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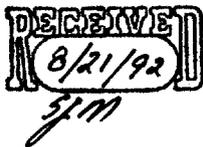
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Robert T. Drew, Ph.D.
Director
Health and Environmental Sciences Department



July 22, 1992

D492000055

FYI Coordinator
OTS Document Processing Center (TS-790)
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8492000055

Dear FYI Coordinator:

In accordance with API's policy of providing the federal government with copies of research designed to determine whether any chemical substance or mixture manufactured, processed or distributed by API member companies may cause risk of injury to health or the environment, we are enclosing copies of the following interim reports:

(Identification no.: FYI-not assigned) Effects of Dermal Exposure to Petroleum Middle Distillates on Irritation and Epidermal Hyperplasia in Mouse Skin (Subprotocol 1).

(Identification no.: FYI-not assigned) Effects of Dermal Exposure to Petroleum Middle Distillates on Irritation and Epidermal Hyperplasia in Mouse Skin. Final histopathology report from Dermigen. (Subprotocol 1)

These documents do not contain confidential information. We will continue to keep you apprised of the progress of this research. If you have any questions about it, please communicate with me.

Sincerely,

Robert T. Drew, Ph.D.



**EFFECTS OF DERMAL EXPOSURE TO PETROLEUM
MIDDLE DISTILLATES ON IRRITATION AND
EPIDERMAL HYPERPLASIA IN MOUSE SKIN**

Project No.: 6037-001

Test Material: 12-O-tetradecanoylphorbol-13-acetate

Contractor: ManTech Environmental Technology, Inc.
Five Triangle Drive
Research Triangle Park, North Carolina 27709

Sponsor: Dermigen, Inc.
908 North East Third Street
Loop 230
Smithville, Texas 78957

Effects of Dermal Exposure to Petroleum Middle Distillates on Irritation and Epidermal Hyperplasia in Mouse Skin

Study Initiation: February 19, 1992
Initiation of Dosing: March 12, 1992
Completion of In-Life Phase: March 16, 1992

SUMMARY

Three groups of 16 female CD-1 mice each were dosed with either acetone (control) or 6.8 nmoles or 13.6 nmoles of 12-O-tetradecanoylphorbol-13-acetate (TPA). An approximate area of 2.5 X 4 cm of the dorsoscapular area was shaved two days prior to the first dose. Each mouse was dosed with 200 μ l on March 5, 1992 (Thursday), March 9, 1992 (Monday), March 12, 1992 (Thursday) and March 16, 1992 (Monday). All animals were observed for dermal irritancy 24 hours after each dose and 72 hours after the last dose. Signs of gross dermal irritation were observed only in five animals from the high dose TPA (13.6 nmole) group after the second dose and continued to be noted throughout the study. Minimal to moderate erythema was noted in five of the mice with minimal to moderate fissuring noted in two mice. Body weight gains in both the 6.8 and 13.6 nmole groups were significantly higher than the controls. Four mice from each group were sacrificed 6, 24, 48 and 96 hours after receiving the last dose. Thirty minutes prior to scheduled necropsy at 24, 48 and 96 h after the last dose, each animal received bromodeoxyuridine (BrdUrd) intraperitoneally at a concentration of 100 μ g/gm of body weight. Mice scheduled for necropsy were killed by cervical dislocation. Skin from the treated area was collected for ornithine decarboxylase (ODC) activity while skin, pancreas and duodenum were collected for histopathological evaluation. At necropsy, pale thickened skin at the dosing site was noted in the 13.6 nmole group at 24, 48 and 96 hours past the last dose. The histopathological evaluation was performed under a separate contract and not part of this report. ODC activity for the acetone (control) group, the 6.8 nmole group and the 13.6 nmole group was 0.14, 31.72 and 60.5 nmole CO₂/mg protein, respectively at 6 hours after the last dose. There was a 227 and 432 fold increase in ODC activity between the control group, the 6.8 nmole and 13.6 nmole groups, respectively. The difference in ODC activity between the two TPA doses was only approximately 2 fold indicating that both doses are probably near the upper end of the dose response curve. ODC activity was dramatically decreased (less than 1 nmole CO₂/mg protein in the 13.6 nmole group) at 24 hours after the last dose indicating a short duration of ODC activity after termination of dosing.

PERSONNEL

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Mr. Allen Ledbetter

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This report was prepared by Mr. Michael Schlicht, Mr. Allen Ledbetter and Dr. Sheela Sharma

Submitted By:  Date 5/18/92
Dr. Sheela Sharma

Approved By:  Date 5/18/92
Dr. Earl Walborg

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I. Introduction

The purpose of this study is to establish a data base for designing and implementing a comparative study to measure the effects of petroleum middle distillates on dermal irritancy and markers of tumor promotion in mouse skin. These data will be used to design subsequent studies on nonhuman primate skin.

Prior to testing middle distillates, a pilot subprotocol was performed in CD-1 mice consisting of only one positive control (TPA) and one negative control (acetone) to assess dermal irritation and two markers of tumor promotion i.e. sustained epidermal hyperplasia and ornithine decarboxylase (ODC) activity. This report summarizes the in-life portion of the study and ODC analysis of skin samples.

II. Materials and Methods

- A. Test Articles: The test article, 12-O-tetradecanoylphorbol-13-acetate (TPA), CAS # 57716-89-9, Lot # F131, 99+ % purity was received on February 12, 1992 from LC Services, Inc., Woburn, MA. TPA was stored in its original container (glass ampule) and kept frozen (-20°C) until just prior to dosing. Acetone, Lot No. 2435 KAVY (99.6% purity) was received from Mallinckrodt Chemicals Co., stored in a glass amber bottle at room temperature.
- B. Animals: Specific pathogen-free, female CD-1 mice (26-30 days old at the time of receipt) were received in autoclaved, filtered cartons on February 10, 1992 from Charles River Laboratories, Inc., Raleigh, NC. Health records (Appendix 1) of the supplier's breeding colony did not show problems that could affect the results of this study.
- C. Animal Maintenance: The mice were quarantined for three weeks in NTC room # 137. During this time they were observed daily for disease or any condition which would render them unsuitable for inclusion in the study. The mice were housed in stainless steel wire mesh cages (Unifab Cages, Kalamazoo, MI). During the first two weeks of quarantine, the mice were housed three per cage then the mice were housed individually for the remainder of the study. Each cage unit consisted of two rows of six cages each for animal holding. Each cage was eight inches long, five inches wide (40 in²), and six and a half inches high. The cage unit and cage dimensions comply with current ILAR/AAALAC caging requirements. Four animals of each treatment group were placed in individual cages within each cage unit.
- D. Animal Water: City of Durham water (filtered and deionized by ManTech) was provided *ad libitum* to each cage via automatic watering.
- E. Animal Feed: Animals were provided Purina 5002 Certified Rodent Chow (pellets) in stainless steel feeders *ad libitum* during the study. All feed used in this study was from the same lot and used within four months of the milling date. See Appendix 2 for feed analysis.
- F. Animal Room Environment: The animal room was environmentally controlled to maintain temperature in a range of 71 to 74°F and a relative humidity of 62 to 70%. Temperature and humidity were recorded twice daily. Fluorescent lighting was provided automatically on a 12 hour light, 12 hour dark cycle (lights on from 0600-1800). In the room where the dosing or

bromodeoxyuridine (BrdUrd) injections were done, yellow light was used to make sure there was no degradation of the light sensitive chemicals. A back-up electrical generator was on-line to ensure animal room environments were maintained in case of power failure.

- G. Assignment to Groups: At the end of the quarantine period, on March 3, 1992 (Study Day -2), the dorsoscapular region (2.5 x 4 cm) of the back was shaved using an Oster® electric shaver with a No. 40 clipper blade. Shaving was performed in a manner that minimized abrasions, and shaving heads were frequently changed to avoid heating the skin. Multiple shavers were used, and the blades were cleaned with a brush and ethanol. The mice were then weighed and given physical examinations to ensure their suitability as test subjects. The mice were assigned to their respective groups by a stratified random method using a computer. The body weights for the animals ranged from 21.4 to 28.1 g.
- H. TPA Dosage Preparation: Two concentrations of TPA (molecular weight 616.92) were prepared on March 4, 1992 and stored at -20°C. A stock solution of TPA in acetone was prepared and dilutions were made with acetone for the two concentrations needed. The dosing solutions were prepared as follows:

$$\frac{1 \text{ mg TPA}}{1.193 \text{ mL Acetone}} \times \frac{1000 \text{ } \mu\text{g}}{\text{mg}} = \frac{838 \text{ } \mu\text{g}}{\text{mL}} \text{ Stock Solution (A)}$$

Low Dose:

$$\frac{0.1 \text{ mL (A)}}{3.9 \text{ mL Acetone}} = 20.95 \text{ } \mu\text{g/mL} = \frac{6.8 \text{ nmoles}}{200 \text{ } \mu\text{L}}$$

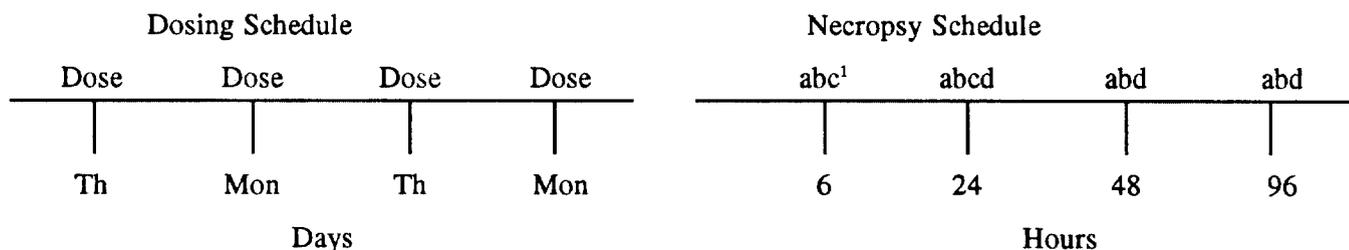
High Dose:

$$\frac{0.2 \text{ mL (A)}}{3.8 \text{ mL Acetone}} = 41.9 \text{ } \mu\text{g/mL} = \frac{13.6 \text{ nmoles}}{200 \text{ } \mu\text{L}}$$

- I. Design of the Study: Groups of 16 female mice were administered TPA dermally to the shaven backs (dorsoscapular area) at concentrations of 6.8 and 13.6 nmoles according to the following schedule. A negative control group was administered acetone and included in the experimental design. All doses were administered dermally at a constant volume of 200 μL per animal using a pipette (Oxford® Labware, St. Louis, MO). The three treatment groups were as follows:

Group	Test Article	Concentration nmoles	Volume μ l	Number of Animals
1	Acetone	0	200	16
2	TPA	6.8	200	16
3	TPA	13.6	200	16

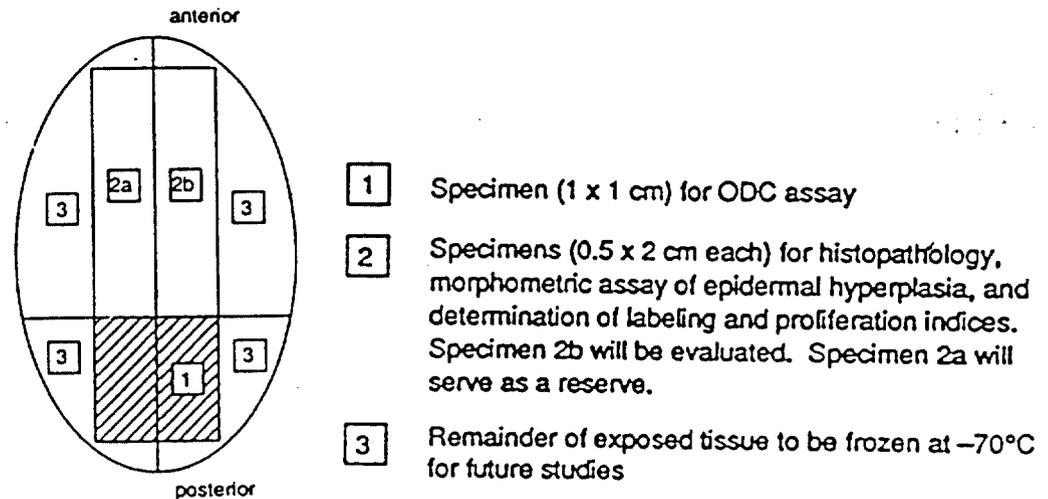
J. **Dosing Schedule:** The first day of dosing was March 5, 1992. Subsequent doses were administered on March 9 and March 12, 1992, and the final dose administered on March 16, 1992. The first three doses were administered between 0800 and 0835. The last doses were administered between 0700 and 0720. On the last dose day, animals (4 per group) scheduled for sacrifice were necropsied 6 h post-dose. The remaining animals (4 per group) were sacrificed at 24 h, 48 h and 96 h post-dose.



¹ a=necropsy performed, b=tissues taken for histology, c=ODC analysis, d=BrdUrd injection

- K. **Body Weights:** All animals were weighed two days prior to the initiation of dosing (Study Day -2) and again on the final dose day (Study Day 12).
- L. **Clinical Observations:** Each animal was observed for signs of morbidity or mortality twice daily on weekdays (am and pm) and once daily on weekends throughout the study.
- M. **Assessment of Gross Dermal Irritancy:** All animals were observed for gross signs of dermal irritancy approximately 24 hours after the dermal dosing and 72 hours following the last dose. The following parameters were assessed: erythema, desquamation, eschar, alopecia, discoloration, fissuring, and ulceration.
- N. **Sacrifice Schedule:** The animals (4 per group) were sacrificed at 6, 24, 48 and 96 h following the last dose. Thirty minutes prior to scheduled necropsy at 24, 48 and 96 h after the last dose, each animal received BrdUrd intraperitoneally at a concentration of 100 μ g/gm of body weight. BrdUrd was dissolved in sterile phosphate-buffered saline one hour before dosing and administered such that the volume for injection ranged from 0.1-0.2 mL.
- O. **Sacrifice and Skin Preparation:** Single groups of four mice each were killed by cervical dislocation. Each group cage cards was removed and placed with the bodies to avoid mixup of animals. The dead mice were then shaved (when required), and a depilatory (Nair™) was applied to the back. The depilatory was removed after six minutes by gentle scrubbing, with a gloved finger, under cold running tap water. The group of four mice was then placed in a pan of ice (dorsal side down), with extra ice covering the ventral side, until necropsy.

