EFFECTS OF TOPICAL EXPOSURE TO ALOE VERA TEST SUBSTANCES ON THE PHOTOCARCINOGENICITY OF SIMULATED SOLAR LIGHT IN SKH-1 MICE

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PATHOLOGY REPORT

PREPARED
BY

TOXICOLOGIC PATHOLOGY ASSOCIATES
JEFFERSON, ARKANSAS

FOR

NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH
3900 NCTR ROAD
JEFFERSON, ARKANSAS 72079

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Protocol Number E2140.05

INTRODUCTION

This report prepared by Toxicologic Pathology Associates for the National Center for Toxicological Research, Jefferson, Arkansas, presents the results of pathology support for Experiment 7 (E2140.05) of Protocol 2140.01. The objective of this portion of the study was to determine if the application of creams containing Aloe vera or Aloe-emodin test substances to the skin of male and female SKH-1 mice alters the tumor incidence induced by simulated solar light (SSL) in the mouse skin.

EXPERIMENT DESIGN AND METHODS

Experiment 2140.05 was a 52-week study to determine the possible enhanced carcinogenicity of SSL in male and female SKH-1 hairless mice treated topically with creams containing 3% or 6% concentrations of Aloe gel, 3% or 6% Aloe whole leaf extract, 3% or 6% charcoal filtered Aloe whole leaf extract or 0.56µg or 5.6µg Aloe-emodin. The number of animals used and their allocation to their respective treatment groups are shown in the following table. The levels of SSL used in this study were No SSL, 6.85, 13.7, or 20.55 milliJuoles per square centimeter (mJ/cm^2). The vehicle cream had a pH of 6.0 and contained no test substances.

Table 1 – Study Design

Test Substance Exposure	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream	36 □ 36 □	36 □ 36 □	36 □ 36 □	36 □ 36 □
Vehicle Control	35* □ 36 □	36 □ 36 □		
Aloe gel 3%		36 □ 36 □		
Aloe gel 6%	36 □ 36 □	36 □ 36 □		
Aloe whole leaf extract 3%		36 □ 36 □		
Aloe whole leaf extract 6%	36 □ 36 □	36 □ 36 □		
Charcoal filtered whole leaf extract 3%		36 □ 36 □		
Charcoal filtered whole leaf extract 6%	36 □ 36 □	36 □ 36 □		
Aloe-emodin 0.56 µg		35* □ 36 □		
Aloe-emodin 5.6 µg	35* □ 36 □	36 □ 36 □		

* One mis-sexed animal was discarded.

Mice were treated topically with cream and exposed to SSL 5 days/week as indicated in the table. Cream formulations were applied to the backs of the mice from the front of the shoulders to the base of the tail. This area is referred to as the "site of application" (SOA). Skin lesions that occurred in the SOA were documented separately from all other areas of the body where no cream was applied. In pathology data tables, areas receiving no cream are referred to as "Skin, Control".

After 40 weeks of treatment, mice were held without treatment for 12 additional weeks prior to scheduled sacrifice. At study termination the surviving animals were euthanized by exposure to carbon dioxide and a complete necropsy was performed. Necropsies were also performed on the 434 females and 434 males that died during the study or that were removed when moribund or when they developed skin masses ≥5 mm in diameter.

Digital photographs of skin were taken after euthanasia but prior to the initiation of necropsy. All protocol specified tissues were examined grossly, removed, and preserved in 10% neutral buffered formalin. Lesion descriptions were recorded on the Individual Animal Necropsy Record (IANR). The protocol designated tissues, including all gross lesions were trimmed, processed, and embedded in infiltrating media (Formula R®), sectioned at approximately 5 microns, and stained with hematoxylin and eosin. All sections of skin and skin lesions were examined microscopically. The photographically documented gross skin lesions were correlated with IANR entries. For consistency in documentation of the sites of the skin lesions, the site of application included the treatment area on the dorsal side from the base of the neck to the beginning of the tail and half-way down each side. In a few cases, special staining procedures were applied to selected lesions to aid in the characterization of pathologic changes. When applicable, non-neoplastic lesions were graded for severity as 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked). Tissues other than skin were not evaluated microscopically per protocol amendment.

RESULTS AND DISCUSSION

Mortality

The pathology results were based on the 648 female and 643 male SKH-1 mice examined histopathologically. Their distribution, including the number of animals in each group that died early, that were removed when moribund, or that were removed when they had skin lesions ≥5 mm in diameter is summarized in Tables 2 and 3. The number of early deaths correlated with the dose of SSL. The largest percentage of early deaths in mice exposed to SSL resulted from removal of animals with skin lesions ≥5 mm in diameter. There was no correlation between treatment group and numbers of mice that died naturally or were removed when moribund and experimental treatment.

Table 2 - Disposition Summary - Females

Test Substance Exposure/ Disposition	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Mice initially in study*	36	36	36	36
Natural Death	1	0	1	0
Moribund	5	1	4	1
Harvest (Skin lesion ≥5 mm)	0	34	7	35
Terminal Sacrifice	30	1	24	0
Examined microscopically	36	36	36	36
Vehicle Control				
Mice initially in study	36	36		
Natural Death	0	0		
Moribund	7	1		
Harvest (Skin lesion ≥5 mm)	1	33		
Terminal Sacrifice	28	2		
Examined microscopically	36	36		
Aloe gel 3%				
Mice initially in study	0	36		
Natural Death	0	0		
Moribund	0	4		
Harvest (Skin lesion ≥5 mm)	0	32		
Terminal Sacrifice	0	0		
Examined microscopically	0	36		
Aloe gel 6%				
Mice initially in study	36	36		
Natural Death	2	1		
Moribund	4	4		
Harvest (Skin lesion ≥5 mm)	0	31		
Terminal Sacrifice	30	0		
Examined microscopically	36	36		
Aloe whole leaf extract 3%				
Mice initially in study	0	36		
Natural Death	0	1		
Moribund	0	3		
Harvest (Skin lesion ≥5 mm)	0	31		
Terminal Sacrifice	0	1		
Examined microscopically	0	36		
Aloe whole leaf extract 6%				
Mice initially in study	36	36		
Natural Death	0	3		
Moribund	2	1		
Harvest (Skin lesion ≥5 mm)	1	32		
Terminal Sacrifice	33	0		
Examined microscopically	36	36		
Charcoal filtered whole leaf extract 3%				
Mice initially in study	0	36		
Natural Death	0	2		
Moribund	0	0		
Harvest (Skin lesion ≥5 mm)	0	34		
Terminal Sacrifice	0	0		
Examined microscopically	0	36		
Charcoal filtered whole leaf extract 6%				
Mice initially in study	36	36		
Natural Death	0	1		
Moribund	4	2		
Harvest (Skin lesion ≥5 mm)	0	33		
Terminal Sacrifice	32	0		
Examined microscopically	36	36		
Aloe-emodin 0.56 µg				
Mice initially in study	0	36		
Natural Death	0	0		
Moribund	0	4		
Harvest (Skin lesion ≥5 mm)	0	32		
Terminal Sacrifice	0	0		
Examined microscopically	0	36		
Aloe-emodin 5.6 µg				
Mice initially in study	36	36		
Natural Death	1	2		
Moribund	2	1		
Harvest (Skin lesion ≥5 mm)	0	33		
Terminal Sacrifice	33	0		
Examined microscopically	36	36		

