[CANCER RESEARCH 53, 53-60, January 1, 1993]

Wavelength Dependence of Skin Cancer Induction by Ultraviolet Irradiation of Albino Hairless Mice[1]

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ABSTRACT

Information on the variation in carcinogenicity with wavelength is crucial in risk assessments for skin cancers induced by UV radiation. Until recently the wavelength (λ) dependencies of other detrimental UV effects, such as sunburn, have been used as substitutes. Direct information on the λ dependency can only be obtained from animal experiments. To this end we accumulated a large data set on skin tumors induced by chronic UV exposure of albino SKH:HR1 mice (14 different broadband UV sources and about 1100 mice); the data come from the Photobiology Unit of the former Skin and Cancer Hospital in Philadelphia and from the Department of Dermatology of the University of Utrecht. The λ dependency was extracted from this data set (a statistically satisfactory description with $\chi^2 = 13.4$, df = 7) and represented by the Skin Cancer Utrecht-Philadelphia action spectrum, *i.e.*, a set of factors to weight the exposures at different wavelengths according to their respective effectivenesses (inversely proportional to the daily exposure required for a median tumor induction time of 300 days). The fits obtained with other already available action spectra proved to be poor ($\chi^2 > 60$, df = 11). The maximum effectiveness was found at 293 nm, and above 340 nm the effectiveness showed a shoulder at about 10^{-4} of the maximum. A sensitivity analysis of the final solution for the λ dependency showed a large margin of uncertainty above 340 nm and an information gap below 280 nm. The large variation in tumor responses in the present data set can be transformed to a coherent, common dose-response relationship by proper spectral weighting with this single action spectrum.

INTRODUCTION

The wavelength (λ) dependence of carcinogenesis by UV radiation is crucial in risk estimates of increased UV exposure, but direct information on this λ dependence has not been fully adequate. Since the 1930s (1) it has been known that the shortest wavelengths in sunlight reaching the earth's surface are the most carcinogenic; filtering through glass, attenuating the UVB[3] range (280–320 nm), abolishes the carcinogenicity in mice. The UVA range (320–400 nm) seemed to do nothing or at least very little. For decades this has been the state of knowledge on the subject, without any real progress.

Projections of a depletion of stratospheric ozone and a related increase in solar UV radiation at the earth's surface aroused much concern, starting in the early 1970s with the prospect of large-scale commercial supersonic stratospheric flights (2). In 1974 Molina and Rowland (3) brought the halocarbons into the limelight as a potential threat to the ozone layer. The recently measured Antarctic "ozone hole" revived interest in the matter, and more recent publicity on winter ozone depletions at intermediate latitudes (around 50°) in the nothern hemisphere increased public concern globally. One of the first consequences that the scientists predicted was an increase of UV-related skin cancers in Caucasians (2). The problem of the λ dependence of UV carcinogenesis, therefore, became very topical. Later on, the demand for information on the subject was further increased by the introduction of commercial "UVA" sources for tanning; was the UVA radiation harmless or not?

In the first risk assessments the λ dependence of carcinogenesis was assumed to be similar to that of other detrimental UV effects, such as erythema (sunburn). Setlow (4) noted that there was little theoretical justification for estimating the carcinogenic efficacy of UV radiation by an erythemal effect. He argued that DNA damage and resulting mutations were the plausible causes of neoplastic transformation. Consequently, he forwarded an estimate of the carcinogenicity that was based on the λ dependence of UV-induced DNA damage after correcting for the transmission of UV radiation through the outermost layer of viable skin (the epidermis). Recent data (5) substantiated this line of reasoning by showing that the mutation spectrum of the *p53* gene in human squamous cell carcinomas has the characteristics of mutation spectra obtained with UVC irradiation.

Direct information on the λ dependence of UV carcinogenesis can only be obtained from animal experiments. It is not possible to extract such information for humans from epidemiological data. Protracted monochromatic irradiation of animals to induce skin cancers is not feasible, and the experiments are therefore carried out with broadband UV sources of various spectral compositions. Over the last decade our groups have performed such experiments in which tumors were induced in hairless SKH:HR1 mice by chronic, daily exposure. Forbes *et al.* (6) at the former Skin and Cancer Hospital in Philadelphia (the "Philadelphia group") carried out a so-called ozone simulation experiment in which solar spectra for various ozone thicknesses were approximated by a filtered xenon arc. The observed differences in carcinogenic responses were due to spectral differences in the UVB range, the most relevant range for the effect of an ozone depletion. This data set provided very limited information on carcinogenesis in the UVA range. Van der Leun *et al.* at the University of Utrecht in the Netherlands (the "Utrecht group") chose a different line of approach and used various broadband UV sources (nearly all fluorescent tubes) to ascertain the spectral differences in the carcinogenic responses (7). The latter data set includes the carcinogenic response to pure UVA sources. The combined data of our groups contain the best available information on λ dependence of UV carcinogenesis, albeit in a very implicit way.

As a first approximation to the problem of defining a biologically meaningful UV dose, one can weight the radiant exposures at the different wavelengths according to the relative effectiveness at these wavelengths and then add these weighted exposures to yield a spectrally weighted UV dose. Such a set of weights for the wavelengths is commonly used in photobiology, and it is called an action spectrum (compare to the Relative Biologic Effectiveness and Quality factor with ionizing radiation). The data of the "ozone simulation"

Received 6/22/92; accepted 10/14/92.

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[1] Financed by a fellowship of the Royal Dutch Academy of Arts and Sciences (F. R. de G.).

