

STANDARDIZED PROTOCOLS FOR PHOTOCARCINOGENESIS SAFETY TESTING

P. Donald Forbes¹, Janusz Z. Beer², Homer S. Black³, Jean-Pierre Cesarini⁴, Curtis A. Cole⁵, Ronald E. Davies⁶, John M. Davitt⁷, Frank deGrujl⁸, John Epstein⁹, Anny Fournanier¹⁰, Adèle Green¹¹, Thomas Koval¹², Ronald D. Ley¹³, Romano Mascotto¹⁴, Warwick Morison¹⁵, Robert Osterberg¹⁶, David Sliney¹⁷, Frederick Urbach¹⁸, Jan C. van der Leun¹⁹, and Antony R. Young²⁰

¹Argus Research, Charles River Laboratories, Inc. Horsham, PA 19044, USA; ²US Food and Drug Administration, Center for Devices and Radiologic Health, Rockville, MD, 20852, USA; ³Veterans Affairs Medical Center and Baylor College of Medicine, Houston, Texas 77030, USA; ⁴Foundation Rothchild, 75019 Paris, France; ⁵Johnson & Johnson Consumer Products Co., Skillman, NJ 08558, USA; ⁶Chisholm Township, Ontario POH 1ZO, Canada; ⁷Regulatory Affairs Services, Silver Spring, MD 20904, USA; ⁸Dermatology, Leiden University Medical Center, Leiden, Netherlands; ⁹450 Sutter St., Suite 1306, San Francisco, CA 94108, USA; ¹⁰Groupe Dermato-Biologie, L'Oreal, 92583 Clichy Cedex, Paris, France; ¹¹Queensland Institute Medical Centre, Brisbane, Queensland 4029, Australia; ¹²NCRP, Suite 800, Bethesda, MD 20814 USA; ¹³University of New Mexico, Albuquerque, NM 87131-5218, USA; ¹⁴Corporate Research and Development, L'Oreal, 92583 Clichy Cedex, Paris, France; ¹⁵10753 Falls Rd., Suite 205, Lutherville, MD 21093, USA; ¹⁶U.S. Food and Drug Administration, Rockville, MD 20857, USA; ¹⁷U.S. Army Environmental Hygiene Agency, Aberdeen Proving Grounds, MD 21010-5422, USA; ¹⁸438 Clairemont Road, Villanova, PA 19085-1706 USA; ¹⁹EcoSys, Utrecht, Netherlands; ²⁰St. John's Institute of Dermatology, King's College, London, SE1 7EH, United Kingdom

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1. ABSTRACT

Solar ultraviolet radiation (UVR) is recognized as a major cause of non-melanoma skin cancer in man. Skin cancer occurs most frequently in the most heavily exposed areas and correlates with degree of outdoor exposure. The incidence of skin cancer is also increased by contact with photosensitizing drugs and chemicals such as psoralens, coal tars and petroleum stocks. Other substances which do not act as photosensitizers, such as immunosuppressants taken by organ transplant recipients, also increase the risk of skin cancer. The U.S. Food and Drug Administration requests, on a case-by-case basis, that risk of enhanced photocarcinogenesis is assessed for many classes of drugs. Health Canada's Therapeutic Products Programme has issued a Notice of Intent to regulate pharmaceutical products which may enhance carcinogenicity of the skin induced by ultraviolet radiation. Other national regulatory agencies review such data when they exist, but their own requirements emphasize batteries of short-term *in vitro* and *in vivo* tests. While they may support drug development strategies, short-term tests have yet to be validated as predictors of the ability of drugs or chemicals to enhance photocarcinogenesis. Published protocols now describe study designs and procedures capable of determining whether test agents enhance the rate of formation of UVR-induced skin tumors.

2. INTRODUCTION

Studies in photobiology and photomedicine tend to confirm that several basic principles about

photocarcinogenesis are shared by man and mouse. Both species develop non-melanoma skin cancers in heavily UVR-exposed areas, with the response related to time and intensity of exposures. Both species exhibit DNA damage and mutations in the *p53* tumor suppressor region, along with evidence of altered immune function.

UVR with wavelengths shorter than 320 nanometers causes sunburn, and can cause basal cell and squamous cell cancers in humans. In laboratory studies, both sunburn and photocarcinogenesis are dose-dependent (1-5). Doses of UVR which cause sunburn (prolonged cutaneous inflammation, scaling and vascular ectasias) are associated with increased risk of skin cancer in man. These similarities provide part of the basis for estimating risks to man from changes in earth level sunlight (6) and from man-made sources (7, 8).