*Additional animals were used as sentinels for microbiological surveillance.

Table 3 - Disposition Summary - Males

Test Substance Exposure/Disposition	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Mice initially in study*	36	36	36	36
Natural Death	1	0	2	0
Moribund	0	0	6	3
Accidentally killed	0	0	1	0
Harvest (Skin lesion ≥5 mm)	0	36	5	33
Terminal Sacrifice	35	0	22	0
Examined microscopically	36	36	36	36
Vehicle Control				
Mice initially in study	35**	35****		
Natural Death	0	1		
Moribund	5	3		
Harvest (Skin lesion ≥5 mm)	0	31		
Terminal Sacrifice	30	0		
Examined microscopically	35	35		
Aloe gel 3%				
Mice initially in study	0	36		
Natural Death	0	0		
Moribund	0	5		
Harvest (Skin lesion ≥5 mm)	0	31		
Terminal Sacrifice	0	0		
Examined microscopically	0	36		
Aloe gel 6%				
Mice initially in study	36	36		
Natural Death	0	0		
Moribund	1	1		
Accidentally killed	1	0		
Harvest (Skin lesion ≥5 mm)	0	35		
Terminal Sacrifice	34	0		
Examined microscopically	36	36		
Aloe whole leaf extract 3%				
Mice initially in study	0	36		
Natural Death	0	2		
Moribund	0	1		
Harvest (Skin lesion ≥5 mm)	0	32		
Terminal Sacrifice	0	1		
Examined microscopically	0	36		
Aloe whole leaf extract 6%				
Mice initially in study	36	36		
Natural Death	2	1		
Moribund	4	2		
Harvest (Skin lesion ≥5 mm)	0	32		
Terminal Sacrifice	30	1		
Examined microscopically	35***	36		
Charcoal filtered whole leaf extract 3%				
Mice initially in study	0	36		
Natural Death	0	1		
Moribund	0	2		
Harvest (Skin lesion ≥5 mm)	0	33		
Terminal Sacrifice	0	0		
Examined microscopically	0	36		
Charcoal filtered whole leaf extract 6%				
Mice initially in study	36	36		
Natural Death	2	0		
Moribund	3	4		
Harvest (Skin lesion ≥5 mm)	1	32		
Terminal Sacrifice	30	0		
Examined microscopically	36	36		
Aloe-emodin 0.56 µg				
Mice initially in study	0	35**		
Natural Death	0	2		
Moribund	0	3		
Harvest (Skin lesion ≥5 mm)	0	30		
Terminal Sacrifice	0	0		
Examined microscopically	0	35		
Aloe-emodin 5.6 µg				
Mice initially in study	35**	36		
Natural Death	0	1		
Moribund	7	0		
Harvest (Skin lesion ≥5 mm)	1	34		
Terminal Sacrifice	27	1		
Examined microscopically	35	36		

*Additional animals were used as sentinels for microbiological surveillance.
** One animal was mis-sexed.
*** One animal was autolyzed NTT (no tissue taken).
**** One animal was died prior to dosing and was discarded per PI request.

Gross Observations

All gross observations were recorded on the Individual Animal Necropsy Record. A microscopic finding was recorded with the corresponding gross observation for skin when possible. The gross observations for skin are listed in Appendix V along with microscopic correlates.

Histopathology

Microscopic findings are summarized by treatment group and atomic site in Pathology Report 2 (Neoplastic), and Pathology Report 3 (Non-neoplastic). They are also tabulated by individual animal in Pathology Reports 4 (Neoplastic) and 9 (Non-neoplastic). These compilations are in Appendices I-IV, respectively, of this report.

Neoplastic Findings

Prolonged exposure of SKH-1 mice to SSL results in the development of three morphologic types of squamous cell neoplasia in the epidermis. A morphologic description of squamous cell papilloma, squamous cell carcinoma *in situ*, and squamous cell carcinoma with criteria used to differentiate these neoplasms is as follows:

SQUAMOUS CELL PAPILLOMA: Grossly these tumors were often “wart-like” lesions. Microscopically, papillomas were solitary focal, arborized projections elevated above the skin surface consisting of a core of fibrovascular tissue contiguous with the dermis and covered by thickened, often hyperkeratotic stratified squamous epithelium. The thickness of the epithelium was quite variable. The epithelial cells were orderly in their arrangement although they were often more numerous and crowded than in normal epidermis. In most instances papillomas were broad based lesions (sessile). A few classical pedunculated papillomas with the arborized projections arising from a single stalk did occur but the incidence of this more classical morphology was much lower than the incidence of sessile papillomas.

