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PHOTOCARCINOGENESIS: MEASURING THE REPRODUCIBILITY OF A BIOLOGIC RESPONSE TO ULTRAVIOLET RADIATION EXPOSURE IN MICE

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1. ABSTRACT

New drugs undergo safety evaluations of many types. For some drugs, a photocarcinogenesis study forms one of the elements in the overall toxicology package. Photocarcinogenesis studies are designed to evaluate a drug's ability to modify the growth and development of ultraviolet radiation (UVR)-induced skin tumors in albino hairless mice. "Exposure control" groups in such studies receive the UVR, either alone, or in combination with the "vehicle" or carrier associated with each study. This report presents skin tumor data from control groups compiled from nine consecutive studies conducted at this testing facility. The endpoints evaluated included median tumor onset, mortality-free prevalence and tumor yield. "Historical control data" are considered essential for designing, monitoring, interpreting and evaluating studies of a given type. In addition, a compilation of such control data can illustrate trends or provide measures of reproducibility more reliably than can individual studies. This data set shows how clearly the UVR-induced skin tumor onset time is dependent on UVR dose, how skin tumors develop sooner in female mice than in male mice at a low UVR exposure dose, and that topical administration of certain vehicle formulations can enhance photocarcinogenesis.

2. INTRODUCTION

The causative relationship between sunlight exposure and skin tumors is well known (1, 2). Less well known is the interactive effect of compounds that enhance sunlight-induced skin tumor development. Psoralens (3-13), antibiotics (14-16), retinoids (17-19), and immunosuppressants (20-24) are among the compounds for which enhancement of photocarcinogenesis has been demonstrated in animal models. In man, one psoralen (8-methoxypsoralen) has been demonstrated to enhance skin tumor production among patients who are also exposed to long wave ultraviolet radiation (UVA, 315 – 400 nm) in a treatment modality referred to as PUVA (25). Among patients undergoing immunosuppressant therapy after transplantation, an increase in skin tumor incidence has been reported and this increase has been considered possibly related to enhancement of sunlight-induced skin tumor development (26).

For about twenty years, regulatory agencies in the United States and other countries have been concerned about the enhancement of ultraviolet radiation (UVR)-induced skin tumors and have requested photosafety data on compounds that meet stated criteria (27). Typically, these studies involve administration of the compound and simulated sunlight exposure using hairless mice as the test system or animal model (28-32).

Over the past twelve years, our testing facility has conducted approximately seventeen of these studies with some modifications in the experimental procedures occurring over the first few years of this period. Since 1996

the procedures have been virtually unchanged. The purpose of this paper is to present skin tumor data from the control groups in all completed studies conducted since 1996.

3. MATERIALS AND METHODS

Data from all nine studies conducted by the testing facility from 1996 to the present were compiled. All studies were conducted in compliance with U.S. Food and Drug Administration *Good Laboratory Practice Regulations*; Final Rule. 21 CFR Part 58.

3.1. Animals and Husbandry

Albino hairless mice (Crl:SKH1-*hr*BR; Charles River Laboratories, Inc., Portage, Michigan) were used in all studies. Each group within a study included thirty-six male and thirty-six female mice. The mice were approximately 8 weeks of age and typically weighed 27 to 32 g (males) or 21 to 26 g (females) at the start of formulation administration and UVR exposure. The mice were acclimated to the housing rooms for two or three weeks before study start. Mice were individually housed in stainless steel cages especially designed to allow for UVR exposure of free moving mice and were permanently identified using a tail tattoo (AIMS Animal Identification and Marking System, AIMS, Inc., Piscataway, New Jersey, AIMS Black Pigment #242). Each animal room was independently supplied with at least ten changes per hour of 100% fresh air that passed through 99.97% HEPA filters. Room temperature was maintained at 74°F to 82°F (temperature appropriate for hairless mice) and monitored constantly. Room humidity was monitored constantly and maintained at 30% to 70%. Fluorescent "gold" lamps (F40GO or equivalent) were used to illuminate the housing rooms and an automatically controlled light cycle of 12-hours light:12-hours dark was maintained. Mice were given Certified Rodent Diet® #5002 (PMI Nutrition International) and water *ad libitum.* Animal use and housing were compliant with the *Guide for the Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, National Research Council (NIH 86-28, 1985; National Academy of Sciences 1996). Each study protocol was reviewed and approved by the Testing Facility's Institutional Animal Care and Use Committee.