Studies have also shown that the risk for sunlight-induced skin cancer in humans can be modified by such environmental factors as chemicals and drugs. For example, although the medical use of therapeutic coal tar appears to provide little additional skin cancer risk, multiple, aggressive squamous cell cancers have been reported in a substantial number of roofers and oil field workers (9-11). In the third decade of the prospective study on psoralen-UVA (PUVA) therapy for psoriasis, the skin cancer risk continues to increase for the treated population, even for those whose PUVA ended many years earlier (12-16). A dramatic increase in skin cancer risk was noted in

chronically immune-suppressed organ transplant recipients (17). In general, these clinical observations had been anticipated by a substantial body of prior laboratory animal studies (9, 10, 18-22).

Agents that enhance photocarcinogenesis can be defined as chemicals or treatments which increase the rate at which specified doses of UVR cause skin cancer under laboratory conditions. Examples include emollients that may alter the transmission of UVR into and through the skin (23), the tumor promoter croton oil (24), topical and systemic retinoids (25), psoralens such as 8-methoxypsoralen (8-MOP) (26), and the more phototoxic fluoroquinolone antimicrobials (27). For most of the agents that enhance photocarcinogenesis in mice there is as yet no direct evidence of a comparable effect in man. The regulatory approval for such products may require precautionary measures such as labeling for the benefit of users (28).

Consistent with the clinical situation, agents that enhance experimental photocarcinogenesis do not operate *via* a single mechanism. For example, carcinogens, tumor promoters, mitogens, immune suppressors and photosensitizers may all increase the rate of tumor formation caused by UVR exposure. Ideally, photocarcinogenesis safety testing should be independent of mechanism. Likewise, the ideal test would assess the ability of test agents (TA) to increase, or decrease, the rates of skin tumor formation in mice that are exposed to UVR.

The U.S. Food and Drug Administration requests, on a case-by-case basis, that risk of enhanced photocarcinogenesis be assessed for many classes of drugs (28). Health Canada's Therapeutic Products Programme has issued a Notice of Intent to regulate pharmaceutical products that may enhance carcinogenicity of the skin induced by ultraviolet radiation (29).

Photocarcinogenesis protocols of two basic types have been used for regulatory submission. The first type is designed to answer questions about a drug in the presence of a specific waveband of optical radiation. Examples of studies of this type include psoralen-UVA (PUVA) phototherapy (26), and the comparative effectiveness of several fluoroquinolone antibiotics (27). In this targeted or mechanistic study design, the selection of lamps and exposure conditions tends to minimize the likelihood of tumor production, at least in the absence of a drug effect. The other category of study design attempts to answer the broader question of anticipated human exposure to sunlight plus chemicals or pharmaceuticals. It starts from the proposition that exposure to sunlight involves an inherent risk of skin tumor induction, and that the presence of chemicals or pharmaceuticals may influence that risk. This document emphasizes the testing method that most directly represents anticipated human exposures, in order to provide the most relevant regulatory guidance.

3. A LABORATORY PROTOCOL BASED ON SIMULATED SOLAR UVR

Human skin is subject to exposure to a wide variety of electromagnetic emissions in the home, in the

workplace and in nature, but the source with the greatest photobiologic impact and with the most significant long-term consequences is the sun. By itself, and by interacting with endogenous and exogenous chemical agents, sunlight accounts for the bulk of the photobiological effects in the general population (6, 30).

Although the natural solar spectrum encompasses cosmic radiation, gamma, UVR, visible, IR, and radio frequencies, our principal photochemical and photobiological concerns are with terrestrial optical radiation, i.e., wavelengths 290 - 800 nm (31-33). At the lower end of that range the solar energy distribution is strongly influenced by atmospheric absorption (including the influence of ozone) and solar angle. Studies involving the simulation of solar optical radiation on a laboratory scale have taken advantage of the characteristics of xenon arc emissions (3, 34-37). Since the dose of UVR (without any test article [TA]) used in the standardized model is sufficient to cause tumors in all mice, the test measures the ability of the TA to affect the *rate* of tumor formation. The certainty of tumor formation increases the statistical power of the test and greatly reduces the number of animals required for reliable testing. The data produced by this test are used to calculate mortality-corrected prevalence as well as tumor yield (i.e., average tumors per survivor).