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[3] The abbreviations used are: UVB, 280–320 nm; UVC, 100–280 nm; UVA, 320–400 nm; SCUP, Skin Cancer Utrecht-Philadelphia; t_{50}, median tumor induction time (the time until 50% of the mice in a group bear carcinomas or precursors).

experiment by itself proved to be insufficient to determine a unique broadband action spectrum, according to earlier calculations of ours[4] and an attempt by Rundel (8). Cole *et al.* (9) of the Philadelphia group found that from a set of available action spectra their data on carcinogenesis was best fitted using an action spectrum (MEE48) for edema in the SKH:HR1 mice. More recently Kelfkens *et al.* (10) provided additional data around 330 nm, and this proved to be a critical addition to the data of the Utrecht group. De Gruijl and Van der Leun (11) found that none of the available action spectra yielded a statistically adequate description of the data from the Utrecht group, including an action spectrum for carcinogenesis of the Utrecht group (12) derived from earlier experiments. The inadequacy of the available action spectra is confirmed for the larger data set dealt with in this paper. The conclusion is that a more adequate action spectrum should exist and may be derived from the available data. The latter is the subject of this paper.

MATERIALS AND METHODS

Animals and Their Maintenance. The experiments were carried out between 1978 and 1991 under standardized conditions in order to maximize reproducibility. Both male and female hairless albino SKH:HR1 mice were used, and they entered the experiments at ages varying between 6 and 10 weeks. All mice originated from the breading stock of the former Skin and Cancer Hospital in Philadelphia. The animals were housed individually and had continuous free access to laboratory chow and tap water. The animal rooms were kept at 25 ± 1°C and illuminated with yellow lamps (no UV output) in a 12-h day/night cycle.

Irradiation. In all experiments the animals received chronic daily UV exposure; in Philadelphia the animals were not exposed during weekends because of logistical reasons.

In the "ozone simulation" experiment in Philadelphia the animal cages hung in mobile racks. For the daily UV irradiation these racks were positioned on the perimeter of a circle (4 m diameter) around a 6-kW xenon arc (model Rm-60; Atlas Electric Devices Co.). The various segments of the circle were irradiated differently by inserting in the light path one of 5 different thicknesses of Schott WG320 glass: 0.64, 1.0, 1.3, 2.0, and 3.0 mm. Thus, 5 different solar UV spectra were simulated; a concise characterization of these 5 spectra is presented in the lower part of Table 1. The daily UV exposure (in J/m^2 = irradiance in $J/m^2/s$ × exposure time in s) was adjustable either by varying the exposure time (120, 85.5, 61.2, or 43.6 min) or by inserting neutral density filters (transmission 1.0, 0.76, 0.54, or 0.38) with a 120-min exposure time. Fine adjustments in the irradiance were made by positioning a rack closer to or further away from the xenon arc. In this exposure geometry the animals were irradiated sideways: the sides of the cages facing the xenon arc consisted of open gratings with no further obstacles to the penetration of the UV radiation. (For further details on this experiment the reader is referred to Ref. 6.)

Since the UV radiation is almost fully absorbed by the skin, the UV (surface) exposure is often referred to as the (spectrally unweighted) UV dose.

In the experiments of the Utrecht group the cages were kept stationary under the UV lamps. The top gratings of the cages allowed a good dorsal exposure of the animals, from which they could not shield themselves. The lamps were switched on and off automatically. The daily exposure times were kept constant, and a certain daily exposure for a group was attained by adjusting the irradiance electrically (dimmer circuit) and/or by inserting neutral density filters (perforated metal sheets).

The Utrecht group has used 3 lamps with mainly UVA output, 5 lamps which are mainly effective in the UVB, and one germicidal TUV lamp with the main output at 254 nm in the UVC (100–280 nm). In Table 1 these lamps are briefly characterized in terms of bandwidth and wavelength of maximum output. All of these lamps are fluorescent tubes, with the exception of the TUV lamp, which is a tube without a fluorescent coating, and the HPA lamp, which is a metal-iodine doped high-pressure mercury lamp. All lamps were manufactured by Philips Lighting BV, with the exception of the FS40 sunlamp, which was manufactured by Westinghouse, Inc. The TLX, EFL330, and the TL01* are not commercially available and have been custom made by Philips. The asterisk denotes filtering of the lamp. The TL09 and the EFL330 were filtered with ordinary window glass to attenuate further the UVB remnants from these lamps. The TL01* had a glass envelope which differed from the commercially available TL01. The TLX and the HPA lamps were filtered with liquid filters in quartz (TLX) or glass (HPA) containers. (For more details on these UV sources and the exposure set-up the reader is referred to Refs. 10–16.)

The daily exposure time with lamps emitting sufficient UVB or UVC radiation was 75 min, while the daily exposure times with lamps lacking significant UVB or UVC output had to be extended to 2 h (HPA) or even 12 h (EFL330*, TL09*) for sufficient UVA exposures. Spreading a certain exposure over a longer period per day was reported to increase the carcinogenicity, *i.e.*, shorten the tumor induction times (17, 18). According to Kelfkens *et al.* (18) a daily dose delivered in 12 h is somewhat more effective (25 ± 4%) than one delivered in 75 min. We have corrected the EFL330* and TL09* exposures for this effect.