3.2. Design of Studies

The design of studies that evaluate the influence of formulation administration on UVR induced skin tumors (i.e., photocarcinogenesis) has been described (28, 32). The design is briefly described here.

The studies consisted of five to eight groups of hairless mice. Formulation administration and simulated sunlight exposure (SSE) were conducted for forty consecutive weeks and the mice were evaluated weekly throughout this period and for twelve or thirteen weeks following completion of formulation administration and SSE. All mice were exposed to SSE and test article or vehicle was administered to all but two groups in each study. The route of administration was either topical or oral (gavage). The two groups in each study that only received SSE were included as calibration groups. Data generated from the two calibration groups in each study are presented here as historical control data. Additionally, the historical control data include the results from the group in each study that received vehicle administration and SSE. "Vehicle" in each case refers to one of several proprietary formulations provided by the study sponsors.

One of the SSE calibration groups and the vehicle-treated group in each study received a dose of radiation of approximately 600 Robertson-Berger Units (RBU) per week and the other SSE calibration group typically received 1200 RBU per week. [The RBU is a measure of biological effectiveness for ultraviolet radiation (UVR); 400 RBU approximates one minimal erythema dose in previously untanned human skin.] In one study (Study ID I) vehicle was not administered to mice irradiated with the low SSE dose and in another study (Study ID F) the high SSE calibration was less than 1200 RBU per week. Therefore, skin tumor data for these two occurrences were not included in this compilation. In three studies (Study ID A, C and G) the route of administration was oral (gavage) and in all other studies the route of administration was topical.

Table 1 includes the weekly formulation administration and SSE exposure regimen. On Monday, Wednesday and Friday of each week, vehicle was administered one-half to one hour before SSE exposure; on Tuesday and Thursday of each week, the vehicle was administered one-half to one hour after SSE exposure. The alternating regimen of formulation administration and UVR exposure is used to address the possible interactive effect(s) of photolability on a test article (i.e., the alternating regimen allows for detection of modification of photocarcinogenesis with test articles which are activated, deactivated or unmodified by SSE).

3.3. Source of Irradiation

A 6.5 kilowatt xenon long arc, water cooled burner was vertically suspended within an octagonal metal frame holding one optical filter on each side. Each filter (15 cm by 15 cm, 1 mm thick; Schott WG 320 doped glass) was held approximately 20 cm from the burner. During exposure, the racks holding the mouse cages were located approximately 2.25 meters from the UVR source. Each rack of cages was irradiated through one filter; all racks of cages in each study were irradiated simultaneously from one xenon arc. Each rack was monitored by a customized detector system, which recorded both intensity and SSE dose (in RBU). A typical emission spectrum for this type of light source is included in Figure 1.

3.4. In Life Observations

The mice were observed twice daily for mortality and morbidity. Clinical observations were performed weekly and body weights were recorded weekly or monthly. Skin tumor data were recorded weekly using a specially designed computer system that captured the anatomical position, size and fate of each tumor (Tumor Tracker System, Argus Research Laboratories, a Division of Charles River Laboratories, Horsham, Pennsylvania).