For the UVR source used in the study, the emission characteristics (spectral power data), and the integrated energy within specified wave bands, must be documented on a regular basis (e.g., monthly, or after 100 hours of operation, whichever is first). This involves standard procedures for implementing a spectroradiometer calibrated against a NIST-traceable light source, plus a broad-band radiometer and photometer calibrated against both the standard lamp and the laboratory UVR source. The utility of the broad-band meters is principally in making initial estimates of biological effectiveness, plus the critical real-time monitoring of radiation exposures.

As a part of the comprehensive documentation on the exposure conditions, all optical filters (inherent to the fixture, or interposed in the beam) must be specified. Since filters supplied by manufacturers are notoriously variable, their transmission spectra should be determined independently using the laboratory's spectroradiometer "difference mode", or using a spectroradiometer, or both.

Any broad-band radiometer or photometer (or exposure monitoring system) used in these studies is to be calibrated (and re-calibrated on a regular basis) against both a traceable standard lamp and against the laboratory radiation source. Proper instrument maintenance includes annual confirmation of the spectral sensitivity of radiometers using a monochromator, either in the user's laboratory or by the instrument manufacturer.

Since some variation is inevitable, the laboratory must define the acceptability criteria for its own operating conditions, both in terms of internal reproducibility, and in terms of comparison with values in published literature and in international (e.g., CIE, IEC, COLIPA) standards and guidelines.

Table 1. Definitions of Broad Band UVR “Dose” Effectiveness Terms

TERM ⁽¹⁾	UVR QUANTITY	COMMENT
Standard Erythema Dose (SED) ⁽²⁾	100 (J/m ²) _e ⁽³⁾	Approximates 0.5 MEDi
Minimal Erythema Dose (MED, Instrumental) (MEDi)	200 (J/m ²) _e ⁽⁴⁾	Analogous to standard instrumental photometric quantities.
Minimal Erythema Dose (MED, Observational)	Individually Determined	Any measured quantity that produces the defined erythema response in skin.
Robertson-Berger Unit (RBU) ⁽⁵⁾	0.5 (J/m ²) _e	400 RBU ~ 200 (J/m ²) _e ~ 1.0 MEDi ⁽⁶⁾
Sunburn Unit (SU) ⁽⁷⁾	200 (J/m ²) _e	1 SU ~ 1 MEDi ~ 2.0E+03 Finsen-seconds

¹ Terms that are found in the published literature on photobiology and photocarcinogenesis. For additional information on definitions and sources, see reference 54.² CIE Standard DS 007.2/E, 1997 (reference 49) ³ The subscript e (for *effective*) indicates that the determined values are multiplied by the specified weighting function. ⁴ 200 (J/m²)_e represents the dose of radiation from a polychromatic source needed to produce a threshold erythema equivalent to that from 200 J/m² of UVR at wavelength 296.5 nm. Instrumental equivalence is based on the use of a defined weighting function such as the CIE “action spectrum” for erythema (reference 55). For derivation of instrumental MED, see reference 47.⁵ Reference 45.⁶ ≈ (Approximations): The cited calculations (references 45-47) were based on erythema weighting functions in use prior to the adoption of the CIE standard erythema action spectrum in 1997 (reference 49). ⁷ Reference 47

4. RECOMMENDED STUDY DESIGN

A standardized *in vivo* photocarcinogenesis safety protocol uses the hairless (hr/hr) mouse which is immunocompetent, comparable with other mice in repairing DNA damage, and develops principally solar keratoses, carcinoma-in-situ, and squamous-cell carcinoma after repeated exposure to UVR (18,19, 38-41). The lack of hair simplifies the application of drugs and UVR, and the observation of tumor formation.

Experimental variability is reduced by good husbandry practices, such as documented monitoring of temperature and humidity, environmental lighting and photoperiod. Study records include drinking water analyses and certificates describing the closed-formula (natural ingredient rations), semi-defined (purified), or chemically-defined diets (42). Diet may have a profound influence upon photocarcinogenesis, as well as upon the TA influence on overall response (43,44). Study records also include identity, purity, and stability of the TA, and documentation of lamp emission spectra and radiometric monitoring of the delivered radiation. UVR dose records must include the definitions of the terms used to describe both absolute energy and biologic effectiveness or its instrumental analog (31, 45-49). Table 1 summarizes conventional dose terminology. For studies intended as part of a regulatory submission, a Quality Assurance audit program is essential for meeting the Good Laboratory Practice guidelines applicable to the jurisdiction where approval is sought.