- P. **Necropsy:** The prosector team was given only one group of four mice at a time to avoid mixing up tissues. Each prosector received two tissue cassettes with a matching number of the mouse they were necropsying. An approximate area of 2.5 X 4 cm of the dorsoscapular area was removed, spread out on a cutting board, and a template was placed on top of the skin to guide the exact size measurement. Using a razor to cut downward, the skin was divided according to the following diagram:



In addition to the above skin sections, the pancreas and a section of duodenum were collected. The abdominal cavity was opened. The stomach was located and approximately 2-3 cm of duodenum, proximal to the stomach, was removed with the pancreas attached. The duodenum and pancreas samples were divided in half with each half going into one of the cassettes along with skin samples 2a and 2b. The cassettes and the remaining carcass were placed in 10% buffered formalin and delivered to Veritas Laboratories, Inc., for histologic processing and histopathologic evaluation.

The two skin specimens 3 were quick frozen on a pre-made block of frozen optimal cutting temperature (OCT) compound (Tissue Tek[®], Miles, Inc., Elkhart, IN). The tissue was spread out, covered with more OCT compound and quick frozen in liquid nitrogen. These specimens, with their labels, were then placed in a freezer (-70°C) and will be retained at ManTech for two years for other future evaluations.

ODC Assay of Skin Specimens: Skin specimen 1 was collected and used to evaluate ODC activity at 6 and 24 h post-exposure. The skins from the four mice from each group at each necropsy period were placed together in a beaker of ice and pooled for analyses.

- Q. **ODC Analysis:** See Appendix 3 for collection and preparation of epidermal homogenization methods and ODC assay.
- R. **Statistical Methods:** The difference in variance was analyzed in body weights, body weight gains and ODC data. Body weights and body weight gain data were also analyzed for difference between groups using *Dunnnett's Test* (Computational Handbook of Statistics, James L. Bruning and B.L. Kintz, 2nd Edition, 1977). Due to the wide range of ODC values between the acetone and TPA groups, the *Mann Whitney U-Test* was deemed inappropriate for analyzing the data.

III. Results

- A. Animal Survival: All mice survived until the scheduled necropsies.
- B. Body Weights: Individual body weights and body weight gains are presented in Table 1. Group mean body weights and body weight gain values are presented in Table 2. There was a statistically significant increase of body weight gains in the TPA dosed animals when compared to the acetone treated (control) animals. This difference in the body weight gain is not clear but may be explained due to the increase in the cell proliferation at the dose site.
- C. Clinical Observations: One high dose TPA animal (No. 48) was observed to have red tinged urine during 72 h post-exposure. No other treatment-related clinical signs of toxicity, with the exception of dermal irritancy, were observed during the study.
- D. Dermal Irritancy: Individual dermal irritancy scoring values are presented in Table 3 with total irritancy scores summarized in Table 4. Signs of gross dermal irritation were observed only in five animals from the high dose TPA (13.6 nmole) group after the second dose and continued to be noted throughout the study. Minimal to moderate erythema was noted in five of the mice with minimal to moderate fissuring noted in two mice. Three to four animals in each treatment group showed varying degrees of hair regrowth, however this was not considered to be the result of treatment.
- E. Necropsy: At the 6-h necropsy, one control mouse (#34) had mild focal redness in skin area 2. At the 24-h necropsy, three mice from the TPA high dose group had thickened skin which was either pale or had pale areas at the dosing site. The thickened area covered the entire dosing region in two of the three mice and 50% of the dosing region in the third mouse. At the 48-h necropsy, two mice from the high dose TPA group had raised areas on the lower back with one of the mice also having a raised area on the shoulder. One of the two mice also had a slightly reddened area near the shoulders. At the 72-h necropsy, one control mouse had a 2 mm X 2 cm red area up the middle of the back. Also at the 72-h necropsy one mouse from the TPA high dose group had a brown scab area (0.5 X 0.25 cm) over the hip on the dorsal surface. The mouse also had several pale, slightly thickened raised areas (1 X 1 cm) in the dosed area.
- F. ODC Results: The results of ODC assay of skin samples from animals treated with acetone and two doses of TPA (4 repeat doses) are given below. Table 5 shows ODC levels at 6 hours (0.14, 31.72 and 60.50 nmoles CO₂/mg protein for the acetone, 6.8 nmole and 13.6 nmole groups, respectively) after the final dosing. There is a striking increase (227 fold) in ODC activity in the low dose (6.8 nmoles) TPA group when compared to the acetone control group. But when the TPA dose was increased two fold (13.6 nmoles), there is no major increase in ODC activity indicating that the selected TPA doses are probably at the top range of the dose response curve. Table 6 indicates the ODC levels 24 hours after the last dosing. As expected, there is a dramatic decrease in ODC levels with some residual activity in TPA treated animals. It is clear that even after repeated dosing, the maximum induction of ODC appears to be only for a short duration even though there was continued hyperplasia for a longer duration. Since both the selected TPA doses are at the maximum dose-response range, we may need to select doses in the half maximal range of the dose-response curve to obtain significant differences between treatment groups.
- G. Protocol Deviations:
1. At necropsy, the Nair™ was left on the backs of the mice six minutes instead of the 3-5 minutes as stipulated by the protocol and SOP.
 2. The mice were not ear tagged as stipulated by the protocol, but they were identified by their cage cards.

TABLES

TABLE 1

INDIVIDUAL BODY WEIGHTS AND BODY WEIGHT GAINS (grams)

Animal No.	Group	---Body Weights---		Weight Gain
		Day 0	Day 12	
1	Control	24.9	26.9	2.0
2	Control	27.2	27.3	0.1
3	Control	25.5	24.7	-0.8
4	Control	24.8	26.2	1.4
5	Control	23.8	25.5	1.7
6	Control	23.1	23.4	0.3
7	Control	24.3	23.8	-0.5
8	Control	27.0	27.6	0.6
9	Control	22.2	23.2	1.0
10	Control	22.1	24.0	1.9
11	Control	26.3	28.4	2.1
12	Control	26.2	25.9	-0.3
13	Control	25.3	25.5	0.2
14	Control	28.0	30.4	2.4
15	Control	25.7	26.8	1.1
16	Control	24.3	23.7	-0.6
17	Low Dose	25.3	28.4	3.1
18	Low Dose	26.3	26.8	0.5
19	Low Dose	26.6	28.3	1.7
20	Low Dose	25.2	26.5	1.3
21	Low Dose	23.4	24.8	1.4
22	Low Dose	23.9	27.4	3.5
23	Low Dose	22.9	26.0	3.1
24	Low Dose	25.5	27.0	1.5
25	Low Dose	27.5	28.8	1.3
26	Low Dose	24.3	26.0	1.7
27	Low Dose	24.2	25.7	1.5
28	Low Dose	27.4	27.9	0.5
29	Low Dose	21.4	23.4	2.0
30	Low Dose	24.8	27.0	2.2
31	Low Dose	26.2	25.8	-0.4
32	Low Dose	26.0	27.7	1.7
33	High Dose	25.4	29.4	4.0
34	High Dose	25.1	27.3	2.2
35	High Dose	25.3	27.7	2.4
36	High Dose	23.5	26.0	2.5
37	High Dose	26.3	28.4	2.1
38	High Dose	24.5	26.3	1.8
39	High Dose	26.0	28.1	2.1
40	High Dose	23.2	25.7	2.5
41	High Dose	27.4	27.6	0.2
42	High Dose	26.1	26.9	0.8
43	High Dose	24.2	27.3	3.1
44	High Dose	22.0	24.9	2.9
45	High Dose	24.6	25.1	0.5
46	High Dose	22.9	26.4	3.5
47	High Dose	28.1	31.8	3.7
48	High Dose	26.5	30.2	3.7

TABLE 2
MEAN BODY WEIGHTS AND BODY WEIGHT GAINS (grams)

<u>Group</u>	<u>Body Weights</u>		<u>Weight Gain</u>
	<u>Day 0</u>	<u>Day 12</u>	
Control	25.0±1.71	25.8±2.02	0.79±1.06
Low Dose	25.1±1.66	26.7±1.42	1.66±1.01 ¹
High Dose	25.1±1.66	27.4±1.86 ¹	2.38±1.14 ²

¹ Significant (Dunnett's Test) from control at P=0.05

² Significant (Dunnett's Test) from control at P=0.01

TABLE 3
 INDIVIDUAL DERMAL IRRITANCY SCORES

Dermigen: 910801
 Test Agent: Acetone

Date: 03/06/92
 Time Postdose: 24h 1st Dose

Observation	Animal Number																Total
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Eschar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Alopecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Ulceration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16

Dermigen: 910801
 Test Agent: Acetone

Date: 03/10/92
 Time Postdose: 24h 2nd Dose

Observation	Animal Number																Total
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Eschar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Alopecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Ulceration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Hair Regrowth	0	0	0	0	0	0	2	0	0	1	0	0	0	0	0	1	3/16

Scoring
 0 = no signs
 1 = minimal
 2 = moderate
 3 = marked

TABLE 3 (con'd)

INDIVIDUAL DERMAL IRRITANCY SCORES

Dermigen: 910801
 Test Agent: Acetone

Date: 03/13/92
 Time Postdose: 24h post 3rd Dose

Observation	Animal Number																Total
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Eschar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Alopecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Ulceration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Hair Regrowth	0	0	0	0	0	0	3	0	0	3	0	0	0	0	0	2	3/16

Dermigen: 910801
 Test Agent: Acetone

Date: 03/19/92
 Time Postdose: 72h post 4th Dose

Observation	Animal Number				Total
	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	
Erythema	0	0	0	0	0/4
Edema	0	0	0	0	0/4
Desquamation	0	0	0	0	0/4
Eschar	0	0	0	0	0/4
Alopecia	0	0	0	0	0/4
Discoloration	0	0	0	0	0/4
Fissuring	0	0	0	0	0/4
Ulceration	0	0	0	0	0/4
Hair Regrowth	0	2	0	3	2/4

Scoring
 0 = no signs
 1 = minimal
 2 = moderate
 3 = marked

TABLE 3 (con'd)

INDIVIDUAL DERMAL IRRITANCY SCORES

Dermigen: 910801
 Test Agent: TPA
 Dose Concentration: 6.8 n mole

Date: 03/06/92
 Time Postdose: 24h 1st Dose

Observation	Animal Number																Total
	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>29</u>	<u>30</u>	<u>31</u>	<u>32</u>	
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Eschar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Alopecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Ulceration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16

Dermigen: 910801
 Test Agent: TPA
 Dose Concentration: 6.8 n mole

Date: 03/10/92
 Time Postdose: 24h 2nd Dose

Observation	Animal Number																Total
	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>29</u>	<u>30</u>	<u>31</u>	<u>32</u>	
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Eschar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Alopecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Ulceration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16

Scoring
 0 = no signs
 1 = minimal
 2 = moderate
 3 = marked

TABLE 3 (con'd)

INDIVIDUAL DERMAL IRRITANCY SCORES

Dermigen: 910801
 Test Agent: TPA
 Dose Concentration: 6.8 n mole

Date: 03/13/92
 Time Postdose: 24h post 3rd Dose

Observation	Animal Number																Total
	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>29</u>	<u>30</u>	<u>31</u>	<u>32</u>	
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Eschar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Alopecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Ulceration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Hair Regrowth	0	0	0	0	0	0	0	1	1	0	0	0	2	0	0	0	3/16

Dermigen: 910801
 Test Agent: TPA
 Dose Concentration: 6.8 n mole

Date: 03/19/92
 Time Postdose: 72h post 4th Dose

Observation	Animal Number				Total
	<u>29</u>	<u>30</u>	<u>31</u>	<u>32</u>	
Erythema	0	0	2	0	1/4
Edema	0	0	0	0	0/4
Desquamation	0	0	0	0	0/4
Eschar	0	0	0	0	0/4
Alopecia	0	0	0	0	0/4
Discoloration	0	0	0	0	0/4
Fissuring	0	0	0	0	0/4
Ulceration	0	0	0	0	0/4
Hair Regrowth	2	1	0	0	2/4

Scoring
 0 = no signs
 1 = minimal
 2 = moderate
 3 = marked

TABLE 3 (con'd)

INDIVIDUAL DERMAL IRRITANCY SCORES

Dermigen: 910801
 Test Agent: TPA
 Dose Concentration: 13.6 n mole

Date: 03/06/92
 Time Postdose: 24h 1st Dose

Observation	Animal Number															Total	
	<u>33</u>	<u>34</u>	<u>35</u>	<u>36</u>	<u>37</u>	<u>38</u>	<u>39</u>	<u>40</u>	<u>41</u>	<u>42</u>	<u>43</u>	<u>44</u>	<u>45</u>	<u>46</u>	<u>47</u>		<u>48</u>
Erythema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Eschar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Alopecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Ulceration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16

Dermigen: 910801
 Test Agent: TPA
 Dose Concentration: 13.6 n mole

Date: 03/10/92
 Time Postdose: 24h 2nd Dose

Observation	Animal Number															Total	
	<u>33</u>	<u>34</u>	<u>35</u>	<u>36</u>	<u>37</u>	<u>38</u>	<u>39</u>	<u>40</u>	<u>41</u>	<u>42</u>	<u>43</u>	<u>44</u>	<u>45</u>	<u>46</u>	<u>47</u>		<u>48</u>
Erythema	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	3/16
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Eschar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Alopecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Fissuring	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Ulceration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Hair Regrowth	0	0	0	0	0	0	0	0	1	0	2	0	0	0	0	0	2/16