Table 1. Weekly regimen of vehicle administration and UVR exposure

MONDAY		TUESDAY		WEDNESDAY		THURSDAY		FRIDAY		
VEH PRE UVR	UVR (RBU)	UVR (RBU)	VEH POST UVR	VEH PRE UVR	UVR (RBU)	UVR (RBU)	VEH POST UVR	VEH PRE UVR	UVR (RBU)	RBU PER WEEK
Group 1 VEH	120	120	Group 1 VEH	Group 1 VEH	120	120	Group 1 VEH	Group 1 VEH	120	600
Group 2 NONE	120	120	Group 2 NONE	Group 2 NONE	120	120	Group 2 NONE	Group 2 NONE	120	600
Group 3 NONE	240	240	Group 3 NONE	Group 3 NONE	240	240	Group 3 NONE	Group3 NONE	240	1200

A representative weekly regimen of formulation administration and solar simulation exposure in studies designed to assess the modification of photocarcinogenesis. Typically, these studies include five or more groups and include groups of mice that are administered various dosages of a test article. The table presented here only includes a vehicle-treated group and the two UVR calibration groups. Shading emphasizes the alternating sequence (vehicle applied pre- or post- UVR) of treatments. Abbreviations: UVR:Ultraviolet Radiation (Solar simulated light exposure), RBU:Robertson-Berger Units (a measure of effectiveness for UVR; 400 RBU approximates one minimal erythema dose in previously untanned human skin); 400 RBU is also the instrumental equivalent of 2 SED (Standard Erythema Dose; see reference 35); VEH:Vehicle formulation.

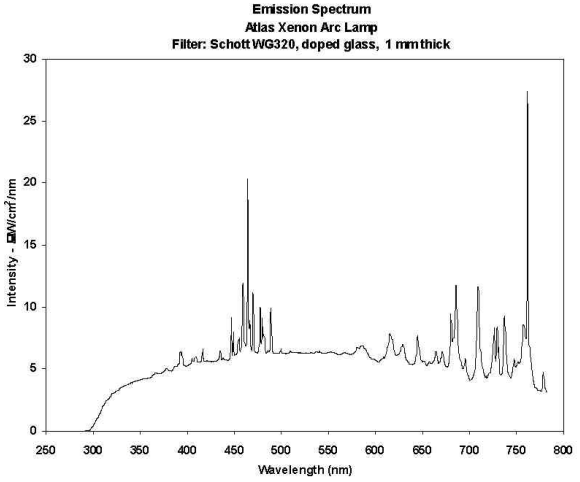

Figure 1. A typical emission spectrum for the 6.5 kilowatt xenon long arc lamp with a 1 mm Schott WG 320 doped glass filter.

During the conduct of the study, individual mice with 10 mm tumors (planar diameter) were sacrificed. A group of mice was sacrificed when both male and female mice met the following criteria. Fewer than one-half of the mice survived and more than one-half of the surviving mice had tumors of at least 4 mm (planar diameter). At the end of fifty-two or fifty-three weeks all surviving mice were sacrificed. All animals were euthanized by carbon dioxide asphyxiation. Necropsies were performed on all mice.

3.5. Data Analyses

Only skin tumor data are presented here. Data were transferred from the Tumor Tracker System to a computer program designed to tabulate, plot and statistically analyze the skin tumor data (i.e., the RoeLee system, P. N. Lee Statistics and Computing LTD, Sutton, United Kingdom). Separate calculations were performed for each of the following size-based acceptance criteria:

all observed tumors;

tumors at least 1 mm in maximum planar diameter;

tumors at least 2 mm in maximum planar diameter; and

tumors at least 4 mm in maximum planar diameter.

In reporting results, the following descriptive parameters were used.

1. "Median Onset" or “Unbiased Median Week to Tumor”: The time at which one-half of the members of the groups have acquired one or more qualifying tumors.

2. "Mortality-free Prevalence": The proportion of mice in a group exhibiting one or more qualifying tumors, as a function of time, and adjusted for the effects of competing mortality. This descriptor is the complementary probability to the Kaplan-Meier "probability of survival without a tumor" and is derived from calculations of the Kaplan-Meier type (33).

3. "Tumor Yield": The number of tumors present, divided by the number of surviving mice (i.e., average number of tumors per mouse).