SQUAMOUS CELL CARCINOMA *IN SITU*: Grossly these lesions were usually small raised nodules one to several millimeters in diameter. Microscopically they were discrete nodules involving the squamous epithelium. In some cases the epithelium was elevated; other lesions were depressed below the adjacent epidermis, compressing the dermis and resulting in a cup-shaped lesion. Superficial ulceration was common. In all cases, the border of the tumor was sharply demarcated from the dermis. This was the principal feature that differentiated squamous cell carcinoma *in situ* from squamous cell carcinoma. Within the proliferating epithelium, nuclei were often atypical and were usually arranged in a disorderly fashion. Mitotic figures were numerous and, in many cases, were large and bizarre. Keratinization of individual cells was common and many individual epithelial cells were dysplastic. Abundant keratin was often present. There was usually an inflammatory reaction in the underlying dermis that consisted of lymphocyte, plasma cell, and polymorphonuclear leukocyte infiltration of variable severity. Squamous cell carcinomas *in situ* were frequently multiple and were often seen in mice that also had squamous cell carcinomas and/or squamous cell papillomas.

SQUAMOUS CELL CARCINOMA: Grossly squamous cell carcinomas were nodular masses with irregular surfaces that were often crateriform and ulcerated. Microscopically, squamous cell carcinomas were characterized by downward projecting sheets, nests, and anastomosing cords of neoplastic squamous cells that extended into the dermis. Some tumor cells penetrated the panniculus carnosus sheet of skeletal muscle and invaded the subcutaneous tissue to a variable degree. Often large masses of keratin occupied the central crateriform depression of

the mass. Concentrically arranged masses of keratin (epithelial pearls) were often present in variable numbers throughout the mass. Keratinization of individual cells, nuclear atypia, and numerous large bizarre mitotic figures were common. The degree of differentiation of squamous cell carcinomas was quite variable; some were well-differentiated while others were very aplastic. Many of these neoplasms produced large amounts of keratin. Some of the invasive squamous cell carcinomas appeared to arise within squamous cell carcinomas *in situ* suggesting that there was a progression from carcinoma *in situ* to squamous cell carcinoma. The distinction between the two was sometimes difficult. Squamous cell carcinomas were sometimes multiple and found in mice that also had squamous cell carcinomas *in situ* and/or squamous cell papillomas.

Incidence of Neoplasia in Skin

The incidence of squamous cell neoplasms of the skin of SKH-1 mice exposed to SSL and treated with no cream, vehicle cream, or creams containing Aloe test materials is presented in Table 4 (females) and Table 5 (males).

Table 4
Incidence of Skin Neoplasms in SKH-1 Mice
Females

Test Substance Exposure/ Tumor Type (SOA*)	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Squamous Cell Papilloma	0	47%	44%	44%
Squamous Cell Carcinoma *in situ*	0	78%	25%	64%
Squamous Cell Carcinoma	0	64%	6%	47%
Vehicle Control				
Squamous Cell Papilloma	0	58%		
Squamous Cell Carcinoma *in situ*	0	83%		
Squamous Cell Carcinoma	0	69%		
Aloe gel 3%				
Squamous Cell Papilloma	na**	69%		
Squamous Cell Carcinoma *in situ*	na	89%		
Squamous Cell Carcinoma	na	61%		
Aloe gel 6%				
Squamous Cell Papilloma	0	81%		
Squamous Cell Carcinoma *in situ*	0	78%		
Squamous Cell Carcinoma	0	64%		
Aloe whole leaf extract 3%				
Squamous Cell Papilloma	na	72%		
Squamous Cell Carcinoma *in situ*	na	86%		
Squamous Cell Carcinoma	na	56%		
Aloe whole leaf extract 6%				
Squamous Cell Papilloma	3%	81%		
Squamous Cell Carcinoma *in situ*	0	78%		
Squamous Cell Carcinoma	0	50%		
Charcoal filtered whole leaf extract 3%				
Squamous Cell Papilloma	na	83%		
Squamous Cell Carcinoma *in situ*	na	89%		
Squamous Cell Carcinoma	na	42%		
Charcoal filtered whole leaf extract 6%				
Squamous Cell Papilloma	0	86%		
Squamous Cell Carcinoma *in situ*	0	81%		
Squamous Cell Carcinoma	0	47%		
Aloe-emodin 0.56 µg				
Squamous Cell Papilloma	na	75%		
Squamous Cell Carcinoma *in situ*	na	75%		
Squamous Cell Carcinoma	na	61%		
Aloe-emodin 5.6 µg				
Squamous Cell Papilloma	0	81%		
Squamous Cell Carcinoma *in situ*	0	86%		
Squamous Cell Carcinoma	0	53%		

* SOA = Site of application **no animals in the group

Table 5
Incidence of Skin Neoplasms in SKH-1 Mice
Males

Test Substance Exposure/ Tumor Type (SOA*)	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Squamous Cell Papilloma	0	61%	14%	28%
Squamous Cell Carcinoma *in situ*	0	86%	19%	75%
Squamous Cell Carcinoma	0	53%	0	72%
Vehicle Control				
Squamous Cell Papilloma	0	46%		
Squamous Cell Carcinoma *in situ*	0	74%		
Squamous Cell Carcinoma	0	43%		
Aloe gel 3%				
Squamous Cell Papilloma	na**	53%		
Squamous Cell Carcinoma *in situ*	na	78%		
Squamous Cell Carcinoma	na	50%		
Aloe gel 6%				
Squamous Cell Papilloma	0	69%		
Squamous Cell Carcinoma *in situ*	0	81%		
Squamous Cell Carcinoma	0	56%		
Aloe whole leaf extract 3%				
Squamous Cell Papilloma	na	56%		
Squamous Cell Carcinoma *in situ*	na	81%		
Squamous Cell Carcinoma	na	47%		
Aloe whole leaf extract 6%				
Squamous Cell Papilloma	0	75%		
Squamous Cell Carcinoma *in situ*	0	78%		
Squamous Cell Carcinoma	0	42%		
Charcoal filtered whole leaf extract 3%				
Squamous Cell Papilloma	na	53%		
Squamous Cell carcinoma *in situ*	na	81%		
Squamous Cell Carcinoma	na	39%		
Charcoal filtered whole leaf extract 6%				
Squamous Cell Papilloma	0	56%		
Squamous Cell carcinoma *in situ*	0	72%		
Squamous Cell Carcinoma	0	56%		
Aloe-Emodin 0.56 µg				
Squamous Cell Papilloma	na	77%		
Squamous Cell Carcinoma *in situ*	na	69%		
Squamous Cell Carcinoma	na	60%		
Aloe-Emodin 5.6 µg				
Squamous Cell Papilloma	0	53%		
Squamous Cell Carcinoma *in situ*	0	83%		
Squamous Cell Carcinoma	0	64%		

* SOA = Site of application ** no animals in the group

The incidence of squamous cell papillomas, squamous cell carcinomas *in situ*, and squamous cell carcinomas was highly dependent on exposure to SSL. Except for one squamous cell papilloma in a female mouse treated with 6% Aloe whole leaf extract with no SSL exposure, no squamous cell neoplasms developed in groups that were not exposed to SSL. The incidence of squamous cell neoplasms was lower in the no cream control group exposed to 6.85 mJ/cm^2 SSL compared to the no cream control groups exposed to 13.7 or 20.55 mJ/cm^2 SSL.

