TRADE SECRET

Study Title

H-24256: Local Lymph Node Assay (LLNA)

Laboratory Project ID: DuPont-3548

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STUDY COMPLETED ON: November 15, 1999

PERFORMING LABORATORY: E. I. du Pont de Nemours and Company
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WORK REQUEST NUMBER: [redacted]

SERVICE CODE NUMBER: [redacted]

CERTIFICATION

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

Issued by Study Director: Gregory S. Ladics 15-Nov-1999

Gregory S. Ladics, Ph.D., D.A.B.T.
Senior Research Scientist

Date

Company Sanitized. Does not contain TSCA CBI

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STUDY INFORMATION

9th Collective Nomenclature: [redacted]

Synonyms/Codes: [redacted]
- H-24256
- [redacted]

Haskell Number: 24256

CAS Registry Number: [redacted]

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

Composition: [redacted]

Purity: [redacted]

Known Impurities: [redacted]

Physical Characteristics: [redacted]

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: October 27, 1999 / (see report cover page)

In-Life Initiated/Completed: October 27, 1999 / November 2, 1999

STUDY PERSONNEL

Study Director: Gregory S. Ladics, Ph.D., D.A.B.T.

Management: Judith C. Stadler, Ph.D.

Primary Technician: Charlene Smith, S.A.

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-24256 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), 5%, 10%, 25%, 50%, or 100% H-24256 on both ears. Dimethylsulfoxide 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-24256. Under the conditions of this study, H-24256 was not a dermal sensitizer.

INTRODUCTION

The purpose of this study was to examine the dermal sensitization potential of H-24256 using the mouse local lymph node assay (LLNA). Following the topical application of the test article to the dorsal side of both ears, the dermal sensitization potential of the test article was evaluated by measuring the proliferation of lymphocytes (via radiolabel uptake) obtained from the auricular lymph nodes (i.e., the lymph nodes that drain the ears). Because H-24256 is a [redacted] and did not appear to have severe skin irritating capability (pH ~8), the 100% concentration was used as the high dose. For subsequent dilutions, the test article was found to be soluble in dimethlysulfoxide (DMSO).

MATERIALS AND METHODS

A. Test Species

The Local Lymph Node Assay screen with H-24256 was conducted in female CBA/JHsd mice.

On 19-Oct-1999, 47 female CBA/JHsd mice with an assigned birth date of 27-Aug-1999, were received from Harlan Sprague Dawley, Frederick, Maryland.

The CBA/JHsd mouse was selected to conduct the LLNA because Haskell Laboratory has an extensive historical database with this strain. Furthermore, this strain has undergone extensive interlaboratory validation with the LLNA.[1,2,3]

B. Test Substance

The test substance, H-24256, was supplied by the sponsor as an [redacted]. The test substance was mixed before each dose was removed. 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.

C. Body Weight Measurements

All mice were weighed on test day 0 and prior to sacrifice on test day 5.

D. Clinical Observations

Cage-site examinations to detect moribund or dead mice and abnormal behavior and appearance among mice were conducted at least once daily throughout the study. At every weighing, each mouse was individually handled and examined for abnormal behavior and appearance.

E. Local Lymph Node Assay

The test substance was prepared as a solution in DMSO except the 100% concentration. Twenty-five μl of H-24256 was administered topically to the dorsum of each ear of mice (6 per group) for 3 consecutive days (test days 0-2) at dosages of 0% (DMSO vehicle), 5%, 10%, 25%, 50%, and 100%. One group of 6 female mice was dosed with 25% hexylcinnamaldehyde (HCA) 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. 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.

F. Statistical Analyses

Significance will be judged at $p < 0.05$ except for Jonckheere-Terpstra trend test which will be judged at $p < 0.01$.

<table>
<tr><th rowspan="2">Parameter</th><th rowspan="2">Preliminary Test</th><th colspan="2">Method of Statistical Analysis</th></tr>
<tr><th>If preliminary test is not significant</th><th>If preliminary test is significant</th></tr>
<tr><td rowspan="2">Body Weight
Body Weight Gain
Lymph Node DPM Data[a,b]</td><td rowspan="2">Levene's test[4] for homogeneity and Shapiro-Wilk test[5] for normality[c]</td><td>One-way Analysis of Variance;[6] followed, with Dunnett's test[7]</td><td>Kruskal-Wallis test;[8] followed, with Dunn's test[9]</td></tr>
<tr><td colspan="2">and/or
Sequential application[10] of the Jonckheere-Terpstra trend test[11]</td></tr>
</table>

a If more than one mouse was eliminated from a group, statistics were not done on the data for that group.

b DPM Positive control data was not included in the statistical analysis.

c If the Shapiro-Wilk test was not significant but Levene's test was significant, a robust version of Dunnett's test 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 observed in cell proliferation measurements at any test concentration. Stimulation indexes were less than 3.0 at all concentrations of H-24256. Under the conditions of this study, H-24256 was not a dermal sensitizer.

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-24256. Under the conditions of this study, H-24256 was not a dermal sensitizer.

RECORDS AND SAMPLE STORAGE

The raw data and final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

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TABLES

TABLE 1

MEAN BODY WEIGHTS (g)

GROUP:	II	IV	VI	VIII	X	XII	XIV
CONCENTRATION (%):	0	5	10	25	50	100	25*
DAYS ON TEST							
0	21.6 (1.7)[a]	21.6 (1.8)	22.0 (1.1)	21.6 (0.5)	22.2 (1.3)	22.6 (1.6)	22.1 (1.5)
5	22.0 (1.7)	22.2 (1.8)	22.4 (1.0)	22.6 (0.9)	22.6 (1.1)	23.0 (1.5)	23.0 (1.8)

a Standard Deviation is reported in parenthesis.
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	5	10	25	50	100	25*
DAYS ON TEST							
0-5	0.4 (0.6)[a]	0.7 (0.4)	0.4 (0.5)	1.1 (0.7)	0.5 (0.8)	0.4 (0.6)	0.8 (0.6)

a Standard Deviation is reported in parenthesis.
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% (DMSO)	N/A
IV	5% H-24256	2.00
VI	10% H-24256	0.75
VIII	25% H-24256	0.25
X	50% H-24256	1.00
XII	100% H-24256	0.00
XIV	25% HCA in DMSO (positive control)	3.50

N/A = Not Applicable

n = Number of animals per group.

There were no statistically significant differences at $p > 0.05$