Tumor Observations. The SKH:HR1 mice do not spontaneously develop carcinomas of the skin (10, 13); other benign skin tumors, like papillomas, are very rare and sometimes develop in old animals (13). The animals were checked for tumors every week or every other week, depending on the duration of the experiment. The locations of the tumors on the individual mice were mapped and numbered, and the tumors were followed in their growth pattern by recording diameters, heights, and other characteristic such as vascularisation, crateri-form, papilloma-like (protruding growth), etc. The tumor diameter was used as a convenient variable to measure tumor size: tumor detection thresholds could be set at sub-1-, 1-, 2-, or 4-mm diameter. (Here we will limit ourselves to the data pertaining to 1-mm tumors; for further details see Refs. 6 and 13.)

The day of first exposure is defined as $t = 0$, where t denotes time. The appearance time of a 1-mm tumor is set at

$$\frac{t_i + t_{i-1}}{2}$$

where t_i is the time of the ith checkup in which the tumor was first spotted with a diameter of 1 mm or larger and t_{i-1} is the time of the previous check-up. In the Utrecht data t_{50} is estimated by the median induction time derived from fitting a log-normal distribution to the times until first tumors on the individual mice (13). The data were corrected for animals that died without tumors by using a maximum likelihood method for the fits. In the Philadelphia data t_{50} was estimated from a life table analysis (19).

As found in earlier experiments by Blum (20), there was an inverse relationship between the daily UV exposure and t_{50}, *i.e.*, all animals would eventually develop tumors, but the time in which this occurred was shorter with higher daily exposures. Again in line with the earlier findings of Blum, the SD in the logarithm of time to first tumor was independent of the daily exposure when UVB sources were used: the SD in $\ln(t)$ ranged from 0.10 to 0.18.

It turned out that the SDs were larger with pure UVA than with UVB irradiation: the SDs in $\ln(t)$ for UVA ranged between 0.25 and 0.40. This difference appeared to be due mainly to a difference in the compositions of the tumor populations induced by UVB and UVA radiations (10): with UVB radiation mainly squamous cell carcinomas (and precursors, actinic keratoses) were observed, whereas with UVA radiation relatively more papillomas were observed. Rejection of frank papillomas from the UVA data left tumors (squamous cell carcinomas and precursors) with SDs in $\ln(t)$ of 0.11–0.18, *i.e.*, equal to those found with UVB radiation. The SDs in $\ln(t)$ of the papillomas ranged from 0.3 to 0.7. In sum, if we rejected the papillomas, the UVB and UVA data appeared to show similar statistics for the induction of tumors, *i.e.*, squamous cell carcinomas and precursors.

The UVC data obtained with the TUV lamp pose a problem all by themselves. There appears to be no consistency in the SD of $\ln(t)$: it systematically shifts from 0.4 with the highest daily exposure, 5.6 kJ/m^2, to 0.17 with the lowest daily exposure, 0.18 kJ/m^2. In contrast to the UVB and UVA daily exposures, the daily exposures to UVC radiation were 1 to 30 times larger than those required to cause a barely perceptible acute effect (delayed edema) in naive animals. In the UVA and UVB experiments the daily exposures were all below the threshold for acute effects because the animals could not bear higher

[4] F. R. de Gruijl, unpublished observations.

chronic dose levels of UVB and because such high-dose levels of UVA radiation were too difficult to achieve. The "super-acute" daily exposures in the UVC experiments may have introduced novel effects modifying the tumorigenesis. Whether papillomas somehow affected the UVC data, too, cannot be ascertained because we lack sufficient data on papilloma induction by UVC radiation: the majority of 4-mm tumors (65 of 73) taken post mortem at the end of the experiment were squamous cell carcinomas (15). The authors reported, however, their impression that this sample of post mortem tumors had a much lower percentage of "papilloma-like" tumors than observed in the course of the experiment: a larger portion of the early occurring tumors could have been papillomas. In sum, the statistics for tumorigenesis with UVC radiation seem to differ from that with UV radiation of longer wavelengths.

As the experiments of the Utrecht group have not been carried out simultaneously, repeat experiments (with the FS40 sunlamp and TL09*) have to serve as positive controls to guard against changes in the sensitivity of the mice (12, 21, 22). At this moment such repeat experiments are again being carried out, this time with SKH:HR1 mice purchased from Charles River, Inc., and the earlier results are reproduced within the margins of error.[5]

Equivalent Dose. The data set of the Westinghouse FS40 sunlamp covers the largest range in daily exposures (varied by a factor of 33) and corresponding t_{50}s (74–503 days) obtained with the same spectral distribution. It is, therefore, best suited for a reference data set.

The effect of a daily UV exposure with spectrum i can be measured by its t_{50}, *i.e.*, the time in days until 50% of the mice bear 1-mm tumors. An equivalent daily exposure from a FS40 sunlamp can be found for this t_{50}. By dividing this equivalent daily exposure by 115.4 J/m²/day (the daily UV exposure from an FS40 sunlamp for t_{50}= 300 days) we find the equivalent dose, E_i. Thus E_i is dimensionless and normalized to give $E_i = 1$ for $t_{50} = 300$ days, and it can be directly calculated from t_{50} through the formula (13)

$$E_i = \left(\frac{283}{t_{50} - 17}\right)^{1.61} \qquad \text{(A)}$$

If data on more than one regimen of chronic daily exposure were available for one type of lamp, the geometrical averages for the daily exposure and the equivalent dose were determined to get a single pair of numbers to ascertain the carcinogenicity of this lamp. For instance, experiments with the Xe1.3 (xenon arc with 1.3-mm WG320 filter) were carried out with exposures of 19.30, 13.79, 9.77, and 6.89 kJ/m²/day with equivalent doses (E) of 1.079, 0.825, 0.582, and 0.566, respectively, yielding geometrical averages of 11.57 kJ/m²/day and $E = 0.74$. The relative error in E can be estimated from the error in the mean of the e-based logarithms of the ratios of E over the exposure, which yields 0.087 for the Xe1.3. In case E was not an average, but based on a single value of t_{50} the relative error in E was estimated as 1.61 (the exponent in Equation A) times that in $t_{50} - 17$. The relative error in the calibration measurement of the exposure to a lamp was also incorporated into that of E; for the Xe1.3 this relative error was estimated to be about 0.02. The overall relative error in E for the Xe1.3 was thus estimated to be 0.09 ($=\sqrt{0.087^2 + 0.02^2}$) (see Table 1).