Squamous Cell Papilloma

In male and female mice exposed to 13.7 mJ/cm^2 SSL the incidence of squamous cell papillomas was higher in all groups treated topically with the Aloe test substances compared to the group treated with vehicle control cream.

Squamous Cell Carcinoma *in situ*

No appreciable difference in the incidence of squamous cell carcinoma *in situ* was evident between groups receiving no cream, vehicle cream, or the 3% or 6% Aloe gel, 3% or 6% Aloe whole leaf extract, 3% or 6% charcoal filtered whole leaf extract, or 0.56µg or 5.6µg Aloe-emodin test substances in either sex.

Squamous Cell Carcinoma

In male mice there was a trend toward increased incidence of squamous cell carcinoma associated with all groups treated with Aloe test substances except in the 3% charcoal filtered whole leaf extract group and 6% whole leaf extract group, in which the incidence of squamous cell carcinoma was slightly less than that of the vehicle cream group. In females there was no appreciable difference in the incidence of squamous cell carcinoma between the vehicle control group and those groups treated with Aloe test substances.

There were some differences in the incidence of the above three different morphologic types of squamous neoplasms between Aloe-treated groups but there was no consistent trend showing that treatment with either concentration of the four Aloe test substances resulted in either a higher or a lower incidence of squamous cell neoplasms.

20.55 mJ/cm^2 SSL

Mice exposed to 20.55 mJ/cm^2 SSL were intended to serve as positive controls for this study and were not exposed to any cream formulations. In both females and males exposed to 20.55 mJ/cm^2 SSL, the incidence of squamous cell neoplasms was not appreciably higher than in groups exposed to 13.7 mJ/cm^2 SSL, and was actually lower for most groups. This may have been due to the removal of animals from the study as soon as the largest lesion reached ≥5 mm in diameter. Removal of animals with lesions ≥5 mm in diameter may have allowed less time for the progressive development of squamous cell carcinomas in some of the animals.

None of the mice in groups without SSL exposure developed squamous cell carcinoma *in situ* or squamous cell carcinoma. One female mouse in the No SSL 6% Aloe whole leaf extract group developed a squamous cell papilloma.

Multiplicity of Neoplasia

Multiplicity of squamous cell neoplasms (average number of neoplasms per animal) is presented by treatment group in Table 6 for females and Table 7 for males.

Table 6
Multiplicity of Skin Neoplasms in SKH-1 Mice
(Average number of neoplasms per mouse)
Females

Test Substance Exposure/ Tumor Type (SOA*)	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Squamous Cell Papilloma	0	1.500	0.889	1.111
Squamous Cell Carcinoma *in situ*	0	2.222	0.528	1.639
Squamous Cell Carcinoma	0	0.917	0.056	0.806
Average total treatment-related skin neoplasms**	0	4.639	1.472	3.556
Vehicle Control				
Squamous Cell Papilloma	0	1.667		
Squamous Cell Carcinoma *in situ*	0	2.250		
Squamous Cell Carcinoma	0	0.861		
Average total treatment-related skin neoplasms	0	4.778		
Aloe gel 3%				
Squamous Cell Papilloma	na***	2.917		
Squamous Cell Carcinoma *in situ*	na	3.222		
Squamous Cell Carcinoma	na	0.861		
Average total treatment-related skin neoplasms	na	7.000		
Aloe gel 6%				
Squamous Cell Papilloma	0	2.750		
Squamous Cell Carcinoma *in situ*	0	2.556		
Squamous Cell Carcinoma	0	0.806		
Average total treatment-related skin neoplasms	0	6.111		
Aloe whole leaf extract 3%				
Squamous Cell Papilloma	na	2.611		
Squamous Cell Carcinoma *in situ*	na	3.667		
Squamous Cell Carcinoma	na	0.722		
Average total treatment-related skin neoplasms	na	7.000		
Aloe whole leaf extract 6%				
Squamous Cell Papilloma	0.028	3.111		
Squamous Cell Carcinoma *in situ*	0	2.250		
Squamous Cell Carcinoma	0	0.611		
Average total treatment-related skin neoplasms	0.028	5.972		
Charcoal filtered whole leaf extract 3%				
Squamous Cell Papilloma	na	3.000		
Squamous Cell Carcinoma *in situ*	na	3.944		
Squamous Cell Carcinoma	na	0.583		
Average total treatment-related skin neoplasms	na	7.528		
Charcoal filtered whole leaf extract 6%				
Squamous Cell Papilloma	0	3.528		
Squamous Cell Carcinoma *in situ*	0	3.028		
Squamous Cell Carcinoma	0	0.722		
Average total treatment-related skin neoplasms	0	7.278		
Aloe-emodin 0.56 µg				
Squamous Cell Papilloma	na	2.750		
Squamous Cell Carcinoma *in situ*	na	2.417		
Squamous Cell Carcinoma	na	0.750		
Average total treatment-related skin neoplasms	na	5.917		
Aloe-emodin 5.6 µg				
Squamous Cell Papilloma	0	2.583		
Squamous Cell Carcinoma *in situ*	0	3.111		
Squamous Cell Carcinoma	0	0.917		
Average total treatment-related skin neoplasms	0	6.611		