Scoring
 0 = no signs
 1 = minimal
 2 = moderate
 3 = marked

TABLE 3 (con'd)
 INDIVIDUAL DERMAL IRRITANCY SCORES

Dermigen: 910801
 Test Agent: TPA
 Dose Concentration: 13.6 n mole

Date: 03/13/92
 Time Postdose: 24h post 3rd Dose

Observation	Animal Number																Total
	<u>33</u>	<u>34</u>	<u>35</u>	<u>36</u>	<u>37</u>	<u>38</u>	<u>39</u>	<u>40</u>	<u>41</u>	<u>42</u>	<u>43</u>	<u>44</u>	<u>45</u>	<u>46</u>	<u>47</u>	<u>48</u>	
Erythema	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	2	4/16
Edema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Desquamation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1/16
Eschar	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1/16
Alopecia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Discoloration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Fissuring	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	2/16
Ulceration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/16
Hair Regrowth	1	0	0	0	0	0	0	0	2	0	2	0	1	0	0	1	5/16

Dermigen: 910801
 Test Agent: TPA
 Dose Concentration: 13.6 n mole

Date: 03/19/92
 Time Postdose: 72h post 4th Dose

Observation	Animal Number				Total
	<u>45</u>	<u>46</u>	<u>47</u>	<u>48</u>	
Erythema	0	0	0	2	1/4
Edema	0	0	0	0	0/4
Desquamation	0	0	0	0	0/4
Eschar	0	0	0	2	1/4
Alopecia	0	0	0	0	0/4
Discoloration	0	0	0	0	0/4
Fissuring	0	0	0	0	0/4
Ulceration	0	0	0	0	0/4
Hair Regrowth	2	0	0	2	2/4

Scoring
 0 = no signs
 1 = minimal
 2 = moderate
 3 = marked

Table 4
TOTAL DERMAL IRRITANCY SCORES

<u>Animal #</u>	<u>24h post 1st Dose</u>	<u>24h post 2nd Dose</u>	<u>24h post 3rd Dose</u>	<u>72h post 4th Dose</u>
<i>Acetone</i>				
1	0	0	0	
2	0	0	0	
3	0	0	0	
4	0	0	0	
5	0	0	0	
6	0	0	0	
7	0	0	0	
8	0	0	0	
9	0	0	0	
10	0	0	0	
11	0	0	0	
12	0	0	0	
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0
16	0	0	0	0
<i>TPA Low Dose</i>				
17	0	0	0	
18	0	0	0	
19	0	0	0	
20	0	0	0	
21	0	0	0	
22	0	0	0	
23	0	0	0	
24	0	0	0	
25	0	0	0	
26	0	0	0	
27	0	0	0	
28	0	0	0	
29	0	0	0	0
30	0	0	0	0
31	0	0	0	0
32	0	0	0	0
<i>TPA High Dose</i>				
33	0	1	1	
34	0	0	0	
35	0	0	0	
36	0	0	0	
37	0	0	0	
38	0	0	0	
39	0	0	0	
40	0	0	0	
41	0	0	0	
42	0	0	0	
43	0	1	1	
44	0	0	3	
45	0	1	2	0
46	0	0	0	0
47	0	0	0	0
48	0	2	7	4

¹Maximum possible total score per animal per scoring period is 24

TABLE 5

ODC ACTIVITY- 6 HOURS POST-DOSING

ODC Assay (Repeat) Dermigen Project Subprotocol I

Date Dosing Initiated: 5 MAR 92

Date of Sacrifice: 16 MAR 92

Sacrifice timepoint : 6 hrs.

Date Entered: 10 APR 92

Entered By: KRG

Sample No.	DPM Counts	Mean	- Bkgrd	nmol CO ₂ Released	Protein (MG)	ODC Activity		Std. Dev.	Acceptable Range Mean + 2 Std. Dev.
						nmol CO ₂ / mg Protein	Mean		
Buffer	1 35.6	44.17	-8.57	-0.002					
	2 32.8		-11.37	-0.003					
	3 64.1		19.93	0.005					
Acetone	4 59.4		15.23	0.004	0.030	0.12			
	5 64.8		20.63	0.005		0.16			
	6 62.1		17.93	0.004		0.14	0.14	0.02	0.099 - 0.184
TPA	7 11550.9		11506.73	2.720	0.100	27.20			
6.8 nmol	8 17448.5		17404.33	4.113		41.13	31.72	8.16	15.396 - 48.035
	9 11389.8		11345.63	2.682		26.82			
TPA	10 31003.7		30959.53	7.317	0.150	48.78			
13.6 nmol	11 44763.4		44719.23	10.569		70.46	60.50	10.95	38.605 - 82.387
	12 39547.9		39503.73	9.337		62.24			

Total Counts Added: 442567.5

TABLE 6

ODC ACTIVITY 24 HOURS POST-DOSING

ODC Assay (Repeat) Dermigen Project Subprotocol 1

Date Dosing Initiated: 5 MAR 92

Date of Sacrifice: 17 MAR 92

Sacrifice timepoint : 24 hrs.

Date Entered: 10 APR 92

Entered By: KRG

Sample No.	DPM Counts	Mean	- Bkgd	nmol CO ₂ Released	Protein (MG)	ODC Activity		Std. Dev.	Acceptable Range Mean \pm 2 Std. Dev.
						nmol CO ₂ / mg Protein	Mean		
Buffer	1 35.6	44.17	-8.57	-0.002					
	2 32.8		-11.37	-0.003					
	3 64.1		19.93	0.005					
Acetone	4 54.1		9.93	0.002	0.030	0.08			
	5 59.9		15.73	0.004		0.12	0.08	0.048	-0.021 - 0.173
	6 47.6		3.43	0.001		0.03			
TPA	7 252.4		208.23	0.049	0.100	0.49			
6.8 nmol	8 166.3		122.13	0.029		0.29	0.49	0.194	0.098 - 0.873
	9 330		285.83	0.068		0.68			
TPA	10 795.4		751.23	0.178	0.150	1.18			
13.6 nmol	11 520.7		476.53	0.113		0.75	0.97	0.216	0.534 - 1.400
	12 657		612.83	0.145		0.97			

Total Counts Added: 442567.5

APPENDICES

APPENDIX 2
 FEED ANALYSIS

CERTIFICATION PROFILE

Based on analysis of a composite sample, each package contains not more than these maximum concentrations of the following substances:

<u>Heavy Metals</u>	<u>Maximum Concentration</u>
Arsenic	1.0 ppm
Cadmium5 ppm
Lead	1.5 ppm
Mercury2 ppm
Aflatoxin	5 ppb
<u>Chlorinated Hydrocarbons and PCB</u>	
Mirex02 ppm
Aldrin03 ppm
Dieldrin03 ppm
Endrin03 ppm
Heptachlor03 ppm
Heptachlor Epoxide03 ppm
Lindane05 ppm
Chlordane05 ppm
Alpha BHC05 ppm
Beta BHC05 ppm
Delta BHC05 ppm
HBC05 ppm
DDT Related Substances15 ppm
Methoxychlor5 ppm
PCB15 ppm
<u>Organophosphates</u>	
Thimet5 ppm
Diazinon5 ppm
Disulfoton5 ppm
Methyl Parathion5 ppm
Malathion5 ppm
Parathion5 ppm
Thiodan5 ppm
Ethion5 ppm
Trithion5 ppm
<u>Drugs and Estrogens</u>	

This product is manufactured in a plant where antibiotics and synthetic estrogens are strictly prohibited. Routine monitoring for over a decade has not shown any detectable levels of these substances. No drugs or synthetic estrogens are permitted in manufacturing, storage, or warehousing to avoid any contamination of Lab Chows® diets.

Other Contaminants

If additional contaminant assays are needed, these can be obtained by ordering such analysis prior to manufacture. Costs of these additional assays will be charged based on current analysis rates at time of assay.

QUALITY CONTROLLED BY PURINA RESEARCH

APPENDIX 2 (con'd)

FEED ANALYSIS

Purina Mills, Inc.
1401 S. Hanley Road
St. Louis, Missouri 63166

RT LAB NUMBER 169860

PAGE 2

CERTIFIED RODENT CHOW CHECKERS
LOT NUMBER AUG 23 91 1A

PESTICIDE & PCB (CONTINUED)

CHLORDANE.....	LESS THAN 0.02	LINDANE.....	LESS THAN 0.02
DDE.....	LESS THAN 0.02	METHOXYCHLOR.....	0.16
DDT..(TOTAL).....	LESS THAN 0.02	MIREX.....	LESS THAN 0.02
DIELDRIN.....	LESS THAN 0.02	PCB.....	LESS THAN 0.15

AFLATOXIN

TOTAL: LESS THAN 5 PPB

FOR ADDITIONAL INFORMATION, PLEASE CONTACT:

- (1) FOR ASSAY METHODOLOGY - MICHAEL J. MURPHY 314-982-3562
- (2) FOR NUTRITIONAL INTERPRETATION-DR DAN HOPKINS 314-768-4576
- (3) ALL OTHER QUESTIONS-RICHMOND, IN., MANUFACTURING PLANT 317-962-9561

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed. The use of the term "Less Than" does not imply that traces of analyte were present.

APPENDIX 3

ODC ANALYSIS PROCEDURES

Assay of ODC Activity Materials and Methods

1. Preparation of Epidermal Homogenate

Fresh skin specimen (1 cm²) from acetone treated and TPA treated animals was spread on a cold glass plate kept on ice and the epidermis were scraped by quick firm strokes. The scrapings from each treatment group (4 replicates) were placed in a single tube containing ODC homogenization fluid, pH 7.2 and homogenized for 15 seconds twice with a 15 second interval in between. The homogenized samples were centrifuged at 15,000 rpm for 30 minutes and the supernates were frozen at -70° C in two equal aliquots.

2. ODC Enzyme Assay

The ODC activity was measured by determining the liberation of ¹⁴CO₂ from ¹⁴C-Ornithine. Each sample including buffer was done in triplicate. Samples were thawed and kept on ice. An aliquot (100 μl) from each sample was used for protein determination using Bio-Rad Protein Assay Kit. Enzyme activity is expressed as nmol CO₂ liberated/ mg protein. The following steps were used for determination of ODC activity.

- a. Place 200 μl of CO₂ trap solution (ethanolamine: methoxy ethanol, 2:1 v/v)/ well.
- b. Add 100 μl of sample to reaction tube.
- c. Add 50 μl of reaction mixture (0.1 M Tris-HCL, 0.2 M L-Ornithine, 0.1 M Dithiothreitol, 4 mM Pyridoxal-5-phosphate, 20 mM EDTA, tetrasodium salt).
- d. At 30 second intervals, add 50 μl of ¹⁴C-Ornithine (0.25 μCi), cap tube, place at 37° C in a shaking water bath and incubate for 60 minutes.
- e. Stop reaction at 30 second intervals by adding 500 μl of 2 M citric acid using a 1 ml syringe and allow the tubes to sit for two hours.
- f. Transfer well to scintillation vial containing 1 ml of 95% ethanol and 4 ml of 989 scintillation cocktail. Mix and count each sample for 5 minutes.
- g. Calculate nmoles of CO₂ produced:

$$\frac{{}^{14}\text{C in sample X Total Ornithine (hot and cold)}}{\text{Total } {}^{14}\text{C added}} = \frac{\text{nmoles CO}_2}{\text{mg protein}}$$

18 MAY 1992

EFFECTS OF DERMAL EXPOSURE TO PETROLEUM MIDDLE DISTILLATES ON IRRITATION AND EPIDERMAL HYPERPLASIA IN MOUSE SKIN

Subprotocol 1 Report

Project Number: 5037-001

Submitted to

**Dermigen, Inc.
908 North East Third Street, Loop 230
Smithville, TX 78957**

ManTech Environmental Technology, Inc.
A ManTech International Company

CONTAINS NO CB



EFFECTS OF DERMAL EXPOSURE TO PETROLEUM MIDDLE
DISTILLATES ON IRRITATION AND EPIDERMAL HYPERPLASIA IN
MOUSE SKIN

FINAL HISTOPATHOLOGY REPORT

Submitted To: Dermigen
PO Box 727
908 NE 3rd St. (Loop 230)
Smithville, Texas 78957

Performed By: John W. Sagartz, D.V.M.
Veritas Laboratories, Inc.
The Nova Building
1238 Anthony Road
Burlington, NC 27215

Dermigen Protocol Number: 910801

Veritas Project Number: 201-910801

Sub-Protocol 1

Veritas Accession Number: 1705

June 26, 1992

Dermigen Approval


6/29/92

INTRODUCTION

The general objective of this protocol is the establishment of a data base for designing and implementing a comparative study to extend the effects of petroleum middle distillates on markers of tumor promotion in mouse skin to the human situation. Specifically, Sub-protocol 1 was designed as a pilot study using 48 CD-1 mice which were sacrificed at 6, 24, 48, and 96 hours following dermal exposure to acetone (vehicle control) and TPA (positive control) at concentrations of 6.8 and 13.6 nmol/200 microliters. The experimental design is summarized as shown:

<u>STARPATH Group No.</u>	<u>Treatment</u>	<u>Post-Exposure Duration</u>	<u>No. of Mice</u>
1	Acetone	6 hour	4
2	TPA, 6.8 nmol	6 hour	4
3	TPA, 13.6 nmol	6 hour	4
4	Acetone	24 hour	4
5	TPA, 6.8 nmol	24 hour	4
6	TPA, 13.6 nmol	24 hour	4
7	Acetone	48 hour	4
8	TPA, 6.8 nmol	48 hour	4
9	TPA, 13.6 nmol	48 hour	4
10	Acetone	96 hour	4
11	TPA, 6.8 nmol	96 hour	4
12	TPA, 13.6 nmol	96 hour	4
Total No. of Mice			= 48

MATERIALS AND METHODS

The in-life portion of this study was performed at ManTech Environmental Technology, Inc., 2 Triangle Drive, PO Box 12313, Research Triangle Park, NC 27709.

Tissues were collected at necropsy, preserved in formalin and submitted to Veritas Laboratories, Inc., The Nova Building, 1238 Anthony Road, Burlington, NC 27215, for processing and microscopic evaluation.

The following sections were prepared for each mouse:

- 1) Hematoxylin and eosin (H&E). Slides prepared from 6, 24, 48 and 96 hour sacrifices.
- 2) Bromodeoxyuridine (BrDU), primary antibody-mouse monoclonal, IgG, Becton Dickinson, 1:200. Alkaline Phosphotase chromagen. Slides prepared from 24, 48, and 96 hour sacrifices only.