Action Spectrum and Weighted Dose. Action spectra usually consist of dimensionless weights normalized to 1 at the wavelength of maximum effectiveness (λ_{max}). Multiplying the spectrum of irradiation, $S(\lambda)$, in J/m²/nm for each light source by the action spectrum, $A(\lambda)$, and integrating this product over the wavelengths (λ) in nm yields the spectrally weighted dose

$$W = \int A(\lambda)\, S(\lambda)\, d\lambda \qquad \text{(B)}$$

where W is expressed in J/m² of λ_{max}-radiation.

Here, an action spectrum is based on exposure measured in J/m²; this convention is commonly used in studying physiological effects because the readout of (spectro-)radiometers is given in J/s/m²(/nm). In biochemistry and mutation research, however, the action spectra are often given in terms of number of photons. As a photon's energy equals hc/λ (h is Planck's constant and c is the speed of light), an exposure to λ_{max}-radiation in J/m² should be multiplied by $5 \cdot 10^{15}$ (λ_{max} in nm) to convert to quanta/m². The action spectra in this paper should be multiplied by λ_{max}/λ to convert them to quantal action spectra.

Because the irradiation spectra are zero below 250 nm and based on the assumption that $A(\lambda)$ tends to zero for $\lambda > 400$ nm (*i.e.*, no carcinogenicity in the visible), all numerical integrations (summations) are carried out from 250 to 400 nm in steps of 1 nm.

Our aim now is to find the best $A(\lambda)$ by varying $S(\lambda)$ and see whether the calculated values of W (Equation B) match the measured equivalent doses, E (Equation A).

Available Action Spectra. In line with Setlow's arguments (4) mutagenic action spectra can be used as hypothetical approximations for the λ dependence of carcinogenicity. We have used two different action spectra for mutagenesis (23, 24) corrected for either transmission through mouse epidermis[6] or transmission through human epidermis (25).

The erythemal action spectrum for humans has commonly been used to ascertain biologically harmful UV doses. We used the action spectrum pertaining to the erythema 24 h after irradiation (26) and a synthetic version thereof (27).

The edemal action spectrum for the SKH:HR1 mice was reported to give a fair spectral weighting for UV carcinogenesis in these mice (9). This action spectrum was dubbed MEE48, where "48" pertains to the time lapse in hours between the UV irradiation and the assessment of which exposure induced a barely perceptible edema. This action spectrum lacks information on the UVA and therefore had to be extended above 330 nm, as suggested by the authors.

Sterenborg of the Utrecht group carried out a series of experiments to derive the carcinogenic action spectrum for the SKH:HR1 mice. Slaper (12) added data acquired with Philips TL09 lamps and modified this action spectrum in the UVA range.

Fit Procedure. Fitting an action spectrum to the data involves scaling of the spectrally weighted daily dose W_i by multiplication with f to minimize

$$\chi^2 = \sum_i \frac{[\ln(f\, W_i/E_i)]^2}{e_i^2} \qquad \text{(C)}$$

where i numbers the various spectra of irradiation, and e_i is the relative error in E_i (Equation A). The minimum in χ^2 is found for

$$\ln(f) = \frac{-\Sigma_i\, (\ln W_i/E_i)/e_i^2}{\Sigma_i\, 1/e_i^2}$$

Note that if the action spectrum is normalized to 1 at its λ_{max}, then $1/f$ equals the required daily exposure to λ_{max}-radiation to induce tumors in 50% of the mice in 300 days.

As pointed out above, the irradiation set-up in Philadelphia differed from the one in Utrecht. It is therefore to be expected that the stated daily surface exposures (measured in J/m² at the skin level of the mice) will have different efficacies for inducing tumors in the two set-ups. On a weekly basis a daily irradiation of the mice in Philadelphia (5 times a week) is likely to be in the order of 5/7 of the efficacy of a similar daily irradiation in Utrecht (7 times a week). Moreover, the freedom of the animals to vary the body sites with which they face the UV source will further reduce the efficacy of an irradiation in Philadelphia. If we compare the maximum attainable surface exposure at a fixed site to an exposure that has been lowered by random rotation, the efficacy is expected to be reduced by a factor on the order of π. Thus, one would expect the surface exposure in Philadelphia to come out as 0.23 ($=5/7\pi$) times as effective as the one in Utrecht. However, in Philadelphia larger skin areas (1.5–2 times larger) of the mice could be effectively exposed, which should increase the numbers of tumors per mouse (by 1.5–2 times), shorten the t_{50} (by 6–10%), and increase E_i (by 10–16%). But the tumors were not randomly spread over the animals in Philadelphia: they tended to show sites of preference on the individual animals, probably corresponding to preferred positions of the animals in the cages. This effect will tend to increase E_i by increasing the exposure of a certain skin site and only slightly decrease E_i by decreasing the skin area that is effectively exposed.