This laboratory test involves application of two variables: 1) the TA, and 2) the dose of UVR from the xenon arc solar simulator. In order to determine whether the TA affects the rate of UVR-induced carcinogenesis, the TA and UVR are delivered in an alternating sequence, i.e., *before* UVR on specified days, and *after* UVR on the remaining days (50,51). The test includes at least five treatment groups, with others added as required by the specific agent or agents under test (Table 2).

In the expanded study and treatment design (Table 3), groups 1 and 2 receive UVR (120 or 240 Robertson-Berger units, respectively) in the absence of TA in order to assess baseline effects of UVR. These groups provide internal calibration for the study. Mice in the high UVR dose group develop tumors earlier, but all mice are affected by the end of the study. Group 3 is treated with the vehicle plus UVR. Group 7 is optional; it allows the study to assess the effects of a comparator drug. Groups 4, 5 and 6 receive increasing doses of TA and the lower dose of UVR. UVR dose terminology is summarized in Table 1.

Animals are exposed to UVR (with or without TA) five or seven days per week for an extended period such as forty weeks. Observation continues for an additional twelve weeks. Animals are then sacrificed one year after the study is initiated. The occurrence and growth of tumors are monitored by visual examination and recorded manually or with a computerized tumor tracking system. Histological confirmation is not routinely required for tumors observed and recorded during photocarcinogenesis studies, although specific studies may benefit from microscopic examination of tissues taken at necropsy. The cumulative prevalence (proportion of affected mice *vs.* weeks on study) is plotted for each control and treatment group (Figure 1). Groups 1 and 2 are plotted as radiation calibration curves. Each group (3-6) which receives TA in addition to UVR is compared with the two calibration groups (1 and 2). The Peto test determines whether differences in time to tumor between treatment groups are statistically significant (52). If significant differences are found between treatment groups and the corresponding calibration group, then calculating a scaling factor or potency ratio can provide a measure of the effectiveness of each treatment (i.e., an estimated ratio of doses to produce the same response). In Figure 1, group 6 would suggest a potency ratio of nearly two (i.e., the group 6 response is close to that of group 2 which receives twice the UVR dose). In contrast, a sunscreen as the TA may provide a potency ratio of less than one (53).

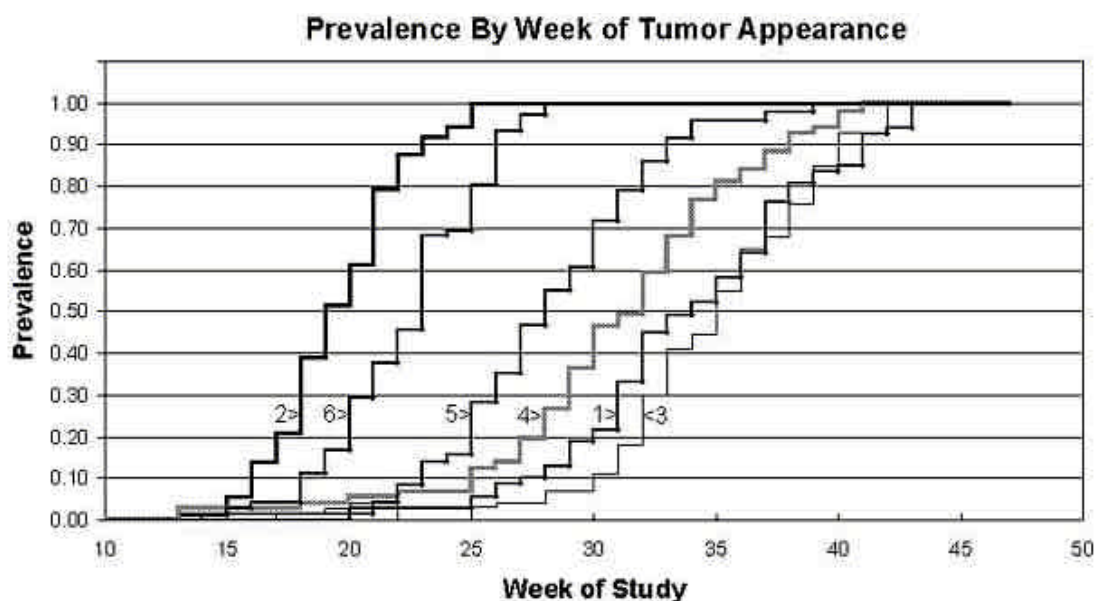


Figure 1. Illustration of possible outcome. Group numbers correspond with those in Table 3.