MATERIALS AND METHODS, (cont'd)

- 3) Proliferating Cell Nuclear Antigen (PCNA), primary antibody-19A2, mouse monoclonal, IgM, Coulter Immunology, 1:400. Alkaline Phosphotase chromagen. Slides prepared from 24, 48, and 96 hour sacrifices only.

The parameters evaluated microscopically were:

- 1) H&E Sections:
 - a) Histopathology, following guidelines stated in: (1) Summaries of E.P.A. Workshops on Carcinogenesis Bioassay Via the Dermal Route, 1987 & 1988, (2) U.S. E.P.A. Atlas of Dermal Lesions, Pesticides and Toxic Substances (TS-796), 20T-2004, August 1990.
 - b) Epidermal Hyperplasia: (1) Epidermal Thickness-average of 10 measurements of the thickness (in micrometers) of non-cornified interfollicular epidermis. (2) Nucleated epidermal cells per 100 micrometers of basement membrane average number of keratinocytes within five 100 micrometer linear segments of interfollicular epidermis.
- 2) BrDU Sections: average number of labeled nuclei per 500 epidermal cell nuclei (labeled and unlabeled), expressed as percent labeled cells.
- 3) PCNA Sections: average number of labeled nuclei per 500 epidermal cell nuclei (labelled and unlabeled), expressed as percent labeled cells.

Statistical Analysis, using the Mann-Whitney Test (GRAPH PAD INSTAT Version 1.0) of variation within and between treatment groups was performed on the following parameters: epidermal thickness, number of nucleated keratinocytes per unit of basement membrane, BrDU labeling index, and PCNA labeling index.

Histopathologic examination was performed by John W. Sagartz, D.V.M. Cell counts on BrDU and PCNA sections were performed by John E. Sagartz, D.V.M. Epidermal hyperplasia measurements were made by Christine G. Ard, BS, HT. Histopathologic results are shown in the following documents attached to this report:

- Individual Animal Histopathologic Observations
- Summary of Histopathologic Observations

GLOSSARY OF HISTOPATHOLOGIC TERMS

Acute Inflammation - infiltrates of polymorphonuclear leukocytes admixed with lower relative numbers of mononuclear lymphohistiocytic cells; accompanied by hyperemia and fluid exudate.

Edema - widening of intercellular spaces due to increased tissue fluid.

Epidermal hyperplasia - increased thickness of noncornified epidermis due to increased cell numbers.

Necrosis - cell death characterized by nuclear fragmentation (Karyorrhexis), nuclear shrinkage (Pyknosis), and disappearance of nuclei (Karyolysis).

Orthokeratosis - form of hyperkeratosis in which there is an anuclear increase in the stratum corneum of epidermis.

Parakeratosis - form of hyperkeratosis in which there is retention of nuclei in the stratum corneum.

Ulceration - loss of epidermis with exposure to the dermis.

DISCUSSION

SIX HOUR SACRIFICE (GROUP 1 COMPARED TO GROUPS 2 AND 3)

HISTOPATHOLOGY, H&E

EPIDERMIS

There were no epidermal lesions in Group 1.

Hyperplasia was observed in all mice in Groups 2 and 3. Erosion was noted in one Group 3 mouse. Epidermitis was found in one Group 2 mouse. Orthokeratosis was noted in two Group 2 mice and in all Group 3 mice. Parakeratosis was seen in three Group 2 mice and in three Group 3 mice. Intraepithelial pustules were identified in three Group 2 mice and in three Group 3 mice. One Group 2 mouse had ulceration. Spongiosis/hydropic degeneration was found in one Group 3 mouse. All of the above changes in Groups 2 and 3 are considered to be treatment-related.

DERMIS

One Group 1 mouse had an inflammatory infiltrate.

Inflammation and edema were found in all mice of Groups 2 and 3. Both changes are regarded as treatment-related.

EPIDERMAL THICKNESS

The mean epidermal thicknesses are as shown:

<u>Group No.</u>	<u>Mean Thickness (micrometers)</u>
1	10.90
2	50.50
3	56.25

The increased epidermal thicknesses in Groups 2 and 3 are regarded as treatment-related and correspond to the epidermal hyperplasia noted earlier.

DISCUSSION, SIX HOUR SACRIFICE, (cont'd)

NUCLEATED EPIDERMAL CELLS

The mean numbers of nucleated epidermal cells per 100 micrometers of basement membrane are:

<u>Group No.</u>	<u>Mean No. of Nucleated Epidermal Cells</u>
1	18.60
2	41.65
3	36.80

The increased mean numbers of nucleated epithelial cells are considered to be exposure-related and correspond to the epidermal hyperplasia noted earlier.

DISCUSSION, (cont'd)

TWENTY-FOUR HOUR SACRIFICE (GROUP 4 COMPARED TO GROUPS 5 AND 6)

HISTOPATHOLOGY, H&E

EPIDERMIS

There were no epidermal lesions in Group 4.

Hyperplasia and orthokeratosis were seen in all mice of Groups 5 and 6. Necrosis was noted in one Group 6 mouse. Parakeratosis was observed in one Group 5 mouse and in two Group 6 mice. Intraepithelial pustules were found in two Group 5 mice and in three Group 6 mice. These changes are considered to be treatment-induced.

DERMIS

There were no dermal lesions in Group 4.

Inflammation and edema were noted in all mice of Groups 5 and 6. The incidences of these changes are considered to be treatment-related.

EPIDERMAL THICKNESS

The mean epidermal thicknesses are as follows:

<u>Group No.</u>	<u>Mean Thickness (micrometers)</u>
4	12.20
5	41.80
6	50.90

The increased mean epidermal thicknesses in Groups 5 and 6 are considered to be treatment-related and correspond to the epidermal hyperplasia identified on histopathologic examination.

DISCUSSION, TWENTY-FOUR HOUR SACRIFICE, (cont'd)

NUCLEATED EPIDERMAL CELLS

The mean numbers of nucleated epidermal cells per 100 micrometers of basement membrane are as shown:

<u>Group No.</u>	<u>Mean No. of Nucleated Epidermal Cells</u>
4	19.70
5	37.00
6	31.95

The increased mean numbers of nucleated epidermal cells in Groups 5 and 6 are regarded as treatment-induced and correspond to the epidermal hyperplasia and increased epidermal thicknesses previously reported.

BRDU SECTIONS

The mean percentages of labeled nuclei in Groups 4, 5, and 6 are as follows:

<u>Group No.</u>	<u>Mean % Labeled Nuclei</u>
4	2.7
5	11.75
6	15.6

The increased numbers of BrDU-labeled nuclei in Groups 5 and 6 are regarded as exposure-related and correspond to the increased epidermal hyperplasia, epidermal thicknesses, and numbers of epidermal nuclei observed earlier.

PCNA SECTIONS

The mean percentages of labeled nuclei in Groups 4, 5, and 6 are as shown:

<u>Group No.</u>	<u>Mean % Labeled Nuclei</u>
4	17.6
5	27.55
6	27.95

The increased numbers of PCNA-labeled nuclei in Groups 5 and 6 are considered to be treatment-induced and correspond to the increased incidences of epidermal hyperplasia, epidermal thicknesses, numbers of epidermal nuclei, and numbers of BrDU-labeled nuclei reported above.

DISCUSSION, (cont'd)

FORTY-EIGHT HOUR SACRIFICE (GROUP 7 COMPARED TO GROUPS 8 AND 9)

HISTOPATHOLOGY, H&E

EPIDERMIS

There were no epidermal lesions in Group 7.

Hyperplasia and orthokeratosis were observed in all mice in Groups 8 and 9. Parakeratosis was found in one Group 8 mouse and in one Group 9 mouse. Intraepithelial pustules were seen in one Group 8 mouse and in one Group 9 mouse. Spongiosis/hydronic degeneration was noted in one Group 8 mouse. All of the foregoing changes are considered as treatment-induced.

DERMIS

There were no dermal lesions in Group 7.

Inflammation and edema were seen in all mice of Groups 8 and 9. The frequencies of this change are regarded as exposure-related.

EPIDERMAL THICKNESS

The mean epidermal thicknesses are:

<u>Group No.</u>	<u>Mean Thickness (micrometers)</u>
7	11.20
8	44.30
9	57.40

The increased mean epidermal thicknesses in Groups 8 and 9 are considered to be exposure-induced and correspond to the epidermal hyperplasia recorded on histopathologic examination.

NUCLEATED EPIDERMAL CELLS

The mean numbers of nucleated epidermal cells per 100 micrometers of basement membrane are as follows:

<u>Group No.</u>	<u>Mean No. of Nucleated Epidermal Cells</u>
7	17.70
8	38.30
9	40.10

DISCUSSION, FORTY-EIGHT HOUR SACRIFICE, (cont'd)

NUCLEATED EPIDERMAL CELLS, (cont'd)

The increased mean numbers of nucleated epidermal cells in Groups 8 and 9 are regarded as treatment-related and correspond to the epidermal hyperplasia and increased epidermal thicknesses noted earlier.

BRDU SECTIONS

The mean percentages of labeled nuclei in Groups 7, 8, and 9 are:

<u>Group No.</u>	<u>Mean % Labeled Nuclei</u>
7	3.15
8	7.75
9	9.6

The increased numbers of BrDU-labeled nuclei in Groups 8 and 9 are considered to be the results of exposure and correspond to the increased epidermal hyperplasia, epidermal thicknesses, and numbers of epidermal nuclei noted previously.

PCNA SECTIONS

The mean percentages of labeled nuclei in Groups 7, 8, and 9 are as shown:

<u>Group No.</u>	<u>Mean % Labeled Nuclei</u>
7	12.75
8	24.65
9	29.5

The increased numbers of PCNA-labeled nuclei in Groups 8 and 9 are regarded as exposure-induced and correspond to the increased epidermal hyperplasia, epidermal thicknesses, numbers of epidermal nuclei, and numbers of BrDU-labelled nuclei recorded previously.

DISCUSSION, (cont'd)

NINETY-SIX HOUR SACRIFICE (GROUPS 10, 11, AND 12)

HISTOPATHOLOGY, H&E

EPIDERMIS

There were no epidermal lesions in Group 10.

Hyperplasia and orthokeratosis were found in all mice in Groups 11 and 12. Both of these changes are considered to be treatment-related.

DERMIS

There were no dermal lesions in Group 10.

Inflammation was seen in all mice of Groups 11 and 12. Edema was reported in three mice of Group 11 and in all mice of Group 12. The increased incidences of this change are regarded as exposure effects.

EPIDERMAL THICKNESS

Mean epidermal thicknesses are as follows:

<u>Group No.</u>	<u>Mean Thickness (micrometers)</u>
10	13.00
11	32.30
12	35.60

The increased mean epidermal thicknesses in Groups 11 and 12 are regarded as exposure-related and correspond to the epidermal hyperplasia noted on histopathologic examination.

NUCLEATED EPIDERMAL CELLS

The mean numbers of nucleated epidermal cells per 100 micrometers of basement membrane are:

<u>Group No.</u>	<u>Mean No. of Nucleated Epidermal Cells</u>
10	20.40
11	30.70
12	27.90

DISCUSSION, NINETY-SIX HOUR SACRIFICE, (cont'd)

NUCLEATED EPIDERMAL CELLS, (cont'd)

The increased mean numbers of nucleated epidermal cells in Groups 11 and 12 are considered to be treatment-induced and correspond to the epidermal hyperplasia and increased epidermal thicknesses reported above.

BRDU SECTIONS

The mean percentages of labeled nuclei in Groups 10, 11, and 12 are as shown:

<u>Group No.</u>	<u>Mean % Labeled Nuclei</u>
10	2.05
11	6.15
12	7.05

The increased numbers of BrDU-labelled nuclei in Groups 11 and 12 are regarded as treatment effects and correspond to the increased epidermal hyperplasia, epidermal thicknesses, and numbers of epidermal nuclei identified earlier.

PCNA SECTIONS

The mean percentages of labeled nuclei in Groups 10, 11, and 12 are:

<u>Group No.</u>	<u>Mean % Labeled Nuclei</u>
10	15.25
11	26.30
12	31.15

The increased numbers of PCNA-labeled nuclei in Groups 11 and 12 are considered to be exposure-induced and correspond to the increased epidermal hyperplasia, epidermal thicknesses, numbers of epidermal nuclei, and numbers of BrDU-labelled nuclei reported earlier.

CONCLUSIONS

Under the conditions of this experiment the following treatment-related changes were observed:

EPIDERMIS

Epidermal hyperplasia - 6, 24, 48 and 96 hour sacrifices, both TPA groups.

Necrosis - 24 hour sacrifice only, TPA, 13.6nmol group only.

Epidermitis - 6 hour sacrifice, TPA, 6.8nmol.

Orthokeratosis - 6, 24, 48, and 96 hour sacrifices both TPA groups.

Parakeratosis - 6, 24, and 48 hour sacrifices, both TPA groups.

Intraepithelial Pustules - 6, 24, and 48 hour sacrifices, both TPA groups.

Spongiosis/hydropic degeneration - 6 hour sacrifice only, TPA, 13.6nmol group.

Ulceration - 6 hour sacrifice only, TPA, 6.8nmol group only.

Increased epidermal thickness - 6, 24, 48, and 96 hour sacrifices, both TPA groups.

Increased numbers of nucleated epidermal cells per unit length - 6, 24, 48, and 96 hour sacrifices, both TPA groups.

Increased labeling indices by incorporations of Bromodeoxyuridine (BrDU) - 24, 48, and 96 hour sacrifices, both TPA groups.

Increased proliferation indices using the proliferating cell nuclear antigen (PCNA) - 24, 48, and 96 hour sacrifices, both TPA groups.

DERMIS

Inflammation & Edema - 6, 24, 48, and 96 hour sacrifices, both TPA groups.

SUMMARY COMMENT

The results of this study indicate that the epidermal hyperplasia identified histopathologically was accompanied by corresponding increases in: (1) epidermal thickness, (2) numbers of nucleated epidermal cell per unit length, (3) percentages of BrDU-labeled epidermal cell nuclei, and (4) percentages of PCNA-labeled epidermal cell nuclei. It is important to note that these interrelated changes were apparent at 24 hours post-exposure (BrDU and PCNA studies were not performed at the six hours sacrifice). It appears therefore, that the essential information on the status of the epidermis can be obtained as early as 24 hours post-exposure.