[5] R. J. W. Berg and Y. Sontag, personal communication.

[6] H. J. C. M. Sterenborg, unpublished results.

By considering the Utrecht and the Philadelphia data as two separate data sets we can allow for a constant relative difference in the E_is, *i.e.*, different f values (Equation C) for Philadelphia and Utrecht. The ratio of these two f values, a Philadelphia/Utrecht match factor, is expected to come out somewhere around 0.25, or higher than that, owing to preferential exposure of skin sites of the animals in Philadelphia.

Search for a New Action Spectrum. Based on the assumptions that the action spectrum $A(\lambda)$ is greater than or equal to 0 for all wavelengths and that it is "smooth," we can write (see also Ref. 8)

$$A(\lambda) = \exp(P_n(\lambda)) \qquad (D)$$

where $P_n(\lambda)$ is a Lagrange polynomial of the order n, *i.e.*,

$$P_n(\lambda) = \sum_j a_j \Pi_i \frac{\lambda - \lambda_i}{\lambda_j - \lambda_i}$$

with i and j running from 1 through $n + 1$, and $i = j$ is excluded from the repeated multiplication Π_i. A polynomial of sufficient order can approximate a continuous function to any desired level of accuracy over a limited interval of the argument, λ. Note that $P_n(\lambda_j) = a_j$, *i.e.*, the polynomial is defined at $n + 1$ wavelengths λ_j by the corresponding values of a_j. The $n + 1$ wavelengths for these "tie points" can be chosen freely; the choice does not affect the final solution of $P_n(\lambda)$. This theoretical prediction has actually been experimentally verified with the algorithm we used. In our experience the fit algorithm with a Lagrange polynomial is very stable, *i.e.*, it shows a good convergence toward the final solution in 10–20 iterations (the computations were carried out on a mainframe Cyber 932 of the Control Data Corporation using the local mathematical Fortran library ACCULIB of the University of Utrecht).

By starting with $n = 0$, *i.e.*, $A(\lambda)$ = constant, one can fit an action spectrum to the data by minimizing χ^2 (Equation C). Then n is increased in steps of 1 until a statistically sound fit to the data is obtained; with m different input spectra and $n + 1$ parameters for the polynomial $P_n(\lambda)$, one finds a χ^2 with $m - n - 1$ degrees of freedom ($m - n - 2$ if both Utrecht and Philadelphia data are used and a match factor between the two has to be derived). The criteria for accepting a fit from a series of increasing n are: n should be as small as possible for χ^2 to lie in the 95% confidence range, and the next larger value of n should not yield a significant improvement of the fit according to an F test (28).

The reader is referred to Appendix A for more particulars on the fit algorithm and on the calculation of the errors in the coefficients a_j, and Appendix B gives information on a λ-dependent sensitivity analysis of the final solution for the action spectrum.

RESULTS

Fig. 1 depicts the basic input data, t_{50} for carcinomas *versus* the daily UV exposure. From the wide spread in daily UV exposures one can see that there are large differences in carcinogenicity among the lamps: a TL09* requires about 10,000 times more radiant energy than a TLX to induce carcinomas in 50% of the mice in about 300 days. Table 1 gives a summary of the data on the UV lamps: a spectral characterization, the daily UV exposure (geometrically averaged for a lamp if experimental results were available on more than one regimen of chronic daily exposure), and the corresponding equivalent dose (calculated from t_{50}, Equation A, and geometrically averaged if applicable). The equivalent doses are given for total tumor induction as well as more specifically (by exclusion of frank papillomas) for the induction of squamous cell carcinomas and precursors. The latter restriction affects only the equivalent doses of the UVA sources (main output wavelengths > 320 nm). For more information the reader is referred to the relevant references given under "Materials and Methods."

Table 2 summarizes the performances of various existing action spectra on the present data on tumor induction. For reasons we will go into in the next paragraph, the data of the UVC source (TUV) are left out. The values of χ^2 show that none of these action spectra yields a satisfactory result. In spite of the fact that all of these action spectra bear a crude resemblance to one another (*i.e.*, high effectiveness below 300 nm and a steep roll-off above 300 nm) their values of χ^2 differ widely. The MEE48, extended above 330 nm at the level of 10^{-4} of its maximum, and the earlier carcinogenic action spectrum by Sterenborg and Slaper perform best with χ^2 = 68.5 and 62.3, respectively (11 degrees of freedom). The best-fitting action spectrum based on mutation induction, given in Table 2, is found by combining the action spectrum of Peak *et al.* (24) with the transmission through a 70-μm (human) epidermis (25); second best is the action spectrum by Jones *et al.* (23) corrected for transmission through mouse epidermis (25–30 μm) with a χ^2 of 280. The performances of most of these action spectra are poorer still for the data pertaining to carcinomas: only the