For the sake of brevity, the results reported here were limited to parameters for tumor size categories that were considered most illustrative of skin tumor induction and development.

4. RESULTS

Tables 2, 3 and 4 include unbiased median week to tumor (i.e., median onset) data for skin tumors at least 1, 2 and 4 mm in diameter, respectively. For skin tumors ≥ 1 mm, median week to tumor ranged from 34.00 through 43.00 (sexes combined) in the groups of mice exposed to 600 RBU/Week without vehicle administration (Table 2). For the same tumor size category (sexes combined), median week to tumor ranged from 20.50 through 26.00 in the groups of mice exposed to 1200 RBU/Week. The means of the median week to tumor values (tumors ≥ 1 mm) were 39.28 and 24.25 for mice (sexes combined) exposed to 600 and 1200 RBU/Week, respectively. For

Table 2. Unbiased median weeks to tumor for skin tumors = 1 mm

STUDY ID	Unbiased Median Week to Tumor - Sexes Combined - Skin Tumors ³ 1 mm UVR Exposure (RBU/Week)			Unbiased Median Week to Tumor - Males - Skin Tumors ³ 1 mm UVR Exposure (RBU/Week)			Unbiased Median Week to Tumor - Females - Skin Tumors ³ 1 mm UVR Exposure (RBU/Week)		
	600	600	1200	600	600	1200	600	600	1200
	Formulation Administration			Formulation Administration			Formulation Administration		
	(Vehicle)	None	None	(Vehicle)	None	None	(Vehicle)	None	None
A	43.00	42.00	26.00	44.00	44.00	26.30	43.00	40.00	26.00
B	28.00	40.00	25.00	27.00	42.00	26.00	28.00	37.50	24.00
C	42.00	41.00	25.00	42.50	41.00	25.00	42.00	41.00	25.00
D	32.00	43.00	24.50	33.50	46.50	25.00	31.00	40.50	24.00
E	26.00	37.00	20.50	25.00	38.25	24.00	27.00	35.50	20.00
F	33.75	42.00	N/A	33.50	44.50	N/A	36.00	42.00	N/A
G	36.00	39.00	25.00	38.00	39.00	25.00	35.00	36.50	24.00
H	37.00	35.50	24.00	37.00	37.00	25.00	36.50	34.00	23.50
I	N/A	34.00	24.00	N/A	34.00	24.00	N/A	33.00	25.00
MEAN	34.72	39.28	24.25	35.06	40.69	25.04	34.81	37.78	23.94
STD. DEV.	6.07	3.15	1.65	6.75	4.00	0.82	5.92	3.25	1.78

Unbiased median week to tumor (skin tumors ≥ 1 mm in planar diameter) in nine studies conducted to assess the modification of UVR-induced skin tumors in hairless mice. The median values are for male and female mice combined (i.e., sexes combined) and male and female mice handled separately. Abbreviations: UVR: Ultraviolet radiation, RBU:Robertson-Berger units, N/A: Not applicable, STD. DEV. :Standard deviation