Evaluated by:


John W. Sagartz, D.V.M.,
Diplomate, A.C.V.P.

JWS:rl

SUMMARY OF HISTOPATHOLOGIC
OBSERVATIONS, MOUSE SKIN

SUMMARY OF HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

GROUP MEAN SUMMARIES

Group ID Acetone Species: Mouse
 Duration (Hours) 6 (Gr.1) 24 (Gr.4) 48 (Gr.7) 96 (Gr.10)

EPIDERMIS

Inflammatory/Degenerative Changes (means)

1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>

Inflammatory/ Degenerative Score	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
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Proliferative Changes

7. Orthokeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
8. Parakeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
9. Hyperplasia	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
10. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
11. Epidermal thickness (um)*	<u>10.93</u>	<u>12.18</u>	<u>11.18</u>	<u>13.00</u>
12. Epi. Cells per 100 um **	<u>18.60</u>	<u>19.70</u>	<u>17.65</u>	<u>20.40</u>
Proliferative Score	<u>29.53</u>	<u>31.88</u>	<u>28.83</u>	<u>33.40</u>

DERMIS

14. Inflammatory Infiltrates	<u>0.25</u>	<u>0</u>	<u>0</u>	<u>0.25</u>
15. Edema	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
16. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
Dermal Score	<u>0.25</u>	<u>0</u>	<u>0</u>	<u>0.25</u>

SCORE 0 = Regular/Normal, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe.

Epidermal thickness is average of ten micrometer-based measurements per specimen.

* Epidermal Cell per 100u is average of five micrometer-based measurements per specimen.

SUMMARY OF HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

GROUP MEAN SUMMARIES

Group ID TPA 6.8	Species: <u>Mouse</u>		
Duration (Hours)	<u>6 (Gr.2)</u>	<u>24 (Gr.5)</u>	<u>48 (Gr.8)</u> <u>96 (Gr.11)</u>

EPIDERMIS

Inflammatory/Degenerative Changes (means)

. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
1. Ulceration	<u>0.50</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0.50</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0.25</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>1.50</u>	<u>0.75</u>	<u>0.25</u>	<u>0</u>

Inflammatory/ Degenerative Score	<u>2.50</u>	<u>0.75</u>	<u>0.50</u>	<u>0</u>
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Proliferative Changes

7. Orthokeratosis	<u>0.75</u>	<u>1.50</u>	<u>1.50</u>	<u>1.25</u>
8. Parakeratosis	<u>0.75</u>	<u>0.25</u>	<u>0.25</u>	<u>0</u>
. Hyperplasia	<u>2.00</u>	<u>2.00</u>	<u>1.75</u>	<u>1.50</u>
10. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
11. Epidermal thickness (um)*	<u>50.50</u>	<u>41.81</u>	<u>44.25</u>	<u>32.25</u>
12. Epi. Cells per 100 um **	<u>41.65</u>	<u>37.00</u>	<u>38.30</u>	<u>30.65</u>
Proliferative Score	<u>95.65</u>	<u>82.55</u>	<u>86.05</u>	<u>65.65</u>

DERMIS

14. Inflammatory Infiltrates	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>1.50</u>
15. Edema	<u>1.75</u>	<u>1.00</u>	<u>1.25</u>	<u>0.75</u>
16. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
Dermal Score	<u>3.75</u>	<u>3.00</u>	<u>3.25</u>	<u>2.25</u>

SCORE 0 = Regular/Normal, 1 = Minimal, 2 - Mild, 3 = Moderate, 4 = Marked, 5 = Severe.

Epidermal thickness is average of ten micrometer-based measurements per specimen.

* Epidermal Cell per 100u is average of five micrometer-based measurements per specimen.

SUMMARY OF HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

GROUP MEAN SUMMARIES

Group ID TPA 13.6 Species: Mouse
 Duration (Hours) 6 (Gr.3) 24 (Gr.6) 48 (Gr.9) 96 (Gr.12)

EPIDERMIS

Inflammatory/Degenerative Changes (means)

1. Erosion	<u>0.25</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0.50</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0.50</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>0.63</u>	<u>1.50</u>	<u>0.50</u>	<u>0</u>
Inflammatory/ Degenerative Score	<u>1.38</u>	<u>2.00</u>	<u>0.50</u>	<u>0</u>

Proliferative Changes

7. Orthokeratosis	<u>0.63</u>	<u>2.00</u>	<u>1.75</u>	<u>1.25</u>
8. Parakeratosis	<u>0.75</u>	<u>0.75</u>	<u>0.25</u>	<u>0</u>
9. Hyperplasia	<u>2.00</u>	<u>2.00</u>	<u>2.25</u>	<u>1.75</u>
10. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
1. Epidermal thickness (um)*	<u>56.25</u>	<u>50.87</u>	<u>57.37</u>	<u>35.56</u>
2. Epi. Cells per 100 um **	<u>36.80</u>	<u>31.95</u>	<u>40.10</u>	<u>27.90</u>
Proliferative Score	<u>96.43</u>	<u>87.57</u>	<u>101.72</u>	<u>66.46</u>

DERMIS

14. Inflammatory Infiltrates	<u>2.00</u>	<u>2.00</u>	<u>2.50</u>	<u>2.25</u>
15. Edema	<u>2.00</u>	<u>1.25</u>	<u>1.50</u>	<u>1.50</u>
16. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
Dermal Score	<u>4.00</u>	<u>3.25</u>	<u>4.00</u>	<u>3.75</u>

SCORE 0 = Regular/Normal, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe.

* Epidermal thickness is average of ten micrometer-based measurements per specimen.

* Epidermal Cell per 100u is average of five micrometer-based measurements per specimen.

INDIVIDUAL ANIMAL HISTOPATHOLOGIC
OBSERVATIONS, MOUSE SKIN

INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

Group ID <u>1, Acetone</u> Duration (Hours) <u>6</u>	Species: <u>Mouse</u>				<u>Mean Grade</u> <u>Group</u>
	<u>Animal Numbers</u>				
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
10. Parakeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
11. Hyperplasia	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>11.75</u>	<u>10.75</u>	<u>12.25</u>	<u>9.00</u>	<u>10.94</u>
14. Epi. Cells Per 100 um **	<u>20.80</u>	<u>19.40</u>	<u>16.80</u>	<u>17.40</u>	<u>18.60</u>
15.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
16.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>0</u>	<u>1</u>	<u>0</u>	<u>0</u>	<u>0.25</u>
18. Edema	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
21.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

CORE 0 = Regular/Normal, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe.

Epidermal thickness is average of ten micrometer-based measurements per specimen.

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INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

Group ID <u>2</u> , TPA <u>6.8</u> Duration (Hours) <u>6</u>	Species: <u>Mouse</u>				Mean Grade
	<u>Animal Numbers</u>				
	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>Group</u>
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>2.00</u>	<u>0.50</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>2.00</u>	<u>0</u>	<u>0</u>	<u>0.50</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>2.00</u>	<u>2.00</u>	<u>0</u>	<u>2.00</u>	<u>1.50</u>
8.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>1.00</u>	<u>0</u>	<u>0</u>	<u>2.00</u>	<u>0.75</u>
10. Parakeratosis	<u>1.00</u>	<u>1.00</u>	<u>0</u>	<u>1.00</u>	<u>0.75</u>
11. Hyperplasia	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>59.25</u>	<u>56.75</u>	<u>35.00</u>	<u>51.00</u>	<u>50.50</u>
14. Epi. Cells Per 100 um **	<u>49.00</u>	<u>43.60</u>	<u>36.40</u>	<u>37.60</u>	<u>41.65</u>
15.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
16.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
18. Edema	<u>2.00</u>	<u>2.00</u>	<u>1.00</u>	<u>2.00</u>	<u>1.75</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
21.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

SCORE 0 = Regular/Normal, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe.

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INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

Group ID <u>3</u> , TPA <u>13.6</u> Duration (Hours) <u>6</u>	Species: <u>Mouse</u>				Mean Grade
	Animal Numbers				
	<u>33</u>	<u>34</u>	<u>35</u>	<u>36</u>	<u>Group</u>
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>1</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0.25</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>2.00</u>	<u>0</u>	<u>0</u>	<u>0.50</u>
7. Pustule, Intraepithelial	<u>2.00</u>	<u>0</u>	<u>2.00</u>	<u>1.00</u>	<u>1.25</u>
3.					
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>1.00</u>	<u>1.00</u>	<u>2.00</u>	<u>1.00</u>	<u>1.25</u>
10. Parakeratosis	<u>1.00</u>	<u>1.00</u>	<u>1.00</u>	<u>0</u>	<u>0.75</u>
11. Hyperplasia	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>58.25</u>	<u>66.25</u>	<u>55.25</u>	<u>45.25</u>	<u>56.25</u>
14. Epi. Cells Per 100 um **	<u>38.8</u>	<u>37</u>	<u>38.60</u>	<u>32.80</u>	<u>36.80</u>
15.					
16.					
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
18. Edema	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.					
21.					

SCORE 0 = Regular/Normal, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe.

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INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

Group ID <u>4, Acetone</u> Duration (Hours) <u>24</u>	Species: <u>Mouse</u>				Mean Grade
	<u>Animal Numbers</u>				
	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>Group</u>
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
10. Parakeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
11. Hyperplasia	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>12.75</u>	<u>11.75</u>	<u>12.00</u>	<u>12.25</u>	<u>12.19</u>
14. Epi. Cells Per 100 um **	<u>21.80</u>	<u>19.00</u>	<u>21.00</u>	<u>17.00</u>	<u>19.70</u>
15.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
16.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
18. Edema	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
21.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

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INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

	Group ID <u>5</u> , TPA <u>6.8</u> Species: <u>Mouse</u>				<u>Mean Grade</u>
	Duration (Hours) <u>24</u> <u>Animal Numbers</u>				
	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>	<u>Group</u>
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>2.00</u>	<u>1.00</u>	<u>0</u>	<u>0</u>	<u>0.75</u>
8.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>2.00</u>	<u>2.00</u>	<u>1.00</u>	<u>1.00</u>	<u>1.50</u>
10. Parakeratosis	<u>1.00</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0.25</u>
11. Hyperplasia	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>60.25</u>	<u>36.50</u>	<u>41.00</u>	<u>29.50</u>	<u>41.81</u>
14. Epi. Cells Per 100 um **	<u>45.20</u>	<u>36.00</u>	<u>39.00</u>	<u>27.80</u>	<u>37.00</u>
15.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
16.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
18. Edema	<u>1.00</u>	<u>1.00</u>	<u>1.00</u>	<u>1.00</u>	<u>1.00</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
21.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

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INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

Group ID 6, TPA 13.6 Duration (Hours) <u>24</u>	Species: <u>Mouse</u>				Mean Grade
	<u>Animal Numbers</u>				
	<u>37</u>	<u>38</u>	<u>39</u>	<u>40</u>	<u>Group</u>
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>2.00</u>	<u>0</u>	<u>0</u>	<u>0.50</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>0</u>	<u>1.50</u>
8.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
10. Parakeratosis	<u>0</u>	<u>2.00</u>	<u>1.00</u>	<u>0</u>	<u>0.75</u>
11. Hyperplasia	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>42.50</u>	<u>51.50</u>	<u>60.75</u>	<u>48.75</u>	<u>50.88</u>
14. Epi. Cells Per 100 um **	<u>35.80</u>	<u>29.40</u>	<u>32.60</u>	<u>30.00</u>	<u>31.95</u>
15.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
16.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
18. Edema	<u>1.00</u>	<u>2.00</u>	<u>1.00</u>	<u>1.00</u>	<u>1.25</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
21.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

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INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

Group ID 7, Acetone Duration (Hours) 48	Species: <u>Mouse</u>				Mean Grade
	<u>Animal Numbers</u>				
	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>Group</u>
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
8.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
10. Parakeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
11. Hyperplasia	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>10.75</u>	<u>10.50</u>	<u>11.25</u>	<u>12.25</u>	<u>11.19</u>
14. Epi. Cells Per 100 um **	<u>17.40</u>	<u>18.20</u>	<u>17.40</u>	<u>17.60</u>	<u>17.65</u>
15.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
16.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
18. Edema	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
21.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

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INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

Group ID 8, TPA 6.8 Duration (Hours) <u>48</u>	Species: <u>Mouse</u>				Mean Grade
	<u>Animal Numbers</u>				
	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>Group</u>
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>1.00</u>	<u>0</u>	<u>0</u>	<u>0.25</u>
7. Pustule, Intraepithelial	<u>0</u>	<u>1.00</u>	<u>0</u>	<u>0</u>	<u>0.25</u>
8.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>1.00</u>	<u>2.00</u>	<u>1.00</u>	<u>2.00</u>	<u>1.50</u>
10. Parakeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>1.00</u>	<u>0.25</u>
11. Hyperplasia	<u>2.00</u>	<u>2.00</u>	<u>1.00</u>	<u>2.00</u>	<u>1.75</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>39.75</u>	<u>49.00</u>	<u>40.50</u>	<u>47.75</u>	<u>44.25</u>
14. Epi. Cells Per 100 um **	<u>35.20</u>	<u>40.00</u>	<u>37.20</u>	<u>40.80</u>	<u>38.30</u>
15.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
16.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>
18. Edema	<u>1.00</u>	<u>2.00</u>	<u>1.00</u>	<u>1.00</u>	<u>1.25</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
21.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

SCORE 0 = Regular/Normal, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe.

Epidermal thickness is average of ten micrometer-based measurements per specimen.

