text{g}/\text{ml}$ ).
- Lot no.: 08073BV.
- CAS No.: 53-96-3.
- Purity: >99.9% (as reported by Supplier).
- Expires: November 12, 1989.
- Stability in DMSO: Stable for > 3 months, as supported  
by our data base.
- Storage conditions: Stock stored in a lightproof container at  
4°C until diluted. 2-AAF was diluted in DMSO on August 23,  
1989, and stored in a lightproof container at -10°C between  
uses.
- Source: Aldrich Chemical  
940 W. St. Paul Ave.  
Milwaukee, WI 53233.

## ANIMAL HUSBANDRY

### Test Animal Description

A shipment of male Fischer-344 (F-344) rats was received by the SRI Laboratory Animal Medicine Department (LAMD) from the Kingston (K62), NY, facility of Charles River Laboratories, Inc. (RTE 209, Kingston, NY 12484) on July 11, 1989. A sample of 20 randomly selected rats from the shipment weighed between 91.8 and 122.7 g. The rats were born on May 22, 1989.

### Quarantine

Upon receipt of the rat shipment on July 17, 1989, the rats were ear punched for unique identification and quarantined for seven days. They were released from quarantine by the Director of LAMD on July 18, 1989. There were no signs or evidence of significant clinical diseases at any time during the quarantine period or during the course of the study.

### Animal Room Environmental Conditions

Rooms: Building L, Rooms Q14 and B110.

Temperature range: 66° to 74° F

Relative humidity range: 46% to 72%

Light cycle: 12 hours light/12 hours dark.

Cage specification: Rats were housed no more than 3/cage in polycarbonate cages containing hardwood-chip bedding.

Food and Water Supply

Food: Purina Certified Rodent Chow #5002  
Lot Nos. MAR30891A, MAY10892A, and MAY23892A  
Expiration September 30, 1989, November 10,  
1989, and November 23, 1989, respectively  
Ralston Purina Co., St. Louis, MO 63166.

Water: Purified tap water automatic drip ad libitum.  
Water-purity analysis on file.

## EXPERIMENTAL DESIGN

### Test Dates

Study Initiation Date: August 18, 1989

Experimental Start Date: August 23, 1989

Experimental Termination Date: October 5, 1989

Study Completion Date: March 14, 1990

### Dilution of Test Article

In preparation for testing, a solubility test for LP1846 was performed to determine the highest test concentration. Culture medium was used in the solubility test. LP1846 dissolved in media allowed the highest soluble concentration, 500 mg/ml. The highest concentration tested was 5000  $\mu$ g/ml. Immediately before the assay, LP1846 was diluted in media to form a series of concentrations that, when further diluted in culture medium, yielded the appropriate set of test concentrations, ranging from 0.5 to 5000  $\mu$ g/ml.

### Controls

The UDS assay included two control groups: an untreated medium control and a positive control group. The positive control chemical was 2-acetylaminofluorene (2-AAF), a hepatocarcinogen that requires metabolic activation and produces a strong positive response in this assay. For each experiment, two concentrations of 2-AAF were tested, but only one (preferably the higher of the two) was scored; the results are included in Table 1.

### Hepatocyte Isolation

The rats used, one each, in the preliminary and replicate experiments were anesthetized with sodium pentobarbital (0.2 cc/100 g body wt.). Primary cell cultures for an experiment were obtained from the liver of the F-344 rat used in that particular experiment. The liver was perfused in situ with a collagenase solution. The rat did not recover from surgery, expiring during the liver perfusion. The liver was removed. Isolated hepatocytes were combed out of the perfused liver and inoculated into numbered six-well culture dishes (containing coverslips) in Williams' Medium E (WE) supplemented with 2 mM l-glutamine, 50 µg/ml gentamycin sulfate, and 10% fetal bovine serum. After 1.5 to 2.0 hours of incubation in a humidified atmosphere at 37°C, 5% CO<sub>2</sub>, the cultures were washed to remove nonviable cells (those not attached to the coverslips). All washes and subsequent culturing were done in serum-free media.

### Cell Culture Chemical Exposure

In the UDS assay, three cultures were used for each of the ten dilutions of LP1846, the positive control, and the untreated medium control. The maximum concentration of LP1846 tested was 5000 µg/ml, which was the highest concentration designed in this test system. Cultures were exposed simultaneously to concentrations of LP1846 and 10 µCi/ml <sup>3</sup>H-thymidine (specific activity, approximately 80 Ci/mole) for 17- to 21-hours at 37°C, 5% CO<sub>2</sub>. A minimum of three scorable concentrations for the test article and one scorable concentration for the medium control were required for a valid assay. Therefore, at times, some low concentrations were not evaluated if enough higher scorable concentrations were available for UDS evaluation meeting the criteria for a valid assay.