* SOA = Site of application

** Total treatment-related skin neoplasms = squamous cell papillomas + squamous cell carcinoma *in situ* + squamous cell carcinoma

*** No animals in the group

Table 7
Multiplicity of Skin Neoplasms in SKH-1 Mice
(Average number of neoplasms per mouse)
Males

Test Substance Exposure/ Tumor Type (SOA*)	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Squamous Cell Papilloma	0	1.056	0.278	0.472
Squamous Cell Carcinoma *in situ*	0	2.528	0.417	2.778
Squamous Cell Carcinoma	0	0.722	0	1.417
Average total treatment-related skin neoplasms**	0	4.306	0.694	4.667
Vehicle Control				
Squamous Cell Papilloma	0	1.257		
Squamous Cell Carcinoma *in situ*	0	2.257		
Squamous Cell Carcinoma	0	0.657		
Average total treatment-related skin neoplasms	0	4.171		
Aloe gel 3%				
Squamous Cell Papilloma	na***	1.750		
Squamous Cell Carcinoma *in situ*	na	2.472		
Squamous Cell Carcinoma	na	0.750		
Average total treatment-related skin neoplasms	na	4.972		
Aloe gel 6%				
Squamous Cell Papilloma	0	1.583		
Squamous Cell Carcinoma *in situ*	0	2.889		
Squamous Cell Carcinoma	0	0.750		
Average total treatment-related skin neoplasms	0	5.222		
Aloe whole leaf extract 3%				
Squamous Cell Papilloma	na	1.611		
Squamous Cell Carcinoma *in situ*	na	2.583		
Squamous Cell Carcinoma	na	0.611		
Average total treatment-related skin neoplasms	na	4.806		
Aloe whole leaf extract 6%				
Squamous Cell Papilloma	0	2.306		
Squamous Cell Carcinoma *in situ*	0	3.250		
Squamous Cell Carcinoma	0	0.611		
Average total treatment-related skin neoplasms	0	6.167		
Charcoal filtered whole leaf extract 3%				
Squamous Cell Papilloma	na	1.611		
Squamous Cell Carcinoma *in situ*	na	3.528		
Squamous Cell Carcinoma	na	0.556		
Average total treatment-related skin neoplasms	na	5.694		
Charcoal filtered whole leaf extract 6%				
Squamous Cell Papilloma	0	1.444		
Squamous Cell Carcinoma *in situ*	0	2.639		
Squamous Cell Carcinoma	0	0.639		
Average total treatment-related skin neoplasms	0	4.722		
Aloe-emodin 0.56 µg				
Squamous Cell Papilloma	na	1.743		
Squamous Cell Carcinoma *in situ*	na	1.971		
Squamous Cell Carcinoma	na	0.771		
Average total treatment-related skin neoplasms	na	4.486		
Aloe-emodin 5.6 µg				
Squamous Cell Papilloma	0	1.639		
Squamous Cell Carcinoma *in situ*	0	2.556		
Squamous Cell Carcinoma	0	0.889		
Average total treatment-related skin neoplasms	0	5.083		

* SOA = Site of application

** Total treatment-related skin neoplasms= squamous cell papillomas + squamous cell carcinoma *in situ* + squamous cell carcinoma

*** No animals in the group

Squamous Cell Papilloma

In both male and female mice exposed to 13.7 mJ/cm^2 SSL, multiplicity of squamous cell papillomas was distinctly higher in groups exposed to all Aloe test substances than in the groups exposed to the vehicle control or no cream.

Squamous Cell Carcinoma *in situ*

The multiplicity of squamous cell carcinomas *in situ* was higher in all groups of male mice exposed to 13.7 mJ/cm^2 SSL and treated with creams containing Aloe test substances compared to males treated with vehicle control except the 0.56µg Aloe-emodin group which had slightly lower multiplicity than the vehicle control group. This was also true of all groups of females except for the group exposed to 6% whole leaf extract in which the average number of squamous cell carcinomas *in situ* per mouse was the same as that of the vehicle control group.

Squamous Cell Carcinoma

There was a slight trend toward increased multiplicity of squamous cell carcinomas in males treated with 3% or 6% Aloe gel or 0.56µg or 5.6µg Aloe-emodin compared to those treated with vehicle control cream. There was no difference in multiplicity of squamous cell carcinomas in females exposed to 13.7 mJ/cm^2 SSL and treated with Aloe test substances compared to vehicle controls and no cream controls.

When all three types of SSL-induced squamous cell neoplasms (squamous cell papillomas, squamous cell carcinomas *in situ*, and squamous cell carcinomas) are combined, the average number of treatment-related skin tumors of all types per animal was higher in all groups of male and female mice treated with Aloe test substances than in the groups treated with no cream or groups treated with the vehicle control cream. There was a very consistent increase in the multiplicity of squamous cell papillomas and squamous cell carcinomas *in situ* with a smaller increase in multiplicity of squamous cell carcinomas associated with treatment with Aloe test substances in mice exposed to 13.7 mJ/cm^2 SSL. Although higher multiplicity of squamous cell neoplasms was associated with application of creams containing Aloe test substances (compared to vehicle control cream or no cream), there was no significant difference among the different test substances in multiplicity of these neoplasms. A possible reason that the multiplicity of squamous cell carcinomas was not increased to a greater degree by test article treatment is that squamous cell carcinomas tend to be larger lesions than either squamous cell papillomas or squamous cell carcinomas *in situ* and the protocol required removal of animals from the study as soon as the largest lesion reached ≥5 mm in diameter. This requirement may have caused removal of some animals from the study before greater numbers of squamous cell carcinomas could develop.

A few skin neoplasms other than those involving the stratified squamous epithelium of the epidermis were observed in this study. The incidence of these neoplasms was unrelated to treatment groups and occurred in numbers and atomic sites that are probably consistent with the spontaneous rate for mice of this age and genotype.