Table 3. Unbiased median weeks to tumor for skin tumors = 2 mm

STUDY ID	Unbiased Median Week to Tumor - Sexes Combined - Skin Tumors ³ 2 mm UVR Exposure (RBU/Week)			Unbiased Median Week to Tumor - Males - Skin Tumors ³ 2 mm UVR Exposure (RBU/Week)			Unbiased Median Week to Tumor - Females - Skin Tumors ³ 2 mm UVR Exposure (RBU/Week)		
	600	600	1200	600	600	1200	600	600	1200
	Formulation Administration			Formulation Administration			Formulation Administration		
	(Vehicle)	None	None	(Vehicle)	None	None	(Vehicle)	None	None
A	45.50	47.00	27.00	46.00	47.50	28.00	44.50	45.00	27.00
B	31.00	44.50	28.00	30.50	45.00	28.00	31.00	41.50	27.00
C	46.00	48.00	29.00	47.00	48.00	29.00	46.00	47.00	28.00
D	35.00	44.00	28.00	38.00	49.13	28.00	33.00	43.00	27.00
E	28.00	42.00	23.00	28.00	42.00	23.00	29.00	39.00	23.00
F	42.00	45.00	N/A	41.50	45.00	N/A	42.00	42.00	N/A
G	38.50	41.00	27.00	40.00	41.50	27.00	37.00	40.00	28.00
H	39.00	37.50	25.50	38.50	39.00	26.00	40.00	36.00	25.00
I	N/A	36.00	25.00	N/A	36.00	24.00	N/A	36.00	29.00
MEAN	38.13	42.78	26.56	38.69	43.68	26.63	37.81	41.06	26.75
STD. DEV.	6.50	4.06	1.95	6.70	4.40	2.13	6.35	3.75	1.91

Unbiased median week to tumor (skin tumors ≥ 2 mm in planar diameter) in nine studies conducted to assess the modification of UVR-induced skin tumors in hairless mice. The median values are for male and female mice combined (i.e., sexes combined) and male and female mice handled separately. Abbreviations: UVR: Ultraviolet radiation, RBU:Robertson-Berger units, N/A: Not applicable, STD. DEV. :Standard deviation

skin tumors ≥ 2 mm, median week to tumor ranged from 36.00 through 48.00 (sexes combined) in the groups of mice exposed to 600 RBU/Week without vehicle administration (Table 3). For the same tumor size category (sexes combined), median week to tumor ranged from 23.00 through 29.00 in the groups of mice exposed to 1200 RBU/Week. The means of the median week tumor values (tumors ≥ 2 mm) were 42.78 and 26.56 for mice (sexes combined) exposed to 600 and 1200 RBU/Week, respectively. For skin tumors ≥ 4 mm, median week to tumor ranged from 39.00 through 54.00 (sexes combined) in the groups of mice exposed to 600 RBU/Week without vehicle administration (Table 4). For the same tumor size category (sexes combined), median week to tumor ranged from 27.00 through 35.00 in the groups of mice exposed to 1200 RBU/Week. The means of the median week to tumor values (tumors ≥ 4 mm) were 48.00 and 31.06 for mice (sexes combined) exposed to 600 and 1200 RBU/Week, respectively.

If we assume that the medians are representative of tumor growth, the estimated time for transition from a 1 mm to a 2 mm skin tumor (sexes combined) was approximately 3.5 weeks at 600 RBU/Week and approximately 2.3 weeks at 1200 RBU/Week. The time for transition from a 2 mm to a 4 mm skin tumor (sexes combined) was slightly more than 5 weeks at 600 RBU/Week and approximately 4.5 weeks at 1200 RBU/Week.

Vehicle administration shortened median onset of skin tumors in some of the studies. In studies B, D and E, median week to tumor was reduced in mice administered the vehicle and exposed to 600 RBU/Week for the ≥ 1 mm, ≥ 2 mm and ≥ 4 mm tumor size categories, as compared with mice only exposed to 600 RBU/Week (Tables 2 through 4). In study F, median week to tumor was reduced for the ≥ 1 mm category and slightly reduced for the ≥ 2 mm and ≥ 4 mm categories in mice administered the vehicle. In each of the studies that revealed enhancement of photocarcinogenesis in mice administered the vehicle, the route of administration was topical.