**Epidermal Cells per 100u is average of five micrometer-based measurements per specimen.

INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
MOUSE SKIN
STUDY 910801-1

Group ID 9, TPA 13.6 Duration (Hours) 48	Species: <u>Mouse</u>				Mean Grade
	<u>Animal Numbers</u>				
	<u>41</u>	<u>42</u>	<u>43</u>	<u>44</u>	<u>Group</u>
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>0</u>	<u>0</u>	<u>0</u>	<u>2.00</u>	<u>0.50</u>
3.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>1.00</u>	<u>2.00</u>	<u>1.00</u>	<u>3.00</u>	<u>1.75</u>
10. Parakeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>1.00</u>	<u>0.25</u>
11. Hyperplasia	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>3.00</u>	<u>2.25</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>49.00</u>	<u>61.00</u>	<u>52.00</u>	<u>67.50</u>	<u>57.38</u>
14. Epi. Cells Per 100 um **	<u>39.60</u>	<u>35.40</u>	<u>41.80</u>	<u>43.60</u>	<u>40.10</u>
15.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
16.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>2.00</u>	<u>3.00</u>	<u>2.00</u>	<u>3.00</u>	<u>2.50</u>
18. Edema	<u>2.00</u>	<u>1.00</u>	<u>1.00</u>	<u>2.00</u>	<u>1.50</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
21.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

SCORE 0 = Regular/Normal, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe.

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**Epidermal Cells per 100u is average of five micrometer-based measurements per specimen.

INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

Group ID <u>10, Acetone</u> Duration (Hours) <u>96</u>	Species: <u>Mouse</u>				Mean Grade
	Animal Numbers				
	<u>13</u>	<u>14</u>	<u>15</u>	<u>16</u>	<u>Group</u>
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3.					
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
10. Parakeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
11. Hyperplasia	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>15.75</u>	<u>11.75</u>	<u>11.00</u>	<u>13.50</u>	<u>13.00</u>
14. Epi. Cells Per 100 um **	<u>20.00</u>	<u>18.40</u>	<u>18.00</u>	<u>25.20</u>	<u>20.40</u>
15.					
16.					
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
18. Edema	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.					
21.					

SCORE 0 = Regular/Normal, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe.

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**Epidermal Cells per 100u is average of five micrometer-based measurements per specimen.

INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
MOUSE SKIN
STUDY 910801-1

Group ID 11, TPA 6.8 Species: Mouse Mean
Grade
Duration (Hours) 96 Animal Numbers

29 30 31 32 Group

EPIDERMIS

Inflammatory/Degenerative Changes

1. Erosion	0	0	0	0	0
2. Ulceration	0	0	0	0	0
3. Crust Formation	0	0	0	0	0
4. Epidermitis	0	0	0	0	0
5. Necrosis/Acantholysis	0	0	0	0	0
6. Spongiosis/Hydropic Degen.	0	0	0	0	0
7. Pustule, Intraepithelial	0	0	0	0	0
3.					

Proliferative Changes

9. Orthokeratosis	1.00	1.00	1.00	2.00	1.25
10. Parakeratosis	0	0	0	0	0
11. Hyperplasia	1.00	1.00	2.00	2.00	1.50
12. Dyskeratosis	0	0	0	0	0
13. Epidermal Thickness (um)*	20	28.50	50.25	30.25	32.25
14. Epi. Cells Per 100 um **	23.20	30.80	37.60	31.00	30.65
15.					
16.					

DERMIS

17. Inflammatory Infiltrates	1.00	1.00	2.00	2.00	1.50
18. Edema	1.00	0	1.00	1.00	0.75
19. Vasodilatation	0	0	0	0	0
20.					
21.					

SCORE 0 = Regular/Normal, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked,
5 = Severe.

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INDIVIDUAL ANIMAL HISTOPATHOLOGIC OBSERVATIONS
 MOUSE SKIN
 STUDY 910801-1

Group ID 12, TPA 13.6 Duration (Hours) 96	Species: <u>Mouse</u>				Mean Grade
	<u>Animal Numbers</u>				
	<u>45</u>	<u>46</u>	<u>47</u>	<u>48</u>	<u>Group</u>
<u>EPIDERMIS</u>					
<u>Inflammatory/Degenerative Changes</u>					
1. Erosion	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
2. Ulceration	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
3. Crust Formation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
4. Epidermitis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
5. Necrosis/Acantholysis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
6. Spongiosis/Hydropic Degen.	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
7. Pustule, Intraepithelial	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
8.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>Proliferative Changes</u>					
9. Orthokeratosis	<u>1.00</u>	<u>1.00</u>	<u>1.00</u>	<u>2.00</u>	<u>1.25</u>
10. Parakeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
11. Hyperplasia	<u>2.00</u>	<u>1.00</u>	<u>2.00</u>	<u>2.00</u>	<u>1.75</u>
12. Dyskeratosis	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
13. Epidermal Thickness (um)*	<u>31.00</u>	<u>29.25</u>	<u>36.50</u>	<u>45.50</u>	<u>35.56</u>
14. Epi. Cells Per 100 um **	<u>32.00</u>	<u>26.60</u>	<u>25.40</u>	<u>27.60</u>	<u>27.90</u>
15.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
16.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
<u>DERMIS</u>					
17. Inflammatory Infiltrates	<u>2.00</u>	<u>2.00</u>	<u>2.00</u>	<u>3.00</u>	<u>2.25</u>
18. Edema	<u>2.00</u>	<u>1.00</u>	<u>1.00</u>	<u>2.00</u>	<u>1.50</u>
19. Vasodilatation	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>0</u>
20.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>
21.	<u> </u>	<u> </u>	<u> </u>	<u> </u>	<u> </u>

CORE 0 = Regular/Normal, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked, 5 = Severe.

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NUCLEATED CELLS

NUCLEATED CELLS/100 MICRONS-6 HOUR POST-EXPOSURE DURATION

05-14-1992

Column	An#	N	MEAN	SD	SEM	95% Confidence Interval	
A	01	5	20.8	2.588438	1.157585	17.5865	to 24.01345
B	02	5	19.4	2.190887	.9797946	16.6800	to 22.1199
C	03	5	16.8	2.77489	1.240968	13.3550	to 20.24493
D	04	5	17.4	.8944204	.3999969	16.2896	to 18.51039
E	17	5	49	2.915476	1.30384	45.3805	to 52.61946
F	18	5	43.6	6.465296	2.891368	35.5735	to 51.62644
G	19	5	36.4	1.140197	.5099115	34.9844	to 37.81552
H	20	5	37.6	2.792857	1.249003	34.1327	to 41.06723
I	33	5	38.8	4.60434	2.059124	33.0838	to 44.51613
J	34	5	37	5.09902	2.28035	30.6697	to 43.33025
K	35	5	38.6	4.037332	1.80555	33.5877	to 43.6122
L	36	5	32.8	4.65832	2.083264	27.0168	to 38.58314

NUCLEATED CELLS/100 MICRONS-24 HOUR POST-EXPOSURE DURATION

05-14-1992

Column	An#	N	MEAN	SD	SEM	95% Confidence Interval	
A	05	5	21.8	2.588438	1.157585	18.5865	to 25.01345
B	06	5	19	3.872983	1.73205	14.1918	to 23.80817
C	07	5	21	2.12132	.9486833	18.3664	to 23.63354
D	08	5	17	1.870829	.83666	14.6774	to 19.32257
E	21	5	45.2	3.346633	1.49666	41.0452	to 49.35473
F	22	5	36	2.915476	1.30384	32.3805	to 39.61946
G	23	5	39	5.049753	2.258318	32.7309	to 45.26909
H	24	5	27.8	5.674506	2.537716	20.7553	to 34.8447
I	37	5	35.8	2.774879	1.240963	32.3550	to 39.24492
J	38	5	29.4	1.816604	.8124098	27.1447	to 31.65525
K	39	5	32.6	4.037332	1.80555	27.5877	to 37.6122
L	40	5	30	1.581139	.7071068	28.0370	to 31.96293

NUCLEATED CELLS/100 MICRONS-48 HOUR POST-EXPOSURE DURATION

05-14-1992

Column	An.#	N	MEAN	SD	SEM	95% Confidence Interval	
A	09	5	17.4	1.516571	.6782312	15.5172	to 19.28277
B	10	5	18.2	2.949578	1.319091	14.5382	to 21.8618
C	11	5	17.4	2.30217	1.029562	14.5419	to 20.25806
D	12	5	17.6	2.073641	.9273606	15.0256	to 20.17435
E	25	5	35.2	5.449766	2.437209	28.4343	to 41.96569
F	26	5	40	3.391165	1.516575	35.7899	to 44.21001
G	27	5	37.2	3.033142	1.356462	33.4344	to 40.96554
H	28	5	40.8	4.816633	2.154064	34.8203	to 46.77968
I	41	5	39.6	4.827013	2.158706	33.6074	to 45.59257
J	42	5	35.4	3.43512	1.536232	31.1354	to 39.66458
K	43	5	41.8	2.588426	1.157579	38.5865	to 45.01344
L	44	5	43.6	2.408329	1.077037	40.6101	to 46.58986

NUCLEATED CELLS/100 MICRONS-96 HOUR POST-EXPOSURE DURATION

05-14-1992

Column	An.#	N	MEAN	SD	SEM	95% Confidence Interval	
A	13	5	20	1	.4472136	18.7585	to 21.24146
B	14	5	18.4	2.408316	1.077032	15.4101	to 21.38984
C	15	5	18	1.581139	.7071068	16.0370	to 19.96293
D	16	5	25.2	2.489982	1.113554	22.1087	to 28.29123
E	29	5	23.2	3.271087	1.462875	19.1390	to 27.26094
F	30	5	30.8	.8366309	.3741527	29.7613	to 31.83865
G	31	5	37.6	2.302184	1.029568	34.7419	to 40.45808
H	32	5	31	3.082207	1.378405	27.1735	to 34.82645
I	45	5	32	2.54951	1.140175	28.8348	to 35.16513
J	46	5	26.6	1.816587	.8124023	24.3447	to 28.85523
K	47	5	25.4	2.30217	1.029562	22.5419	to 28.25806
L	48	5	27.6	1.816587	.8124023	25.3447	to 29.85523

NUCLEATED CELLS POST EXPOSURE 6 HOUR GROUP 1 TO GROUP 2
05-14-1992

Parameter:	ACETONE	TPA 6.8
Mean:	18.6	41.65
# of points:	4	4
std deviation:	1.84027	5.82492
Std. error:	.9201354	2.91246
Minimum:	16.8	36.4
Maximum:	20.8	49
Median:	18.4	40.6

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

NUCLEATED CELLS POST EXPOSURE 6 HOUR GROUP 1 TO GROUP 3
05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	18.6	36.8
# of points:	4	4
std deviation:	1.84027	2.785694
Std. error:	.9201354	1.392847
Minimum:	16.8	32.8
Maximum:	20.8	38.8
Median:	18.4	37.8

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

NUCLEATED CELLS POST EXPOSURE 6 HOUR GROUP 2 TO GROUP 3
05-14-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	41.65	36.8
# of points:	4	4
std deviation:	5.82492	2.785694
Std. error:	2.91246	1.392847
Minimum:	36.4	32.8
Maximum:	49	38.8
Median:	40.6	37.8

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 5.0
Sum of ranks in smaller group (T) = 15.0

The two-tailed P value is 0.4857 - - 'not significant'.

NUCLEATED CELLS POST EXPOSURE 24 HOUR GROUP 4 TO GROUP 5
05-14-1992

Parameter:	ACETONE	TPA 6.8
Mean:	19.7	37
# of points:	4	4
Std deviation:	2.15096	7.231421
Std. error:	1.07548	3.61571
Minimum:	17	27.8
Maximum:	21.8	45.2
Median:	20	37.5

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

NUCLEATED CELLS POST EXPOSURE 24 HOUR GROUP 4 TO GROUP 6
05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	19.7	31.95
# of points:	4	4
Std deviation:	2.15096	2.918308
Std. error:	1.07548	1.459154
Minimum:	17	29.4
Maximum:	21.8	35.8
Median:	20	31.3

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

NUCLEATED CELLS POST EXPOSURE 24 HOUR GROUP 5 TO GROUP 6
05-14-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	37	31.95
# of points:	4	4
Std deviation:	7.231421	2.918308
Std. error:	3.61571	1.459154
Minimum:	27.8	29.4
Maximum:	45.2	35.8
Median:	37.5	31.3

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 4.0
Sum of ranks in smaller group (T) = 14.0

The two-tailed P value is 0.3429 - - 'not significant'.

NUCLEATED CELLS POST EXPOSURE 48 HOUR GROUP 7 TO GROUP 8
05-14-1992

Parameter:	ACETONE	TPA 6.8
Mean:	17.65	38.3
# of points:	4	4
Std deviation:	.37851	2.579466
Std. error:	.189255	1.289733
Minimum:	17.4	35.2
Maximum:	18.2	40.8
Median:	17.5	38.6

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

NUCLEATED CELLS POST EXPOSURE 48 HOUR GROUP 7 TO GROUP 9
05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	17.65	40.1
# of points:	4	4
Std deviation:	.37851	3.534567
Std. error:	.189255	1.767284
Minimum:	17.4	35.4
Maximum:	18.2	43.6
Median:	17.5	40.7

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

NUCLEATED CELLS POST EXPOSURE 48 HOUR GROUP 8 TO GROUP 9
05-14-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	38.3	40.1
# of points:	4	4
Std deviation:	2.579466	3.534567
Std. error:	1.289733	1.767284
Minimum:	35.2	35.4
Maximum:	40.8	43.6
Median:	38.6	40.7

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 5.0
Sum of ranks in smaller group (T) = 15.0

The two-tailed P value is 0.4857 - - 'not significant'.

NUCLEATED CELLS POST EXPOSURE 96 HOUR GROUP 10 TO GROUP 11
05-14-1992

Parameter:	ACETONE	TPA 6.8
Mean:	20.4	30.65
# of points:	4	4
Std deviation:	3.314606	5.88643
Std. error:	1.657303	2.943215
Minimum:	18	23.2
Maximum:	25.2	37.6
Median:	19.2	30.9

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 1.0
Sum of ranks in smaller group (T) = 11.0

The two-tailed P value is 0.0571 - - 'marginally significant'.

NUCLEATED CELLS POST EXPOSURE 96 HOUR GROUP 10 TO GROUP 12
05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	20.4	27.9
# of points:	4	4
Std deviation:	3.314606	2.877504
Std. error:	1.657303	1.438752
Minimum:	18	25.4
Maximum:	25.2	32
Median:	19.2	27.1

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

NUCLEATED CELLS POST EXPOSURE 96 HOUR GROUP 11 TO GROUP 12
05-14-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	30.65	27.9
# of points:	4	4
Std deviation:	5.88643	2.877504
Std. error:	2.943215	1.438752
Minimum:	23.2	25.4
Maximum:	37.6	32
Median:	30.9	27.1

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 6.0
Sum of ranks in smaller group (T) = 16.0

The two-tailed P value is 0.6857 - - 'not significant'.

