TRADE SECRET

Study Title

H-23745: Local Lymph Node Assay (LLNA)

Laboratory Project ID: DuPont-2642

AUTHOR: Gregory S. Ladics, Ph.D.

STUDY COMPLETED ON: April 7, 1999

PERFORMING LABORATORY: E. I. du Pont de Nemours and Company
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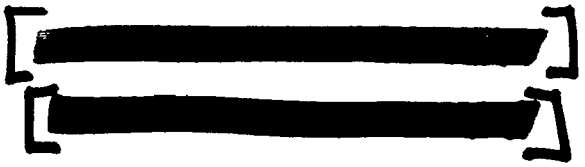

CERTIFICATION

I, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Issued by Study Director: _____ 7-April-1999

Gregory S. Ladics, Ph.D. Date
Senior Research Toxicologist

Company Sanitized. Does not contain TSCA CBI

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STUDY INFORMATION

Substance Tested: [redacted]

Synonyms/Codes: • H-23745
[redacted]

Haskell Number: 23745

Submitter's Notebook Number(s): [redacted]

Composition: [redacted]

Known Impurities: [redacted]

Physical Characteristics: Amber liquid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Sponsor: E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: January 13, 1999 / (see report cover page)

In-Life Initiated/Completed: January 13, 1999 / January 18, 1999

STUDY PERSONNEL

Study Director: Gregory S. Ladics, Ph.D.
Management: Judith C. Stadler, Ph.D.
Primary Technician: Charlene Smith, S.A.

Toxicology Report Preparation: Amy L. Williamson

Laboratory Veterinarian: Wanda L. West, D.V.M., A.C.L.A.M.

SUMMARY

The objective of this study was to evaluate the potential of H-23745 to produce a dermal sensitization response in mice using the local lymph node assay (LLNA). Six groups of female CBA/JHsd mice were dosed for 3 days with 0 (vehicle), 10, 25, 50, 75, and 100% H-23745 on both ears. Acetone:olive oil (4:1 ratio) was used as the diluting vehicle. A seventh group of 6 female mice were dosed for 3 days with 25% Hexylcinnamaldehyde (HCA) as a positive control. On day 5 of the assay, mice received 125-Iododeoxyuridine and were sacrificed 5 hours later. The cell proliferation in the draining auricular lymph nodes of the ears was then evaluated and compared to control.

There were no clinical signs of toxicity observed in the study. There were no statistically significant differences in mean body weights and mean body weight gains between the vehicle control group and any test substance group.

No statistically significant differences were seen in cell proliferation measurements at any test concentration. Stimulation indexes were less than 3.0 at all concentrations.

A 25% concentration of the positive control, HCA, produced a positive dermal sensitization response in mice. Therefore, the LLNA test system was valid for this study with H-23745. Under the conditions of this study, H-23745 was not a dermal sensitizer.

INTRODUCTION

The purpose of this study was to examine the dermal sensitization potential of H-23745 using the mouse local lymph node assay (LLNA).

The LLNA screen with H-23745 [Haskell 23745] [redacted] was conducted in female CBA/JHsd mice (Harlan Sprague Dawley, Frederick, MD).

MATERIALS AND METHODS

A. Test Substance

The test substance, H-23745, was supplied by the sponsor as a amber liquid. Each concentration of test article was prepared in a separate vial and vortexed to mix. The test substance appeared to be stable under the conditions of the study. No evidence of instability, such as a change in color or physical state, was observed.

B. Protocol

The test substance was prepared as a solution in 4:1 acetone:olive oil except the 100% concentration. Twenty-five µl of H-23745 was administered topically per ear to the dorsum of both ears of mice (6 per group) for 3 consecutive days (test days 0-2) at dosages of 0% (4:1 acetone:olive oil vehicle), 10%, 25%, 50%, 75%, and 100%. One group of 6 female mice was dosed with 25% hexylcinnamaldehyde (HCA) in 4:1 acetone:olive oil as a positive control. Test days 3-4 were days of rest, followed by intravenous injection of 2 µCi of 125-Iododeoxyuridine (^{125}IUdR) per mouse on the morning of test day 5. Any mouse which was not properly injected with the appropriate amount of ^{125}IUdR was excluded from study. Approximately 5 hours later, animals were sacrificed, draining auricular lymph nodes were removed, and single cell suspensions were prepared. The single cell suspensions were then incubated at ~4°C overnight. Counts per minute (cpm) data were obtained on test day 6 by counting the single cell suspensions on a gamma counter, and the cpm data were converted to disintegrations per minute (dpm) data.

C. Statistical Analyses

Calculations were done using the dpm data. A Stimulation Index (SI) of greater than or equal to 3.0 is considered a positive response. Data, other than for the positive control, were also analyzed for statistical significance using Jonckheere's Trend Test when a monotone dose response was evident. If a monotone dose response was not evident, either Dunnett's or Dunn's Multiple Comparison Procedure was used.

RESULTS AND DISCUSSION

A. Clinical Signs of Toxicity, Body Weights, and Body Weight Gains
(Tables 1-2)

There were no clinical signs of toxicity observed in the study. There were no statistically significant differences in mean body weights and mean body weight gains between the vehicle control group and any test substance group.

B. Stimulation Index Data
(Table 3)

No statistically significant differences were seen in cell proliferation measurements at any test concentration. Stimulation indexes were less than 3.0 at all concentrations of H-23745. A stimulation index of 5.71 was observed for the positive control HCA.

CONCLUSIONS

A 25% concentration of HCA produced a positive dermal sensitization response in mice. Therefore, the LLNA test system was valid for this study with H-23745. Under the conditions of this study, H-23745 was not a dermal sensitizer.

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

TABLES

TABLE 1

MEAN BODY WEIGHTS (g)

GROUP:	II	IV	VI	VIII	X	XII	XIV*
CONCENTRATION (%):	0	10	25	50	75	100	25
DAYS ON TEST							
0	20.4(0.9)[a]	20.3(0.8)	20.3(0.8)	20.1(0.5)	20.3(0.9)	20.3(0.8)	20.4(0.7)
5	20.9(0.6)	20.8(0.9)	21.0(0.9)	20.5(1.0)	21.0(1.4)	21.0(1.1)	21.3(1.1)

a Standard deviation is reported in parentheses.

Statistical methods: One-way Analysis of Variance and Dunnett's tests were performed on data
There were no statistically significant differences at $p > 0.05$

* HCA (positive control)

TABLE 2

MEAN BODY WEIGHT GAINS (g)

GROUP:	II	IV	VI	VIII	X	XII	XIV*
CONCENTRATION (%):	0	10	25	50	75	100	25
DAYS ON TEST							
0-5	0.5(0.6)[a]	0.5(0.3)	0.6(0.4)	0.4(0.8)	0.8(0.6)	0.7(0.4)	0.8(0.8)

a Standard deviation is reported in parentheses.

Statistical methods: One-way Analysis of Variance and Dunnett's tests were performed on data
There were no statistically significant differences at $p > 0.05$

* HCA (positive control)

TABLE 3

STIMULATION INDEX (SI) DATA

GROUP	MATERIAL TESTED	SI
II	0% (4:1 acetone:olive oil vehicle)	N/A
IV	10% H-23745	0.82
VI	25% H-23745	0.82
VIII	50% H-23745	0.82
X	75% H-23745	0.24
XII	100% H-23745	0.53
XIV	25% HCA in 4:1 acetone:olive oil (positive control)	5.71

N/A = Not Applicable