The data for the separate sexes revealed that skin tumors tended to occur slightly earlier in female mice

Table 4. Unbiased median weeks to tumor for skin tumors = 4 mm

STUDY ID	Unbiased Median Week to Tumor - Sexes Combined - Skin Tumors ³ 4 mm UVR Exposure (RBU/Week)			Unbiased Median Week to Tumor - Males - Skin Tumors ³ 4 mm UVR Exposure (RBU/Week)			Unbiased Median Week to Tumor - Females - Skin Tumors ³ 4 mm UVR Exposure (RBU/Week)		
	600	600	1200	600	600	1200	600	600	1200
	Formulation Administration			Formulation Administration			Formulation Administration		
	(Vehicle)	None	None	(Vehicle)	None	None	(Vehicle)	None	None
A	52.00	52.00	32.00	52.00	52.00	31.00	54.00	52.00	33.00
B	36.50	47.50	31.00	36.00	51.00	31.00	37.50	46.00	32.00
C	51.50	54.00	35.00	51.00	54.00	35.00	52.00	54.00	36.00
D	43.00	52.00	31.50	43.00	53.00	30.00	42.50	49.00	33.00
E	34.50	48.00	27.00	34.00	48.00	27.00	36.50	48.50	26.50
F	45.50	49.00	N/A	43.50	50.00	N/A	46.00	47.00	N/A
G	44.50	46.50	32.00	45.50	48.50	31.00	43.00	46.50	34.00
H	41.00	44.00	30.00	41.00	44.00	30.00	41.50	43.00	32.00
I	N/A	39.00	30.00	N/A	39.00	28.00	N/A	39.00	31.00
MEAN	43.56	48.00	31.06	43.25	48.83	30.38	44.13	47.22	32.19
STD. DEV.	6.30	4.59	2.27	6.38	4.76	2.39	6.28	4.49	2.75

Unbiased median week to tumor (skin tumors ≥ 4 mm in planar diameter) in nine studies conducted to assess the modification of UVR-induced skin tumors in hairless mice. The median values are for male and female mice combined (i.e., sexes combined) and male and female mice handled separately.Abbreviations: UVR: Ultraviolet radiation, RBU:Robertson-Berger units, N/A: Not applicable, STD. DEV. :Standard deviation

Table 5. Maximum number of skin tumors per surviving mice

Study Identification	Maximum Number of Skin Tumors per Surviving Mice - Sexes Combined - (Skin Tumors ³ 1 mm)		
	UVR Exposure (RBU/Week)		
	600	600	1200
		Test Article	
	Vehicle	None	None
A	4.40	4.40	6.90
B	16.73	9.59	14.00
C	4.71	3.85	6.79
D	9.74	4.13	10.43
E	8.00	3.33	12.21
F	9.34	6.31	N/A
G	8.74	6.18	7.24
H	3.78	4.44	5.50
I	N/A	5.68	4.58
MEAN ± STD.DEV.	8.18 ± 4.19	5.32 ± 1.91	8.46 ± 3.36

Maximum number of skin tumors per surviving mice (i.e., tumor yield) in nine studies conducted to assess the modification of UVR-induced skin tumors in hairless mice. The tumor yield values are for male and female mice combined and for skin tumors ≥ 1 mm in planar diameter. UVR: Ultraviolet radiation, RBU:Robertson-Berger units, N/A: Not applicable, STD. DEV. :Standard deviation

exposed to 600 RBU/Week, as compared with male mice (Tables 2 through 4) at least for the two smaller tumor size categories. For the ≥ 1 mm tumor size category, the means for the median week to tumor were 40.69 and 37.78 in male and female mice, respectively (600 RBU/Week, without vehicle administration). For the ≥ 2 mm tumor size category, the means were 43.68 and 41.06 weeks in male and female mice, respectively (600 RBU/Week, without vehicle administration). For the ≥ 4 mm tumor size category, the means were 48.83 and 47.22 weeks in male and female mice, respectively (600 RBU/Week, without vehicle administration). In mice exposed to 1200 RBU/Week, the means for median week to tumor were comparable in male and female mice.