ALL TIME POINTS (6, 24, 48, & 96 hour) COMBINED

NUCLEATED CELLS ACETONE COMPARED TO TPA 13.6
05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	19.0875	33.875
# of points:	16	16
Std deviation:	2.236629	5.931554
Std. error:	.5591571	1.482889
Minimum:	16.8	24.4
Maximum:	25.2	43.6
Median:	18.3	34.1

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 1.0
Sum of ranks in smaller group (T) = 137.0

The two-tailed P value is <0.0001 - - 'extremely significant'.
(The P value is an estimate based on a normal approximation.)

NUCLEATED CELLS ACETONE COMPARED TO TPA 6.8
05-14-1992

Parameter:	ACETONE	TPA 6.8
Mean:	19.0875	36.9
# of points:	16	16
Std deviation:	2.236629	6.516436
Std. error:	.5591571	1.629109
Minimum:	16.8	23.2
Maximum:	25.2	49
Median:	18.3	37.4

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 1.0
Sum of ranks in smaller group (T) = 137.0

The two-tailed P value is <0.0001 - - 'extremely significant'.
(The P value is an estimate based on a normal approximation.)

NUCLEATED CELLS TPA 13.6 COMPARED TO TPA 6.8
05-14-1992

Parameter:	TPA 13.6	TPA 6.8
Mean:	33.875	36.9
# of points:	16	16
Std deviation:	5.931554	6.516436
Std. error:	1.482889	1.629109
Minimum:	24.4	23.2
Maximum:	43.6	49
Median:	34.1	37.4

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 92.5
Sum of ranks in smaller group (T) = 228.5

The two-tailed P value is 0.1873 - - 'not significant'.

EPIDERMAL THICKNESS

EPIDERMAL THICKNESS-6 HOUR POST-EXPOSURE DURATION

05-14-1992

Column	An#	N	MEAN	SD	SEM	95% Confidence Interval	
A	01	10	11.75	1.207615	.3818813	10.8861	to 12.61382
B	02	10	10.75	1.207615	.3818813	9.88618	to 11.61382
C	03	10	12.25	1.844662	.5833333	10.9305	to 13.5695
D	04	10	9	1.290994	.4082483	8.07654	to 9.923458
E	17	10	59.25	6.015028	1.902119	54.9474	to 63.55259
F	18	10	56.75	5.143766	1.626602	53.0706	to 60.42937
G	19	10	35	3.90868	1.236033	32.2040	to 37.7959
H	20	10	51	3.574602	1.130388	48.4430	to 53.55694
I	33	10	58.25	2.898755	.9166667	56.1765	to 60.3235
J	34	10	66.25	8.51877	2.693872	60.1564	to 72.34354
K	35	10	55.25	7.115125	2.25	50.1605	to 60.3395
L	36	10	45.25	4.632314	1.464866	41.9364	to 48.56353

EPIDERMAL THICKNESS-24 HOUR POST-EXPOSURE DURATION

05-14-1992

Column	An#	N	MEAN	SD	SEM	95% Confidence Interval	
A	05	10	12.75	2.486072	.786165	10.9716	to 14.5283
B	06	10	11.75	1.687371	.5335937	10.5430	to 12.95699
C	07	10	12	1.581139	.5	10.869	to 13.131
D	08	10	12.25	1.419116	.4487637	11.2349	to 13.2651
E	21	10	60.25	13.35675	4.223775	50.6958	to 69.80418
F	22	10	36.5	6.687468	2.114763	31.7164	to 41.2836
G	23	10	41	6.03232	1.907587	36.6850	to 45.31496
H	24	10	29.5	3.689324	1.166667	26.861	to 32.139
I	37	10	42.5	6.871843	2.173068	37.5845	to 47.41548
J	38	10	51.5	8.266398	2.614065	45.5869	to 57.41301
K	39	10	60.75	8.823108	2.790112	54.4387	to 67.06123
L	40	10	48.75	5.682576	1.796988	44.6852	to 52.81479

EPIDERMAL THICKNESS-48 HOUR POST-EXPOSURE DURATION

05-14-1992

Column	An.#	N	MEAN	SD	SEM	95% Confidence Interval	
A	09	10	10.75	1.687371	.5335937	9.54301	to 11.95699
B	10	10	10.5	1.581139	.5	9.369	to 11.631
C	11	10	11.25	1.317616	.4166667	10.3075	to 12.1925
D	12	10	12.25	1.844662	.5833333	10.9305	to 13.5695
E	25	10	39.75	9.162878	2.897556	33.1957	to 46.30427
F	26	10	49	8.514693	2.692582	42.9093	to 55.09062
G	27	10	40.5	4.830459	1.527525	37.0447	to 43.95526
H	28	10	47.75	9.238357	2.921425	41.1417	to 54.35826
I	41	10	49	7.283925	2.303379	43.7897	to 54.21024
J	42	10	61	6.258328	1.979057	56.5233	to 65.47662
K	43	10	52	7.434903	2.351123	46.6817	to 57.31824
L	44	10	67.5	4.564355	1.443376	64.2350	to 70.76492

EPIDERMAL THICKNESS-96 HOUR POST-EXPOSURE DURATION

05-14-1992

Column	An.#	N	MEAN	SD	SEM	95% Confidence Interval	
A	13	10	15.75	3.343734	1.057382	13.3582	to 18.1418
B	14	10	11.75	1.207615	.3818813	10.8861	to 12.61382
C	15	10	11	1.748015	.5527708	9.74963	to 12.25037
D	16	10	13.5	1.748015	.5527708	12.2496	to 14.75037
E	29	10	20	3.90868	1.236033	17.2040	to 22.7959
F	30	10	28.5	2.108185	.6666667	26.992	to 30.008
G	31	10	50.25	3.21671	1.017213	47.9490	to 52.55094
H	32	10	30.25	4.632314	1.464866	26.9364	to 33.56353
I	45	10	31	2.934469	.9279607	28.9009	to 33.09905
J	46	10	29.25	3.545341	1.121135	26.7139	to 31.786
K	47	10	36.5	2.41523	.7637627	34.7723	to 38.22763
L	48	10	45.5	6.43342	2.034426	40.8981	to 50.10187

EPIDERMAL THICKNESS POST EXPOSURE 6 HOUR GROUP 1 TO GROUP 2
05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	10.9375	50.5
# of points:	4	4
std deviation:	1.434326	10.89534
Std. error:	.7171631	5.447668
Minimum:	9	35
Maximum:	12.25	59.25
Median:	11.25	53.875

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

EPIDERMAL THICKNESS POST EXPOSURE 6 HOUR GROUP 1 TO GROUP 3
05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	10.9375	56.25
# of points:	4	4
std deviation:	1.434326	8.679478
Std. error:	.7171631	4.339739
Minimum:	9	45.25
Maximum:	12.25	66.25
Median:	11.25	56.75

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

EPIDERMAL THICKNESS POST EXPOSURE 6 HOUR GROUP 2 TO GROUP 3
05-14-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	50.5	56.25
# of points:	4	4
std deviation:	10.89534	8.679478
Std. error:	5.447668	4.339739
Minimum:	35	45.25
Maximum:	59.25	66.25
Median:	53.875	56.75

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 6.0
Sum of ranks in smaller group (T) = 16.0

The two-tailed P value is 0.6857 - - 'not significant'.

EPIDERMAL THICKNESS POST EXPOSURE 24 HOUR GROUP 4 TO GROUP 5
05-14-1992

Parameter:	ACETONE	TPA 6.8
Mean:	12.1875	41.8125
# of points:	4	4
Std deviation:	.4269563	13.17095
Std. error:	.2134781	6.585475
Minimum:	11.75	29.5
Maximum:	12.75	60.25
Median:	12.125	38.75

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

EPIDERMAL THICKNESS POST EXPOSURE 24 HOUR GROUP 4 TO GROUP 6
05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	12.1875	50.875
# of points:	4	4
Std deviation:	.4269563	7.584249
Std. error:	.2134781	3.792125
Minimum:	11.75	42.5
Maximum:	12.75	60.75
Median:	12.125	50.125

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

EPIDERMAL THICKNESS POST EXPOSURE 24 HOUR GROUP 5 TO GROUP 6
05-14-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	41.8125	50.875
# of points:	4	4
Std deviation:	13.17095	7.584249
Std. error:	6.585475	3.792125
Minimum:	29.5	42.5
Maximum:	60.25	60.75
Median:	38.75	50.125

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 3.0
Sum of ranks in smaller group (T) = 13.0

The two-tailed P value is 0.2000 - - 'not significant'.

EPIDERMAL THICKNESS POST EXPOSURE 48 HOUR GROUP 7 TO GROUP 8
05-14-1992

Parameter:	ACETONE	TPA 6.8
Mean:	11.1875	44.25
# of points:	4	4
Std deviation:	.773924	4.800174
Std. error:	.386962	2.400087
Minimum:	10.5	39.75
Maximum:	12.25	49
Median:	11	44.125

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

EPIDERMAL THICKNESS POST EXPOSURE 48 HOUR GROUP 7 TO GROUP 9
05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	11.1875	57.375
# of points:	4	4
Std deviation:	.773924	8.459462
Std. error:	.386962	4.229731
Minimum:	10.5	49
Maximum:	12.25	67.5
Median:	11	56.5

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

EPIDERMAL THICKNESS POST EXPOSURE 48 HOUR GROUP 8 TO GROUP 9
05-14-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	44.25	57.375
# of points:	4	4
Std deviation:	4.800174	8.459462
Std. error:	2.400087	4.229731
Minimum:	39.75	49
Maximum:	49	67.5
Median:	44.125	56.5

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.5
Sum of ranks in smaller group (T) = 10.5

The two-tailed P value is 0.0571 - - 'marginally significant'.

EPIDERMAL THICKNESS POST EXPOSURE 96 HOUR GROUP 10 TO GROUP 11
05-14-1992

Parameter:	ACETONE	TPA 6.8
Mean:	13	32.25
# of points:	4	4
Std deviation:	2.111477	12.80788
Std. error:	1.055738	6.403938
Minimum:	11	20
Maximum:	15.75	50.25
Median:	12.625	29.375

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

EPIDERMAL THICKNESS POST EXPOSURE 96 HOUR GROUP 10 TO GROUP 12
05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	13	35.5625
# of points:	4	4
Std deviation:	2.111477	7.30974
Std. error:	1.055738	3.65487
Minimum:	11	29.25
Maximum:	15.75	45.5
Median:	12.625	33.75

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

EPIDERMAL THICKNESS POST EXPOSURE 96 HOUR GROUP 11 TO GROUP 12
05-14-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	32.25	35.5625
# of points:	4	4
Std deviation:	12.80788	7.30974
Std. error:	6.403938	3.65487
Minimum:	20	29.25
Maximum:	50.25	45.5
Median:	29.375	33.75

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 5.0
Sum of ranks in smaller group (T) = 15.0

The two-tailed P value is 0.4857 - - 'not significant'.

ALL TIME POINTS (6, 24, 48, and 96 hour) COMBINED

EPIDERMAL THICKNESS ACETONE COMPARED TO TPA 6.8

05-14-1992

Parameter:	ACETONE	TPA 6.8
Mean:	11.82813	42.20313
# of points:	16	16
Std deviation:	1.476817	11.90736
Std. error:	.3692043	2.976839
Minimum:	9	20
Maximum:	15.75	60.25
Median:	11.75	40.75

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 136.0

The two-tailed P value is <0.0001 - - 'extremely significant'.
(The P value is an estimate based on a normal approximation.)

EPIDERMAL THICKNESS ACETONE COMPARED TO TPA 13.6

05-14-1992

Parameter:	ACETONE	TPA 13.6
Mean:	11.82813	50.01563
# of points:	16	16
Std deviation:	1.476817	11.50126
Std. error:	.3692043	2.875314
Minimum:	9	29.25
Maximum:	15.75	67.5
Median:	11.75	50.25

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 136.0

The two-tailed P value is <0.0001 - - 'extremely significant'.
(The P value is an estimate based on a normal approximation.)

EPIDERMAL THICKNESS TPA 6.8 COMPARED TO TPA 13.6

05-14-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	42.20313	50.01563
# of points:	16	16
Std deviation:	11.90736	11.50126
Std. error:	2.976839	2.875314
Minimum:	20	29.25
Maximum:	60.25	67.5
Median:	40.75	50.25

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 80.0
Sum of ranks in smaller group (T) = 216.0

The two-tailed P value is 0.0737 - - 'marginally significant'.

BRDU

BRDU (PERCENT LABELLED CELLS) POST EXPOSURE 24 HOUR GROUP 4 TO GROUP 5
05-18-1992

Parameter:	ACETONE	TPA 6.8
Mean:	2.7	11.75
# of points:	4	4
Std deviation:	.7393686	4.84527
Std. error:	.3696843	2.422635
Minimum:	2	5.2
Maximum:	3.6	15.8
Median:	2.6	13

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

BRDU (PERCENT LABELLED CELLS) POST EXPOSURE 24 HOUR GROUP 4 TO GROUP 6
05-18-1992

Parameter:	ACETONE	TPA 13.6
Mean:	2.7	15.6
# of points:	4	4
Std deviation:	.7393686	2.617883
Std. error:	.3696843	1.308942
Minimum:	2	13.6
Maximum:	3.6	19.4
Median:	2.6	14.7

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

BRDU (PERCENT LABELLED CELLS) POST EXPOSURE 24 HOUR GROUP 5 TO GROUP 6
05-18-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	11.75	15.6
# of points:	4	4
Std deviation:	4.84527	2.617883
Std. error:	2.422635	1.308942
Minimum:	5.2	13.6
Maximum:	15.8	19.4
Median:	13	14.7

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 5.0
Sum of ranks in smaller group (T) = 15.0

The two-tailed P value is 0.4857 - - 'not significant'.