Non-neoplastic Findings

The skin of SKH-1 mice has a number of unique histologic characteristics. The epidermis usually consists of one or two layers of squamous cells and, in the untreated animal, is unremarkable. Hair shafts and adnexal structures such as sebaceous glands are either absent

or are atypical in location and development. There are often prominent cystic structures in the dermis, which appear to be remnants of hair follicles. The cysts are usually lined by squamous or low cuboidal epithelium and are either empty or contain small amounts of keratinized debris. Fragmented hair shafts are occasionally present within cysts. A minimal or mild inflammatory change in the dermis characterized by infiltration of lymphocytes, macrophages, an occasional multinucleated foreign body giant cell and a few plasma cells is frequently associated with smaller hair shaft/cystic structures. These infiltrates, which were present in all animals, are probably due to rupture or leakage of cyst contents into the dermis and were coded as inflammation, chronic-active, dermis.

Squamous Hyperplasia

Both exposure to SSL and treatment with the *Aloe vera* cream formulations used in this study were associated with diffuse thickening of the epidermis. This change was documented using the term “squamous hyperplasia”. The severity of squamous hyperplasia was graded according to the number of epithelial cell layers in the epidermis:

Minimal	2-4 cell layers
Mild	4-6 cell layers
Moderate	6-9 cell layers
Marked	> 9 cell layers

A few animals in each group (except males in the 6% Aloe whole leaf extract group) treated topically with cream formulations but no SSL had minimal to mild squamous hyperplasia. In mice that were not exposed to SSL, the incidence and severity of squamous hyperplasia was similar in all groups treated with the different cream formulations except females treated with the vehicle cream or 6% Aloe gel which had higher incidence (Table 8). Squamous hyperplasia was observed in one male and one female treated with no cream and no SSL. In SSL-exposed mice, the incidence and severity of squamous hyperplasia increased with increasing light exposure. The application of vehicle cream or creams containing Aloe test substances did not affect the incidence or severity of SSL-induced squamous hyperplasia in either sex of SKH-1 mice (Tables 8, 9).

Table 8
Incidence and Severity of Squamous Hyperplasia in Skin of SKH-1 Mice - Females

Test Substance Exposure/Lesion (SOA*)	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Squamous Hyperplasia				
Minimal	3%	31%	58%	17%
Mild	0	61%	31%	69%
Moderate	0	8%	0	8%
Vehicle Control				
Squamous Hyperplasia				
Minimal	22%	6%		
Mild	6%	75%		
Moderate	0	17%		
Aloe gel 3%				
Squamous Hyperplasia				
Minimal	na**	3%		
Mild	na	92%		
Moderate	na	0		
Aloe gel 6%				
Squamous Hyperplasia				
Minimal	36%	11%		
Mild	0	64%		
Moderate	0	14%		
Marked	0	3%		
Aloe whole leaf extract 3%				
Squamous Hyperplasia				
Minimal	na	19%		
Mild	na	78%		
Moderate	na	0		
Aloe whole leaf extract 6%				
Squamous Hyperplasia				
Minimal	6%	14%		
Mild	0	61%		
Moderate	0	14%		
Charcoal filtered whole leaf extract 3%				
Squamous Hyperplasia				
Minimal	na	6%		
Mild	na	81%		
Moderate	na	8%		
Charcoal filtered whole leaf extract 6%				
Squamous Hyperplasia				
Minimal	3%	22%		
Mild	0	61%		
Moderate	0	14%		
Aloe-emodin 0.56 µg				
Squamous Hyperplasia				
Minimal	na	17%		
Mild	na	72%		
Moderate	na	6%		
Aloe-emodin 5.6 µg				
Squamous Hyperplasia				
Minimal	3%	22%		
Mild	3%	69%		
Moderate	0	3%		

* SOA = Site of application ** no animals in the group

Table 9
Incidence and Severity of Squamous Hyperplasia in Skin of SKH-1 Mice - Males

Test Substance Exposure/Lesion (SOA*)	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Squamous Hyperplasia				
Minimal	3%	36%	39%	25%
Mild	0	58%	17%	47%
Moderate	0	3%	0	6%
Vehicle Control				
Squamous Hyperplasia				
Minimal	0	29%		
Mild	3%	54%		
Moderate	0	6%		
Aloe gel 3%				
Squamous Hyperplasia				
Minimal	na**	33%		
Mild	na	58%		
Moderate	na	0		
Aloe gel 6%				
Squamous Hyperplasia				
Minimal	3%	28%		
Mild	0	69%		
Moderate	0	3%		
Aloe whole leaf extract 3%				
Squamous Hyperplasia				
Minimal	na	50%		
Mild	na	39%		
Moderate	na	6%		
Aloe whole leaf extract 6%				
Squamous Hyperplasia				
Minimal	0	25%		
Mild	0	69%		
Moderate	0	0		
Charcoal filtered whole leaf extract 3%				
Squamous Hyperplasia				
Minimal	na	50%		
Mild	na	44%		
Moderate	na	3%		
Charcoal filtered whole leaf extract 6%				
Squamous Hyperplasia				
Minimal	0	47%		
Mild	3%	50%		
Moderate	0	0		
Aloe-emodin 0.56 µg				
Squamous Hyperplasia				
Minimal	na	34%		
Mild	na	57%		
Moderate	na	0		
Aloe-emodin 5.6 µg				
Squamous Hyperplasia				
Minimal	3%	28%		
Mild	0	61%		
Moderate	0	0		

* SOA = Site of application **no animals in the group

Focal Atypical Squamous Hyperplasia

Exposure of SKH-1 mice to SSL was also associated with focal nodular thickening of the epidermis that was documented using the term “focal atypical squamous hyperplasia”. Focal atypical squamous hyperplasia was differentiated from simple squamous hyperplasia described above by its nodular characteristic. It was a focal, nodular epidermal thickening, whereas squamous hyperplasia was a diffuse thickening of the epidermis. Focal atypical squamous hyperplasia was almost always detected grossly; simple squamous hyperplasia was usually seen only by microscopic examination.

In focal atypical squamous hyperplasia the squamous cells comprising the nodule were arranged in orderly fashion resembling normal epidermis. However, dysplastic changes including lack of cohesion or orientation, pleomorphism, nuclear atypia, and basal disorganization also occurred. Increased numbers of normal and abnormal mitotic figures were common as were inflammatory changes. Increased amounts of keratin were often present over the superficial layers of the hyperplastic epidermis. This “hyperkeratosis” was not coded separately.