Skin tumor prevalence is depicted graphically in Figures 2 and 3. The prevalence data clearly demonstrates UVR dose-dependence with respect to skin tumor onset. Each curve (or cumulative distribution function) represents the mean prevalence of all studies evaluated. Inspection of the prevalence plots reveals the following salient findings. In mice exposed to 600 RBU/Week (sexes combined): skin tumors first occurred at approximately 22, 28 and 32 weeks for tumor sizes ≥ 1 mm, ≥ 2 mm and ≥ 4 mm, respectively. For the same respective tumor size categories (600 RBU/Week, sexes combined), prevalence reached the 0.5 value approximately in weeks 37, 41 and 47, and at week 52 the prevalence values were at approximately 1.0, 0.9 and 0.6. In mice exposed to 1200 RBU/Week (sexes combined): skin tumors first occurred at approximately 17, 19 and 22 weeks for tumor sizes ≥ 1 mm, ≥ 2 mm and ≥ 4 mm, respectively. For the same respective tumor size categories (1200 RBU/Week, sexes combined), prevalence reached the 0.5 value approximately in weeks 23, 26 and 30 and prevalence values reached or nearly reached 1.0 before week 45.

The maximum tumor yield and the study week in which the maximum tumor yield occurred for the appropriate groups in each study were tabulated (Tables 5 and 6, respectively). In mice exposed to 600 RBU/Week (sexes combined, tumors ≥ 1 mm) without vehicle

Table 6. Week of occurrence of the maximum number of skin tumors per surviving mice

Study Identification	Week of Occurrence of Maximum Number of Skin Tumors per Surviving Mice - Sexes Combined - (Skin Tumors ³ 1 mm)		
	UVR Exposure (RBU/Week)		
	600	600	1200
	Test Article		
	Vehicle	None	None
A	52	52	44
B	45	52	36
C	53	53	37
D	49	53	36
E	42	52	35
F	50	50	N/A
G	48	52	38
H	47	46	34
I	N/A	44	35
MEAN ± STD.DEV.	48 ± 4	50 ± 3	37 ± 3

Week of occurrence of the maximum number of skin tumors per surviving mice in nine studies conducted to assess the modification of UVR-induced skin tumors in hairless mice. The values are for male and female mice combined and for skin tumors ≥ 1 mm in planar diameter. UVR: Ultraviolet radiation, RBU:Robertson-Berger units, N/A: Not applicable, STD. DEV. :Standard deviation

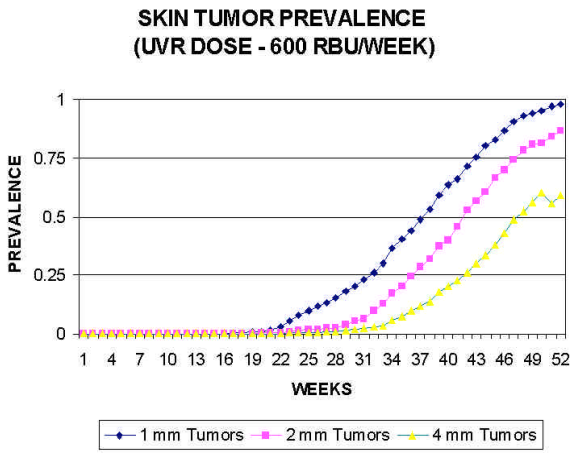

Figure 2. Average skin tumor prevalence for tumors ≥1, ≥2 and ≥4 mm in planar diameter for mice (sexes combined) exposed to simulated sunlight at 600 RBU/Week for the first forty weeks of each study. Each curve (or cumulative distribution function) represents the mean prevalence of the nine studies reported here.

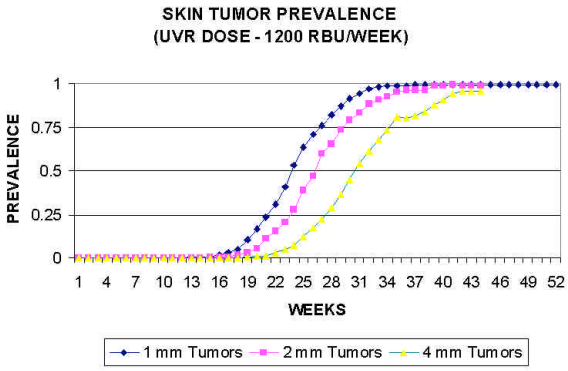

Figure 3. Average skin tumor prevalence for tumors ≥1, ≥2 and ≥4 mm in planar diameter for mice (sexes combined) exposed to simulated sunlight at 1200 RBU/Week for the first forty weeks of each study. Each curve (or cumulative distribution function) represents the mean prevalence of the nine studies reported here.