BRDU (PERCENT LABELLED CELLS) POST EXPOSURE 48 HOUR GROUP 7 TO GROUP 8
05-18-1992

Parameter:	ACETONE	TPA 6.8
Mean:	3.15	7.75
# of points:	4	4
Std deviation:	1.024695	1.47309
Std. error:	.5123473	.7365448
Minimum:	1.8	6
Maximum:	4.2	9.4
Median:	3.3	7.8

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

BRDU (PERCENT LABELLED CELLS) POST EXPOSURE 48 HOUR GROUP 7 TO GROUP 9
05-18-1992

Parameter:	ACETONE	TPA 13.6
Mean:	3.15	9.6
# of points:	4	4
Std deviation:	1.024695	2.698147
Std. error:	.5123473	1.349074
Minimum:	1.8	7
Maximum:	4.2	13.2
Median:	3.3	9.1

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

BRDU (PERCENT LABELLED CELLS) POST EXPOSURE 48 HOUR GROUP 8 TO GROUP 9
05-18-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	7.75	9.6
# of points:	4	4
Std deviation:	1.47309	2.698147
Std. error:	.7365448	1.349074
Minimum:	6	7
Maximum:	9.4	13.2
Median:	7.8	9.1

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 5.0
Sum of ranks in smaller group (T) = 15.0

The two-tailed P value is 0.4857 - - 'not significant'.

BRDU (PERCENT LABELLED CELLS) POST EXPOSURE 96 HOUR GROUP 10 TO GROUP 11
05-18-1992

Parameter:	ACETONE	TPA 6.8
Mean:	2.05	6.15
# of points:	4	4
Std deviation:	.6999994	2.650157
Std. error:	.3499997	1.325078
Minimum:	1	4
Maximum:	2.4	10
Median:	2.4	5.3

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

BRDU (PERCENT LABELLED CELLS) POST EXPOSURE 96 HOUR GROUP 10 TO GROUP 12
05-18-1992

Parameter:	ACETONE	TPA 13.6
Mean:	2.05	7.05
# of points:	4	4
Std deviation:	.6999994	.8544009
Std. error:	.3499997	.4272004
Minimum:	1	6.4
Maximum:	2.4	8.2
Median:	2.4	6.8

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

BRDU (PERCENT LABELLED CELLS) POST EXPOSURE 96 HOUR GROUP 11 TO GROUP 12
05-18-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	6.15	7.05
# of points:	4	4
Std deviation:	2.650157	.8544009
Std. error:	1.325078	.4272004
Minimum:	4	6.4
Maximum:	10	8.2
Median:	5.3	6.8

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 4.0
Sum of ranks in smaller group (T) = 14.0

The two-tailed P value is 0.3429 - - 'not significant'.

ALL TIME POINTS (24, 48, & 96 hour) COMBINED

BRDU (PERCENT LABELLED CELLS) ACETONE COMPARED TO TPA 6.8
05-18-1992

Parameter:	ACETONE	TPA 6.8
Mean:	2.633333	8.55
# of points:	12	12
Std deviation:	.889672	3.867934
Std. error:	.2568262	1.116576
Minimum:	1	4
Maximum:	4.2	15.8
Median:	2.4	7.8

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 1.0
Sum of ranks in smaller group (T) = 79.0

The two-tailed P value is <0.0001 - - 'extremely significant'.
(The P value is an estimate based on a normal approximation.)

BRDU (PERCENT LABELLED CELLS) ACETONE COMPARED TO TPA 13.6
05-18-1992

Parameter:	ACETONE	TPA 13.6
Mean:	2.633333	10.75
# of points:	12	12
Std deviation:	.889672	4.250453
Std. error:	.2568262	1.227
Minimum:	1	6.4
Maximum:	4.2	19.4
Median:	2.4	9.1

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 78.0

The two-tailed P value is <0.0001 - - 'extremely significant'.
(The P value is an estimate based on a normal approximation.)

BRDU (PERCENT LABELLED CELLS) TPA 6.8 COMPARED TO TPA 13.6
05-18-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	8.55	10.75
# of points:	12	12
Std deviation:	3.867934	4.250453
Std. error:	1.116576	1.227
Minimum:	4	6.4
Maximum:	15.8	19.4
Median:	7.8	9.1

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 50.0
Sum of ranks in smaller group (T) = 128.0

The two-tailed P value is 0.2146 - - 'not significant'.

SUBPROTOCOL 1 - DERMIGEN NO. 910801

GROUP - ACETONE

BRDU

Animal #	Sacrifice Day	Raw Count # per 500 Nuclei	Percentage
5	3-17-92	15	3.0%
6	3-17-92	10	2.0%
7	3-17-92	18	3.6%
8	3-17-92	11	2.2%
9	3-18-92	18	3.6%
10	3-18-92	21	4.2%
11	3-18-92	15	3.0%
12	3-18-92	9	1.8%
13	3-20-92	12	2.4%
14	3-20-92	12	2.4%
15	3-20-92	12	2.4%
16	3-20-92	5	1.0%

SUBPROTOCOL 1 - DERMIGEN NO. 910801

GROUP - TPA 6.8 nmol

BRDU

Animal #	Sacrifice Day	Raw Count # per 500 Nuclei	Percentage
21	3-17-92	75	15.0%
22	3-17-92	79	15.8%
23	3-17-92	55	11.0%
24	3-17-92	26	5.2%
25	3-18-92	47	9.4%
26	3-18-92	30	6.0%
27	3-18-92	42	8.4%
28	3-18-92	36	7.2%
29	3-20-92	20	4.0%
30	3-20-92	25	5.0%
31	3-20-92	50	10.0%
32	3-20-92	28	5.6%

SUBPROTOCOL 1 - DERMIGEN NO. 910801

GROUP - TPA 13.6 nmol

BRDU

Animal #	Sacrifice Day	Raw Count # per 500 Nuclei	Percentage
37	3-17-92	76	15.2%
38	3-17-92	68	13.6%
39	3-17-92	97	19.4%
40	3-17-92	71	14.2%
41	3-18-92	41	8.2%
42	3-18-92	66	13.2%
43	3-18-92	35	7.0%
44	3-18-92	50	10.0%
45	3-20-92	32	6.4%
46	3-20-92	36	7.2%
47	3-20-92	32	6.4%
48	3-20-92	41	8.2%

PCNA

PCNA (PERCENT LABELLED CELLS) POST EXPOSURE 24 HOUR GROUP 4 TO GROUP 5
05-18-1992

Parameter:	ACETONE	TPA 6.8
Mean:	17.6	27.55
# of points:	4	4
Std deviation:	2.12916	1.968915
Std. error:	1.06458	.9844577
Minimum:	15.4	25
Maximum:	19.8	29.2
Median:	17.6	28

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

PCNA (PERCENT LABELLED CELLS) POST EXPOSURE 24 HOUR GROUP 4 TO GROUP 6
05-18-1992

Parameter:	ACETONE	TPA 13.6
Mean:	17.6	27.95
# of points:	4	4
Std deviation:	2.12916	5.305019
Std. error:	1.06458	2.65251
Minimum:	15.4	20.4
Maximum:	19.8	32.2
Median:	17.6	29.6

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

PCNA (PERCENT LABELLED CELLS) POST EXPOSURE 24 HOUR GROUP 5 TO GROUP 6
05-18-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	27.55	27.95
# of points:	4	4
Std deviation:	1.968915	5.305019
Std. error:	.9844577	2.65251
Minimum:	25	20.4
Maximum:	29.2	32.2
Median:	28	29.6

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 6.0
Sum of ranks in smaller group (T) = 16.0

The two-tailed P value is 0.6857 - - 'not significant'.

PCNA (PERCENT LABELLED CELLS) POST EXPOSURE 48 HOUR GROUP 7 TO GROUP 8
05-18-1992

Parameter:	ACETONE	TPA 6.8
Mean:	12.75	24.65
# of points:	4	4
Std deviation:	.9848709	3.746559
Std. error:	.4924355	1.873279
Minimum:	11.6	19.4
Maximum:	14	27.8
Median:	12.7	25.7

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

PCNA (PERCENT LABELLED CELLS) POST EXPOSURE 48 HOUR GROUP 7 TO GROUP 9
05-18-1992

Parameter:	ACETONE	TPA 13.6
Mean:	12.75	29.5
# of points:	4	4
Std deviation:	.9848709	7.733034
Std. error:	.4924355	3.866517
Minimum:	11.6	21.2
Maximum:	14	39.8
Median:	12.7	28.5

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

PCNA (PERCENT LABELLED CELLS) POST EXPOSURE 48 HOUR GROUP 8 TO GROUP 9
05-18-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	24.65	29.5
# of points:	4	4
Std deviation:	3.746559	7.733034
Std. error:	1.873279	3.866517
Minimum:	19.4	21.2
Maximum:	27.8	39.8
Median:	25.7	28.5

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 4.0
Sum of ranks in smaller group (T) = 14.0

The two-tailed P value is 0.3429 - - 'not significant'.

PCNA (PERCENT LABELLED CELLS) POST EXPOSURE 96 HOUR GROUP 10 TO GROUP 11
05-18-1992

Parameter:	ACETONE	TPA 6.8
Mean:	15.25	26.3
# of points:	4	4
std deviation:	2.247222	3.814026
Std. error:	1.123611	1.907013
Minimum:	13.8	22.4
Maximum:	18.6	30.8
Median:	14.3	26

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

PCNA (PERCENT LABELLED CELLS) POST EXPOSURE 96 HOUR GROUP 10 TO GROUP 12
05-18-1992

Parameter:	ACETONE	TPA 13.6
Mean:	15.25	31.15
# of points:	4	4
std deviation:	2.247222	3.134218
Std. error:	1.123611	1.567109
Minimum:	13.8	28.4
Maximum:	18.6	35
Median:	14.3	30.6

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 10.0

The two-tailed P value is 0.0286 - - 'significant'.

PCNA (PERCENT LABELLED CELLS) POST EXPOSURE 96 HOUR GROUP 11 TO GROUP 12
05-18-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	26.3	31.15
# of points:	4	4
std deviation:	3.814026	3.134218
Std. error:	1.907013	1.567109
Minimum:	22.4	28.4
Maximum:	30.8	35
Median:	26	30.6

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 2.0
Sum of ranks in smaller group (T) = 12.0

The two-tailed P value is 0.1143 - - 'not significant'.

ALL TIME POINTS (24, 48, & 96 hour) COMBINED

PCNA (PERCENT LABELLED CELLS) ACETONE COMPARED TO TPA 6.8
05-18-1992

Parameter:	ACETONE	TPA 6.8
Mean:	15.2	26.16667
# of points:	12	12
Std deviation:	2.675142	3.2236
Std. error:	.7722469	.9305733
Minimum:	11.6	19.4
Maximum:	19.8	30.8
Median:	14.3	26.9

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 1.0
Sum of ranks in smaller group (T) = 79.0

The two-tailed P value is <0.0001 - - 'extremely significant'.
(The P value is an estimate based on a normal approximation.)

PCNA (PERCENT LABELLED CELLS) ACETONE COMPARED TO TPA 13.6
05-18-1992

Parameter:	ACETONE	TPA 13.6
Mean:	15.2	29.53333
# of points:	12	12
Std deviation:	2.675142	5.340975
Std. error:	.7722469	1.541807
Minimum:	11.6	20.4
Maximum:	19.8	39.8
Median:	14.3	29.2

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 0.0
Sum of ranks in smaller group (T) = 78.0

The two-tailed P value is <0.0001 - - 'extremely significant'.
(The P value is an estimate based on a normal approximation.)

PCNA (PERCENT LABELLED CELLS) TPA 6.8 COMPARED TO TPA 13.6
05-18-1992

Parameter:	TPA 6.8	TPA 13.6
Mean:	26.16667	29.53333
# of points:	12	12
Std deviation:	3.2236	5.340975
Std. error:	.9305733	1.541807
Minimum:	19.4	20.4
Maximum:	30.8	39.8
Median:	26.9	29.2

Unpaired nonparametric test: Are the medians of columns A and column B equal?

Mann-Whitney Two Sample Test:

Mann-Whitney statistic (U) = 37.0
Sum of ranks in smaller group (T) = 115.0

The two-tailed P value is 0.0467 - - 'significant'.

SUBPROTOCOL 1 - DERMIGEN NO. 910801

GROUP - ACETONE

PCNA

Animal #	Sacrifice Day	Raw Count # per 500 Nuclei	Percentage
5	3-17-92	77	15.4%
6	3-17-92	95	19.0%
7	3-17-92	81	16.2%
8	3-17-92	99	19.8%
9	3-18-92	70	14.0%
10	3-18-92	64	12.8%
11	3-18-92	63	12.6%
12	3-18-92	58	11.6%
13	3-20-92	93	18.6%
14	3-20-92	71	14.2%
15	3-20-92	72	14.4%
16	3-20-92	69	13.8%

SUBPROTOCOL 1 - DERMIGEN NO. 910801

GROUP - TPA 6.8 nmol

PCNA

Animal #	Sacrifice Day	Raw Count # per 500 Nuclei	Percentage
21	3-17-92	145	29.0%
22	3-17-92	135	27.0%
23	3-17-92	146	29.2%
24	3-17-92	125	25.0%
25	3-18-92	134	26.8%
26	3-18-92	97	19.4%
27	3-18-92	139	27.8%
28	3-18-92	123	24.6%
29	3-20-92	120	24.0%
30	3-20-92	154	30.8%
31	3-20-92	112	22.4%
32	3-20-92	140	28.0%

SUBPROTOCOL 1 - DERMIGEN NO. 910801

GROUP - TPA 13.6 nmol

PCNA

Animal #	Sacrifice Day	Raw Count # per 500 Nuclei	Percentage
37	3-17-92	155	31.0%
38	3-17-92	102	20.4%
39	3-17-92	141	28.2%
40	3-17-92	161	32.2%
41	3-18-92	199	39.8%
42	3-18-92	148	29.6%
43	3-18-92	106	21.2%
44	3-18-92	137	27.4%
45	3-20-92	144	28.8%
46	3-20-92	142	28.4%
47	3-20-92	175	35.0%
48	3-20-92	162	32.4%