Incidence of Focal Atypical Squamous Hyperplasia

The incidence and percent of animals in each dose group that had one, two, three, four, five, or greater than five focal atypical hyperplastic nodules is presented in Tables 10 and 11.

Table 10
Incidence of Focal Atypical Squamous Hyperplasia in Skin of SKH-1 Mice - Females

Test Substance Exposure/Lesion (SOA*)	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Focal atypical squamous hyperplasia				
One	0	19%	28%	14%
Two	0	22%	6%	17%
Three	0	22%	3%	14%
Four	0	8%	0	19%
Five	0	3%	3%	11%
> Five	0	14%	3%	19%
Vehicle Control				
Focal atypical squamous hyperplasia				
One	0	22%		
Two	0	11%		
Three	0	19%		
Four	0	19%		
Five	0	11%		
> Five	0	14%		
Aloe gel 3%				
Focal atypical squamous hyperplasia				
One	na**	28%		
Two	na	22%		
Three	na	8%		
Four	na	8%		
Five	na	3%		
> Five	na	6%		
Aloe gel 6%				
Focal atypical squamous hyperplasia				
One	0	28%		
Two	0	14%		
Three	0	14%		
Four	0	8%		
Five	0	11%		
> Five	0	6%		
Aloe whole leaf extract 3%				
Focal atypical squamous hyperplasia				
One	na	17%		
Two	na	28%		
Three	na	14%		
Four	na	11%		
Five	na	17%		
> Five	na	6%		
Aloe whole leaf extract 6%				
Focal atypical squamous hyperplasia				
One	0	14%		
Two	0	19%		
Three	0	17%		
Four	0	17%		
Five	0	8%		
> Five	0	6%		
Charcoal filtered whole leaf extract 3%				
Focal atypical squamous hyperplasia				
One	na	33%		
Two	na	25%		
Three	na	25%		
Four	na	3%		
Five	na	0		
> Five	na	3%		
Charcoal filtered whole leaf extract 6%				
Focal atypical squamous hyperplasia				
One	0	17%		
Two	0	17%		
Three	0	8%		
Four	0	11%		
Five	0	3%		
> Five	0	19%		
Aloe-emodin 0.56 µg				
Focal atypical squamous hyperplasia				
One	na	22%		
Two	na	14%		
Three	na	22%		
Four	na	17%		
Five	na	8%		
≥ Five	na	6%		
Aloe-emodin 5.6 µg				
Focal atypical squamous hyperplasia				
One	0	17%		
Two	0	14%		
Three	0	14%		
Four	0	14%		
Five	0	11%		
≥ Five	0	6%		

* SOA = Site of application **no animals in the group

Table 11
Incidence of Focal Atypical Squamous Hyperplasia in Skin of SKH-1 Mice - Males

Test Substance Exposure/Lesion (SOA*)	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Focal atypical squamous hyperplasia				
One	0	14%	17%	19%
Two	0	25%	8%	25%
Three	0	17%	3%	8%
Four	0	8%	3%	17%
Five	0	6%	3%	6%
≥ Five	0	22%	0	14%
Vehicle control				
Focal atypical squamous hyperplasia				
One	0	14%		
Two	0	17%		
Three	0	19%		
Four	0	11%		
Five	0	14%		
≥ Five	0	11%		
Aloe gel 3%				
Focal atypical squamous hyperplasia				
One	na**	6%		
Two	na	28%		
Three	na	14%		
Four	na	11%		
Five	na	8%		
≥ Five	na	11%		
Aloe gel 6%				
Focal atypical squamous hyperplasia				
One	0	11%		
Two	0	28%		
Three	0	17%		
Four	0	11%		
Five	0	14%		
≥ Five	0	14%		
Aloe whole leaf extract 3%				
Focal atypical squamous hyperplasia				
One	na	11%		
Two	na	14%		
Three	na	11%		
Four	na	19%		
Five	na	11%		
≥ Five	na	14%		
Aloe whole leaf extract 6%				
Focal atypical squamous hyperplasia				
One	0	11%		
Two	0	14%		
Three	0	33%		
Four	0	11%		
Five	0	3%		
≥ Five	0	22%		
Charcoal filtered whole leaf extract 3%				
Focal atypical squamous hyperplasia				
One	na	11%		
Two	na	39%		
Three	na	25%		
Four	na	0		
Five	na	8%		
≥ Five	na	11%		
Charcoal filtered whole leaf extract 6%				
Focal atypical squamous hyperplasia				
One	0	14%		
Two	0	6%		
Three	0	11%		
Four	0	28%		
Five	0	11%		
≥ Five	0	14%		
Aloe-emodin 0.56 µg				
Focal atypical squamous hyperplasia				
One	na	14%		
Two	na	14%		
Three	na	14%		
Four	na	23%		
Five	na	9%		
≥ Five	na	11%		
Aloe-emodin 5.6 µg				
Focal atypical squamous hyperplasia				
One	3%	22%		
Two	0	14%		
Three	0	11%		
Four	0	11%		
Five	0	14%		
≥ Five	0	19%		

* SOA = Site of application **no animals in the group

The data in Tables 10 and 11 show that the overall incidence of focal atypical squamous hyperplasia was greater in both female and male mice exposed to 13.7 mJ/cm^2 SSL than in those exposed at the 6.85 mJ/cm^2 level. The incidence was similar in animals exposed to 20.55 mJ/cm^2 or 13.7 mJ/cm^2 SSL. The lesion did not occur in animals not exposed to SSL whether or not they received applications of test or control cream. The data further indicate that in mice exposed to 13.7 mJ/cm^2 SSL, the number of animals with focal atypical squamous hyperplasia was similar in all of the treatment subgroups.

Multiplicity of Focal Atypical Squamous Hyperplasia

The average number of focal atypical squamous hyperplasia lesions per mouse (multiplicity) in each treatment group is shown in Table 12 for females and Table 13 for males.