administration, the maximum tumor yield ranged from 3.33 to 9.59 skin tumors per mouse and the maximum values tended to occur during the last few weeks of each study (i.e., in weeks 50 through 53). In mice exposed to 1200 RBU/Week (sexes combined, tumors ≥ 1 mm), the maximum tumor yield ranged from 4.58 to 14.00 tumors per mouse and the mean week of occurrence of maximum tumor yield was 37. Tumor yield in mice exposed to 1200 RBU/Week was truncated by the early sacrifice of mice because of tumor burden. Typically, none of the mice in the 1200 RBU/Week group survive to scheduled sacrifice in these types of studies. In mice administered vehicle and exposed to UVR at 600 RBU/Week (sexes combined, tumors ≥ 1 mm), the mean maximum skin tumor yield was 8.18, as compared with 5.32 in mice only exposed to UVR at 600 RBU/Week. In mice administered vehicle and exposed to UVR at 600 RBU/Week (sexes combined, tumors ≥ 1 mm), the mean week in which the maximum skin tumor yield occurred was week 48, as compared with week 50 in mice only exposed to UVR at 600 RBU/Week. Therefore, inspection of the maximum tumor yield data clearly indicates that these parameters were also affected by topical administration of some vehicles.

5. DISCUSSION

Compilation of historical control data can be very useful. The data allow for temporal prediction and scrutiny of skin tumor production in ongoing studies and can serve as a source of information in interpreting the results of photocarcinogenesis studies. Additionally, the data can be useful in designing new studies.

Inspection of these data revealed some findings that were anticipated. Median onset of UVR-induced skin tumors was dependent on UVR dose (i.e., skin tumors occurred earlier in mice exposed to the high UVR dose, as compared with mice exposed to the low UVR dose). Prevalence of UVR-induced skin tumors was dependent on UVR dose (i.e., once skin tumors started to occur, the mortality-adjusted proportion of mice with skin tumors at any time point was greater in mice exposed to the high

UVR dose, as compared with mice exposed to the low UVR dose). Yield of UVR-induced skin tumors was dependent on UVR dose (i.e., once skin tumors started to occur, the average number of skin tumors per mouse at any time point was greater in mice exposed to the high UVR dose, as compared with mice exposed to the low UVR dose).

Inspection of these data also revealed findings that were not anticipated. The apparent reduction in tumor onset in female mice as compared with male mice at the low UVR exposure dose was striking when all data were evaluated. The range of influence of the various vehicle formulations (i.e., interaction with the UVR effect) was also unanticipated. Vehicle-induced reduction of tumor onset time occurred in four of the nine studies evaluated and in four of the six studies in which the route of administration was topical. In one study, the median onset was reduced as much as 15 weeks (male mice, tumors ≥ 1 mm). An acceleration in tumor onset of this magnitude is equivalent to doubling the UVR dose. Vehicle effects on photocarcinogenesis have been reported previously (34), but only in terms of tumor multiplicity.

6. CONCLUSION

The conduct of studies designed to assess test article modification of UVR-induced skin tumor development in hairless mice has permitted accumulation of useful data on UVR dosage-dependence and vehicle-effects. In addition to providing useful information for designing, monitoring and interpreting studies, compilation of these data revealed the following salient findings. UVR-induced skin tumor onset in hairless mice is clearly dependent on UVR dose. There is a tendency for skin tumors to develop sooner in female mice than in male mice at the low UVR exposure dose. Topical administration of vehicle formulations can enhance photocarcinogenesis.

7. ACKNOWLEDGEMENTS

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