### Cell Culture Fixation and Staining

After approximately a 19-hour exposure in both experiments, all cultures were washed with culture medium, swelled in 1% sodium

citrate solution, fixed in 1:3 glacial acetic acid/ethanol, and washed with deionized water. The coverslips were mounted to slides, which were then dipped in Kodak NTB-2 emulsion and exposed at -20°C for seven days prior to development. Cells were stained with 1% methyl-green Pyronin Y, dried, coverslipped, and evaluated.

## Criteria for a Valid Assay

### Slide Evaluation

Slides were evaluated for cytotoxicity under a light microscope by the Study Director following the autoradiographic exposure. At this time concentrations determined to be extra were set aside to be archived and not coded. Unscorable slides (if any) were determined according to the criteria for toxicity discussed below. Random-letter codes were generated by a random-letter-generator program on the VAX 8800. The slides selected for scoring were coded by an individual who would not be scoring the experiment. The purpose of the first experiment was to interpret any UDS response and determine cytotoxicity. The Sponsor was informed, via telephone, of the cytotoxicity results in the first experiment. Concentrations for the second experiment were selected at that time. A minimum of three scorable concentrations constitute a valid assay.

### Toxicity

Toxicity resulting from exposure to the test article was evaluated using the following criteria: (a) unscorable slides because of poor or no cell attachment, (b) unscorable slides because of pyknotic cells or other obvious morphologic changes, or (c) unscorable slides due to cellular death, resulting in an uneven or grainy stain.

## Data Collection

### Measurement of UDS

Quantitative autoradiographic grain-counting was accomplished using an ARTEK Model 880 or 980 colony counter interfaced with a Zeiss Universal microscope via an ARTEK TV camera. Data were fed directly into a VAX 8800 computer via an ARTEK BCD-RS232 Omni-Interface. At least 30 morphologically unaltered cells on a randomly selected area of the slide were counted. The highest count from two nuclear-sized areas over the most heavily labeled cytoplasmic areas adjacent to the nucleus was subtracted from the nuclear count to give the net grains/nucleus (NG). The percentage of cells in repair (% IR) indicates the extent of the response throughout the liver (cells in repair are those cells exhibiting at least 5 NG). Routinely, three slides per concentration were scored. If a slide had poor hepatocyte attachment, or was damaged or lost, a different patch (as determined by the microscope vernier coordinates) from another slide from the same concentration was scored. For each experiment, 90 cells were scored for each concentration reported. The data were summarized and the average NG and % IR were calculated for each concentration.

## Data Analysis and Statistical Methods

### Criteria for Interpretation

The UDS data generated were considered acceptable if the vehicle control animal data were within historical ranges (-5 to -15 NG) and if positive controls had significant elevations in NG ( $\geq 5$  NG) and percentage of cells undergoing repair ( $\geq 10\%$  IR).

### Interpretation of UDS Response

Positive. A test article was considered unequivocally positive if the mean NG count for any dose group was greater than 5 NG.

Negative. A test article was considered unequivocally negative if the mean NG count was less than 0 NG and the % IR was less than 10% for all dose groups.

Other. When results fell within 0 to 5 NG, the presence of a dose response relationship, the frequency distribution of cellular responses, increases in the % IR, and reproducibility of data among concentrations were all considered, and the test article was then classified as "negative," "weak positive," or "equivocal." No acceptable statistical tests are available for evaluation of data from UDS assays.

### Statistical Methods

No statistical methods were used for the evaluation of these data.

### Control of Bias

Microscope slides were coded prior to scoring by a person not assigned to score that experiment. At least two individuals scored results from each animal.

### Records to be Maintained

Laboratory notebook No. 9208, the Final Report, client communications, and hard copies of computer-generated data will be stored for at least ten years in the SRI Records Center, Building

B. Microscope slides will be stored in the SRI Slide and Tissue Repository, Building TT-2, for ten years or as long as quality affords evaluation.

### Key Personnel

The following key individuals participated in this study:

Jon C. Mirsalis, Ph.D., D.A.B.T.	Program Director
James P. Bakke, B.S.	Study Director/Project Leader
Carol M. Hamilton, B.A.	Cell Biologist
Kathleen E. Garin, B.S.	Biological Technician
Glenn D. Cunningham	Biological Technician
Kathleen R. Stewart	Microbiologist
Sherry Hanen, B.A., M.P.H.	Director, Quality Assurance
Jill E. Kovach, B.A.	Regulatory Affairs Specialist

### Changes from Original Protocol

The protocol specified that rats would be purchased in the weight range of 150-200 g; however rats were purchased at a weight of approximately 120g. The rats used on this project were maintained within the SRI Animal Care facility until they reached an acceptable size for use in the study.