Table 12
Multiplicity in Focal Atypical Squamous Hyperplasia in Skin of SKH-1 Mice
(Average number of lesions per mouse)
Females

Test Substance Exposure/Lesion (SOA*)	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Focal atypical squamous hyperplasia	0	2.806	0.806	3.778
Vehicle Control				
Focal atypical squamous hyperplasia	0	3.278		
Aloe gel 3%				
Focal atypical squamous hyperplasia	na**	1.778		
Aloe gel 6%				
Focal atypical squamous hyperplasia	0	2.278		
Aloe whole leaf extract 3%				
Focal atypical squamous hyperplasia	na	2.778		
Aloe whole leaf extract 6%				
Focal atypical squamous hyperplasia	0	2.472		
Charcoal filtered whole leaf extract 3%				
Focal atypical squamous hyperplasia	na	1.861		
Charcoal filtered whole leaf extract 6%				
Focal atypical squamous hyperplasia	0	2.722		
Aloe-emodin 0.56 µg				
Focal atypical squamous hyperplasia	na	2.611		
Aloe-emodin 5.6 µg				
Focal atypical squamous hyperplasia	0	2.306		

* SOA = Site of application **no animals in the group

Table 13
Multiplicity of Focal Atypical Squamous Hyperplasia in Skin of SKH-1 Mice
(Average number of lesions per mouse)
Males

Test Substance Exposure/Lesion (SOA*)	No SSL	13.7 mJ/cm^2 SSL	6.85 mJ/cm^2 SSL	20.55 mJ/cm^2 SSL
No Cream				
Focal atypical squamous hyperplasia	0	3.278	0.667	2.917
Vehicle Control				
Focal atypical squamous hyperplasia	0	3.086		
Aloe gel 3%				
Focal atypical squamous hyperplasia	na**	2.611		
Aloe gel 6%				
Focal atypical squamous hyperplasia	0	3.250		
Aloe whole leaf extract 3%				
Focal atypical squamous hyperplasia	na	3.000		
Aloe whole leaf extract 6%				
Focal atypical squamous hyperplasia	0	3.556		
Charcoal filtered whole leaf extract 3%				
Focal atypical squamous hyperplasia	na	2.917		
Charcoal filtered whole leaf extract 6%				
Focal atypical squamous hyperplasia	0	3.278		
Aloe-emodin 0.56 µg				
Focal atypical squamous hyperplasia	na	2.914		
Aloe-emodin 5.6 µg				
Focal atypical squamous hyperplasia	0.029	3.472		

* SOA = Site of application **no animals in the group

Data in Tables 12 and 13 show that multiplicity (average number of lesions per mouse) of focal atypical squamous hyperplasia in both sexes was related to SSL exposure. The multiplicity of this lesion was much lower in animals exposed to 6.85 mJ/cm^2 SSL than in those exposed to either 13.7 or 20.55 mJ/cm^2 SSL. However, there was no appreciable difference in the multiplicity of focal atypical squamous hyperplasia among groups of animals exposed to 13.7 mJ/cm^2 SSL and treated topically with any of the Aloe test substance formulations compared to those treated with the vehicle control cream.

Dysplastic cellular changes in focal atypical squamous hyperplastic nodules were qualitatively similar to those in carcinoma creating frequent difficulty in differentiating marked focal atypical squamous hyperplasia, squamous cell papilloma and squamous cell carcinoma *in situ*. In marked focal atypical squamous hyperplasia the epithelium comprising the nodules retained the characteristic arrangement of normal skin and was often arranged in orderly, although variably thickened rete pegs. Squamous cell papillomas were characterized by orderly arrangement of multiple layers of well-differentiated squamous epithelium covering thin branching strands of collagenous stroma. Squamous cell carcinomas *in situ* lacked orderly arrangement of cells and consisted of masses or sheets of cells lacking normal cohesion and orientation that did not penetrate, although often compressed the underlying dermis. Squamous cell carcinomas lacked normal cellular differentiation and orderly arrangement and infiltrated the dermis and often the subcutis. Thus the proliferative lesions of the SKH-1 mouse epidermis induced by exposure to SSL consisted of a continuum of morphologic alterations beginning with slight focal thickening of the epidermis diagnosed as minimal focal atypical squamous hyperplasia, and progressing with increasing proliferation and dysplastic change to marked focal atypical

squamous hyperplasia, squamous cell papilloma, squamous cell carcinoma *in situ*, and ultimately to squamous cell carcinoma.

Miscellaneous Non-neoplastic Lesions in Skin

There were a number of non-neoplastic lesions in skin that were indirectly associated with SSL exposure. These included abscesses, pyogranulomatous inflammation in epidermis, dermis, and subcutaneous tissue, and necrosis and/or ulceration of the epidermis. All of these changes were probably the result of SSL-induced damage that may have been intensified by self mutilation and/or bacterial infection. The incidence and severity of these secondary lesions are summarized in Pathology Report 3 and tabulated by individual animal in Pathology Report 9. These compilations are in Appendices II and IV, respectively, of this report.

SUMMARY

The results of this 52-week photocarcinogenicity study in male and female SKH-1 hairless mice demonstrated increased incidence of squamous cell papillomas and increased multiplicity of squamous cell papillomas and squamous cell carcinomas *in situ* in male and female mice exposed to 13.7 mJ/cm^2 SSL and treated with creams containing 3% or 6% Aloe gel, 3% or 6% Aloe whole leaf extract, 3% or 6% charcoal filtered Aloe whole leaf extract, or 0.56 µg or 5.6 µg Aloe-emodin compared to mice treated with vehicle control cream. In general, treatment with Aloe test substances in SSL-exposed mice was associated with increased multiplicity but not increased incidence of squamous cell carcinoma *in situ* in both sexes compared to treatment with vehicle control cream. In SSL-exposed male and female mice the incidence and multiplicity of SSL-induced focal atypical squamous cell hyperplasia was unaffected by treatment with Aloe test substances compared to mice treated with vehicle control cream. In mice treated with no cream and exposed to 13.7 mJ/cm^2 SSL versus 20.55 mJ/cm^2 SSL, the incidence and multiplicity of squamous cell neoplasms and of focal atypical squamous cell hyperplasia were not appreciably different and were greater than in mice treated with no cream and exposed to 6.85 mJ/cm^2 SSL.

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Paul W. Mellick, D.V.M., PhD.
Diplomate, A.C.V.P.