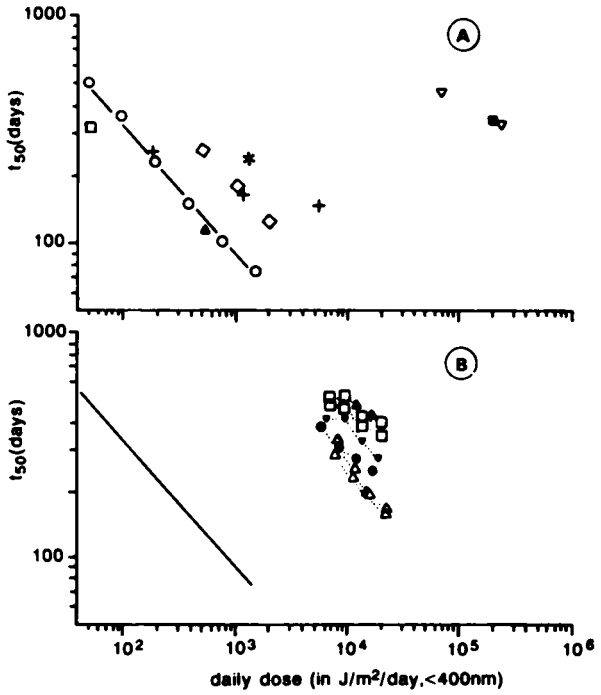

Fig. 1. t_{50} *versus* the daily UV exposure for the Utrecht data (*A*) and the Philadelphia data (*B*). The Utrecht data: +, TUV; □, TLX; ○, FS40; ▲, TL12; ◇, TL01; *, TL01*; ▼, EFL330; ▽, TL09*; ■, HPA*. The Philadelphia data: △, Xe0.64; ●, Xe1.00; ▼, Xe1.30; □, Xe2.00; ▲, Xe3.00.

Table 1 *Characteristics of the UV sources*

UV source	Spectral range in nm, 1% cutoff	Peak(s) in nm	Exposure[a] in J/m²/day[b] (λ < 400 nm)	Equivalent dose[c, d] for tumors (re)[e]	Equivalent dose for carcinomas[c, f] (re)
TUV	253–254	254	1,064	2.45 (0.70)	
TLX	270–305	280	72	0.93 (0.10)	
TL12	275–375	313	555	5.61 (0.10)	
FS40	275–390	313	269	2.33 (0.04)	
TL01	305–367	312	1,040	2.47 (0.09)	
TL01*[g]	306–368	312	1,375	1.68 (0.16)	
EFL330	317–>400	345/365	70,000	0.54 (0.10)	0.45 (0.06)
TL09*[g]	328–>400	358/365	238,000	1.15 (0.13)	0.81 (0.08)
HPA*[g]	332–>400	365/373/385	220,000	1.24 (0.18)	0.83 (0.08)
Xe0.6	295–>400	396	13,890	1.77 (0.07)	
Xe1.0	296–>400	396	10,360	1.00 (0.06)	
Xe1.3	299–>400	396	11,570	0.74 (0.09)	
Xe2.0	301–>400	396	12,090	0.53 (0.11)	
Xe3.0	304–>400	396	14,630	0.48 (0.11)	

[a] A geometrical average in case of data on more than one exposure level (see Fig. 1).
[b] Chronic daily exposure, only on weekdays in Philadelphia.
[c] The equivalent dose represents the measured relative tumorigenicity of a lamp's exposure.
[d] Ascertained from t_{50} through Equation A and geometrically averaged if applicable.
[e] re, relative error.
[f] Stated only if different from that for tumors.
[g] *, filtered.

fit for the mutagenic action through 70-μm epidermis improves somewhat with a χ^2 of 172.5. Apparently, there is room for improvement here, and an effort to extract a better-fitting action spectrum is warranted.

As found earlier, the data from the Philadelphia "ozone simulation" experiment are not sufficient to derive a broadband action spectrum, and the "Utrecht" data provide just enough information to extract an action spectrum extending into the UVA. A problem in the latter data set stems from the UVC data acquired with the TUV lamp. In Fig. 1*A* the points for the TUV lamp do not run parallel to those for the FS40 sumlamp. Consequently the three levels of daily exposure to TUV lamps show a wide systematic shift in the ratios of the E_is against the respective daily exposures, from 0.7 to 7.3/kJ/m^2. The resulting large error in the average E_i (Table 1) leads to an erratic approach to the final solution for the action spectrum as we increase n, the order of the polynomial in Equation D (see the fit procedure given in "Materials and Methods"). Combining this with the previous comments on these UVC data (given in "Materials and Methods") leaves us with rather aberrant data in the UVC. Leaving out these data completely circumvents this problem of erratic convergence: in all cases to be discussed we then find a good steady convergence toward the final solutions. As an example, Fig. 2 shows the sequence of action spectra fitted to the total data set on tumors for n = 2 through 5. The χ^2 of 10.3 with 7 degrees of freedom obtained with n = 4 indicates a good fit; increasing n to 5 does not yield a significant improvement with χ^2 = 9.4 with 6 degrees of freedom. Moreover, we see little difference between the action spectra with n = 4 and n = 5 in Fig. 2. Thus, a good convergence toward the final solution is observed.

Table 2 *Various action spectra with their respective χ^2 values and derived parameters (± error)*

Action spectrum (reference)	χ^2 df = 11[a]	λ_{max}[b] in nm	$1/f$[c] in J/m^2/day	Match factor Philadelphia/ Ultrecht[d]
Mutation (24) + epidermis (25)	256	297	17.8 ± 1.7	0.19 ± 0.03
Erythema, Parrish *et al.* (26)	281	296	37 ± 4	0.29 ± 0.05
CIE, McKinlay-Diffey (27)	323	250–298	39 ± 4	0.38 ± 0.07
MEE48 extended, Cole *et al.* (9)	68.5	297	21.7 ± 1.1	0.30 ± 0.02
Ca in SKH, Sterenborg-Slaper (12)	62.3[e]	302	24.4 ± 1.1	0.21 ± 0.02

[a] P = 0.05 for χ^2 = 19.7 with df = 11, *i.e.*, a larger χ^2 signifies a poor fit to the data.
[b] Wavelength where the action spectrum reaches its maximum.
[c] An estimate of the required daily exposure to λ_{max}-radiation for t_{50} = 300 days.
[d] Estimate of reduced efficacy of UV exposures in the Philadelphia experiments.
[e] df = 11 is not really correct; this action spectrum was derived earlier from a part of the present data.