### Circumstances That May Have Affected Data

The temperature in the animal room dropped below the recommended range of 70 to 74°F, to 66°F. However, this deviation does not effect outcome of the data obtained.

### Scientific Reports

No outside scientists (e.g., pathologists, analytical chemists) were used during the conduct of this study.

## RESULTS AND CONCLUSION

The in vitro rat hepatocyte DNA repair assay was used to measure the ability of LP1846 to induce UDS, an indicator of genotoxicity, following a 17- to 21-hour exposure of hepatocyte cultures. Results of the UDS assay with LP1846 are presented in Table 1. UDS was measured at test compound concentrations between 0.5 and 100  $\mu\text{g/ml}$  in both the preliminary and replicate experiments. UDS was not measured at concentrations ranging from 500 to 5000 and 250 to 500  $\mu\text{g/ml}$  in the preliminary and replicate experiments, respectively, due to cytotoxicity.

The NG counts were negative for both concentrations of the medium control, yielding mean values of -13.6 and -10.7 NG at a level  $\leq 2\%$  IR, in contrast to the strong positive response produced by 2-AAF shown in table 1 (25.8 NG, 93% IR and 27.2 NG, 97% IR).

Concentrations of LP1846 ranging from 0.5 to 100  $\mu\text{g/ml}$  all yielded negative mean NG values ranging from -13.6 to -7.5 NG with % IR values ranging from 0 to 6% IR. A toxic response was observed at the higher concentrations ranging from 250 to 5000  $\mu\text{g/ml}$ .

Therefore, based on our interpretation criteria, LP1846 is negative in the in vitro rat hepatocyte DNA repair assay.

Table 1

INDUCTION OF UNSCHEDULED DNA SYNTHESIS BY LP1846  
IN THE IN VITRO HEPATOCYTE DNA REPAIR ASSAY

Treatment	Concentration [ $\mu\text{g/ml}$ ]	Preliminary Assay		Replicate Assay		
		NG $\pm$ S.E.	Median % IR	NG $\pm$ S.E.	Median % IR	
Control/culture medium	---	-13.6 $\pm$ 3.8	-13.1	-10.7 $\pm$ 1.1	-9.7	0
Positive control/2-AAF	3	25.8 $\pm$ 3.9	26.3	27.2 $\pm$ 5.6	25.6	97
LP1846	0.5	-10.5 $\pm$ 0.2	-9.5	-8.1 $\pm$ 2.3	-7.7	2
	1	-12.0 $\pm$ 2.5	-12.1	-9.4 $\pm$ 1.2	-8.3	0
	5	-12.1 $\pm$ 1.5	-13.1	-8.9 $\pm$ 0.5	-8.3	3
	10	-13.6 $\pm$ 2.8	-12.6	-8.0 $\pm$ 2.7	-7.7	2
	50	-11.5 $\pm$ 2.0	-10.7	-9.9 $\pm$ 0.6	-10.3	0
	100	-10.3 $\pm$ 2.2	-9.5	-7.5 $\pm$ 1.4	-6.6	6
	250	N.T.		Toxic		
500	Toxic		Toxic			
1000	Toxic		N.T.			
2500	Toxic		N.T.			
5000	toxic		N.T.			

NG = mean Net Grains/nucleus

Standard errors (S.E.) represent slide-to-slide variation.

% IR = percentage of cells in repair.

Toxic = cytotoxicity observed; slides unscorable.

N.T. = Not tested at this concentration.

## REFERENCES

- Martin, C.N., A.C. McDermid, and R.C. Garner. 1978. Testing of known carcinogens and noncarcinogens for their ability to induce unscheduled DNA synthesis in HeLa cells. *Cancer Res.* 38, 2621-2627.
- San, R.H.C., and H.F. Stich. 1975. DNA repair synthesis of cultured human cells as a rapid bioassay for chemical carcinogens. *Int. J. Cancer* 16, 284-291.
- Trosko, J.E., and J.D. Yager. 1974. A sensitive method to measure physical and chemical carcinogen-induced "unscheduled DNA synthesis" in rapidly dividing eukaryotic cells. *Exp. Cell Res.* 88, 47-55.
- Williams, G.M. 1977. Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures. *Cancer Res.* 88, 47-55.

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