Table 2. Variables and Treatment Group Numbers

			Test article		
			Test article level		
UVR DOSE	NONE	VEHICLE	High	Mid	Low
UV High	2				
UV Low	1	3	6	5	4
UV Low		7*			

(Study designs may also include more than two UVR dosage levels and more than three dosage levels of the test article, and/or comparator articles). *Group 7: Comparator Article

Table 3. Treatment Groups and UVR Exposure Schedule

MON			TUES			WED			THUR			FRI		
TA PRE UVR	UVR (SED)	UVR (SED)	TA POST UVR	UVR (SED)	UVR (SED)	TA PRE UVR	UVR (SED)	UVR (SED)	TA POST UVR	UVR (SED)	UVR (SED)	TA PRE UVR	UVR (SED)	SED Per WEEK
Group 1 NONE	0.6	0.6	Group 1 NONE	0.6	0.6	Group 1 NONE	0.6	0.6	Group 1 NONE	0.6	0.6	Group 1 NONE	0.6	3.0
Group 2 NONE	1.2	1.2	Group 2 NONE	1.2	1.2	Group 2 NONE	1.2	1.2	Group 2 NONE	1.2	1.2	Group 2 NONE	1.2	6.0
Group 3 VEH	0.6	0.6	Group 3 VEH	0.6	0.6	Group 3 VEH	0.6	0.6	Group 3 VEH	0.6	0.6	Group 3 VEH	0.6	3.0
Group 4 TA	0.6	0.6	Group 4 TA	0.6	0.6	Group 4 TA	0.6	0.6	Group 4 TA	0.6	0.6	Group 4 TA	0.6	3.0
Group 5 TA	0.6	0.6	Group 5 TA	0.6	0.6	Group 5 TA	0.6	0.6	Group 5 TA	0.6	0.6	Group 5 TA	0.6	3.0
Group 6 TA	0.6	0.6	Group 6 TA	0.6	0.6	Group 6 TA	0.6	0.6	Group 6 TA	0.6	0.6	Group 6 TA	0.6	3.0
Group 7 COMP*	0.6	0.6	Group 7 COMP	0.6	0.6	Group 7 COMP	0.6	0.6	Group 7 COMP	0.6	0.6	Group 7 COMP	0.6	3.0

Bold type emphasizes the alternating sequences of treatments (i.e., TA applied pre- or post- UVR). *Comparator drug (optional) Abbreviations: UVR:Ultraviolet Radiation SED: Standard Erythema Dose (an estimate of effectiveness for UVR; the mathematical equivalent of 100 J/m² delivered by a source whose emission spectrum is weighted by the CIE action spectrum for erythema; see also next table). TA:Test Article VEH:Vehicle

The ability to recognize various stages of skin tumor development in live animals must be verified, particularly for a facility undergoing qualification to perform and submit photocarcinogenesis studies, or for purposes of qualifying an alternate animal model. Part of this verification involves the use of histopathological confirmation of skin and tumors taken at specified times and stages in a qualification study.

Within standard or regulated studies, histopathological evaluation provides a body of supporting, rather than primary, data. Histopathological confirmation is obviously possible only for "killed" tissue; histopathological confirmation of sequential stages is obviously not possible during the observation (data-gathering) phase while tumors are appearing, developing, and being mapped on the study animals. Any tissues, including skin tumors, may be preserved at necropsy, and specific studies may benefit from microscopic examination of tissues taken at necropsy. These evaluations need not, and indeed cannot, "confirm" all of the observations made on the changing appearance of the tumors during the study, and the observational data and analyses are not "corrected" on the basis of histopathology evaluations (most of which involve tissues taken at different stages or times).

5. ACKNOWLEDGEMENT

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Send correspondence to: Dr. Paul Donald Forbes, Charles River Laboratories, Discovery and Development Division, 905 Sheehy Drive, Horsham, PA 19044, USA, Tel: 610-647-4509, Fax: 800-878-7435, E-mail: forbes@toxarus.com