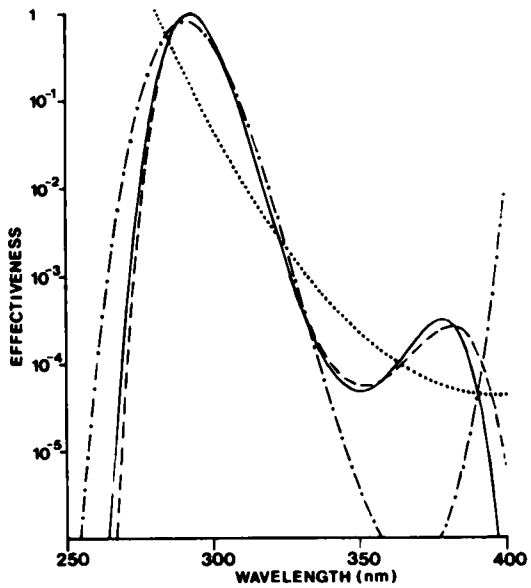

Fig. 2. Action spectra of increasing polynomial order n fitted to the tumor data: ·····, n = 2, χ^2 = 614.8, df = 9; – · –, n = 3, χ^2 = 91.3, df = 8; – – –, n = 4, χ^2 = 10.3, df = 7; ——, n = 5, χ^2 = 9.4, df = 6.

Table 3 *Action spectra derived for tumors or carcinomas*

Natural logarithms of the action spectra, ln A, are given in tie points at 5 wavelengths. As in Table 2, the χ^2 value and derived parameters are given for each action spectrum. The last column defines the SCUP action spectrum.

	Action spectrum for	
	Tumors	Carcinomas
ln A(270 nm)[a]	−10.10 ± 0.91	−10.91 ± 0.94
ln A(302 nm)[a]	−0.86 ± 0.05	−0.86 ± 0.06
ln A(334 nm)[a]	−8.32 ± 0.12	−8.60 ± 0.09
ln A(367 nm)[a]	−9.08 ± 0.21	−9.36 ± 0.16
ln A(400 nm)[a]	−11.92 ± 1.41	−13.15 ± 1.41
χ^2 (df = 7)[b]	10.3	13.4
λ_{max}[c] in nm	293	293
$1/f$ in J/m^2/day	24.3	23.8
Match factor Philadelphia/Utrecht	0.26 ± 0.01	0.27 ± 0.02

[a] These values of ln $A(\lambda_j)$ are equal to the polynomial coefficients a_j of $P_n(\lambda)$ with n = 4 in Equation D, and they fully define the action spectrum.
[b] P = 0.05 for χ^2 = 14.1 with df = 7, *i.e.*, a larger χ^2 signifies a bad fit to the data.
[c] ln $A(\lambda_{max})$ = 0.

Action spectra could be derived either for tumor induction or, more restrictedly, for carcinoma induction, based either on the Utrecht data alone or on the complete data set. Thus 4 action spectra were derived. In all cases the required polynomial order n equaled 4, and the action spectra derived from the Utrecht data alone (with 3 degrees of freedom and χ^2 equal to 2.2 and 2.9, respectively) did not significantly differ from those derived from the entire data set. The particulars of the two action spectra derived from the entire data set are given in Table 3. The match factor between the data sets from Philadelphia and Utrecht equals 0.26–0.27, *i.e.*, the daily UV exposure in Philadelphia has been about 0.26 to 0.27 times as effective as that in Utrecht, which is surprisingly close to the expected 0.25. The action spectrum for carcinomas in Table 3 (last column) is dubbed the SCUP action spectrum.

Although Table 3 gives the estimated errors in 5 tie points for each action spectrum, it is rather difficult to see how well an action spectrum is defined at a certain wavelength. Each tie point determines the total shape of the action spectrum, and an error at a tie point therefore affects the total shape. To provide more direct information on the quality of the definition of the action spectrum at certain wavelengths we have carried out sensitivity analyses on the final solutions we found. Fig. 3 shows the result for the SCUP action spectrum. The *dashed curves* depict the upper and lower boundaries for the displacement of a 5-nm segment of the action spectrum, which will not increase the χ^2 by more than 1.0. Thus, it is clear that the action spectrum is well defined in the UVB range, especially around 311–312 nm, owing to the almost monochromatic TL01. In the UVA range the uncertainty increases substantially. Toward the shorter wavelengths an information gap opens up below 280 nm. Even if we include the rejected UVC data of the TUV lamp, this information gap persists down to 254 nm, where the action spectrum would return to a poorly defined effectivity of approximately 5% (1.5–15%) of the maximum at 293 nm.

The SCUP action spectrum should introduce a coherency by bringing together the input data in common dose-time relationship. To illustrate this point we present the input data from Fig. 1 (excluding the TUV data) after applying the SCUP action spectrum in Fig. 4: plots of t_{50} *versus* the SCUP-weighted daily dose (scaled by f to

estimate the equivalent dose, E). On the whole a good coherency indeed emerges, but for the very low daily doses and long median inducion times (around 500 days, in the order of the average life span of the animals) we see a slight deviation in the Philadelphia data (Fig. 4*B*): the median induction times are shorter than expected. Because the t_{50}s in the Philadelphia data were directly estimated from actuarial tables of the chance of tumor-free survival, and because the number of unaffected survivors became very low after 500 days (typically about 5–8), the resulting estimates of the t_{50}s became increasingly inaccurate (10–20% relative error). Moreover, a bias toward shorter t_{50}s was introduced because at these low daily doses (around $E = 0.4$) some groups only just reached the 50% level, whereas two groups did not.

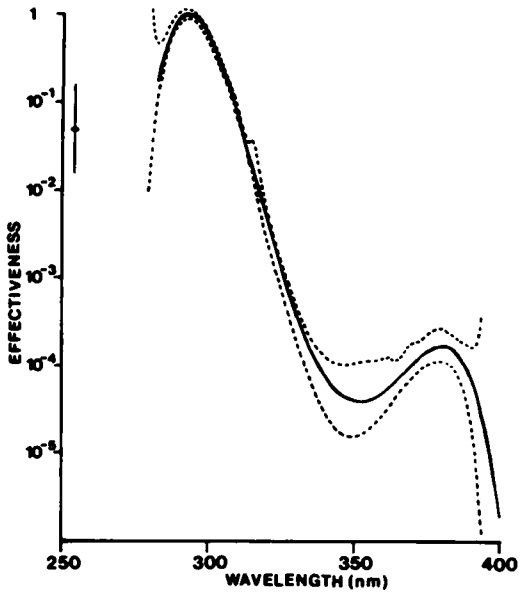

Fig. 3. ——, SCUP action spectrum for skin cancer induction in SKH:HR1 mice; – – – –, upper and lower limits of 5-nm segment displacements which will not increase the χ^2 by more than 1. The range of measured effectivities at 254 nm is depicted separately.

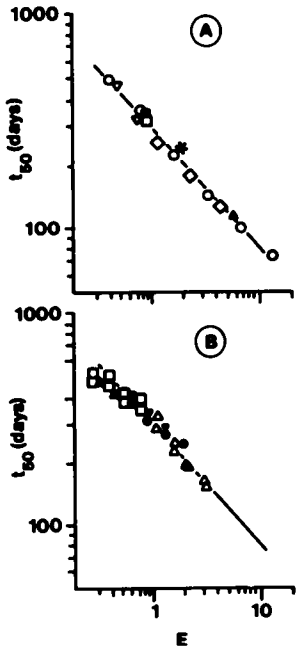

Fig. 4. t_{50} *versus* the SCUP-based equivalent dose, E (the SCUP-weighted daily dose scaled by its f value). *A*, Utrecht data (TUV data exluded); *B*, Philadelphia data. *Symbols*, as in Fig. 1.

Aside from this deviation at low daily doses, the weighting with the SCUP action spectrum brings the data points together in a common (weighted) dose-time relationship, *i.e.*, the SCUP-weighted dose adequately estimates E_i and hence t_{50} through Equation A.

DISCUSSION

A crude similarity between erythemal effectivity (in humans) and carcinogenicity (in mice) was indicated in the experiments by Freeman (29), but the data were insufficient for a proper determination of an action spectrum. The available action spectra for UV carcinogenesis performed poorly on our data set; the MEE48 edema action spectrum and an earlier approximation to the carcinogenic action spectrum (12) came out best, with a χ^2 of 68.5 and 62.3 (df = 11), respectively. The MEE48 action spectrum lacked information in the UVA and had to be extrapolated. The earlier approximation to the carcinogenic action spectrum for hairless SKH:HR1 mice (12) suffered from a large mismatch to the data recently acquired with the custom-made EFL330 lamp (26.2 is the contribution of the EFL330 data to a χ^2 of 62.3, df = 11). In spite of a crude resemblance between all of these action spectra, these action spectra did not automatically match the data equally well: the position of the maximum, the steepness of the roll-off, and the level of the shoulder in the UVA (see Fig. 3) were apparently crucial.

The newly derived SCUP action spectrum is the only one that produces a statistically satisfactory spectral weighting of the present data on carcinomas. A sensitivity analysis of this action spectrum (Fig. 3) to the input data shows that its definition in the UVB is very good and that the uncertainty increases dramatically in the UVA. Below 280 nm the data show an information gap, which was not caused by the exclusion of the data acquired with the TUV lamp (output at 254 nm).

The UVC and UVA ranges posed some problems in the derivation of an action spectrum: the induction kinetics of tumors seemed to differ from that by UVB radiation. By making a distinction between papillomas and carcinomas this problem could be solved for UVA radiation: the data on carcinomas resembled the data acquired with UVB radiation which induced mainly carcinomas. In the Philadelphia data the progressive filtering of the xenon arc led to increasingly lower fractions of UVB radiation and an increasing fraction of UVA radiation. This may have caused a gradual relative increase in papillomas which was not picked up from direct observations (at low doses and long induction times the tumors may remain too small to come to a clear diagnosis on papilloma *versus* carcinoma). This effect may contribute to the deviation observed in Fig. 4*B* at low daily doses. The UVC data acquired with the TUV lamp could not be corrected for papillomas either, partially because of insufficient data on the induced papillomas (only post mortem). The super-acute daily exposures to the TUV lamps may have caused additional aberrant effects. The resulting large (systematic) error in the equivalent dose (Table 1) and an information gap between 255 and 280 nm caused an erratic approach to the final solution for the action spectrum with increasing order n (Equation D). Hence the data of the TUV lamps were rejected.

From the above it is clear that more data on carcinogenesis are needed for wavelengths over 340 nm for a better definition of the action spectrum in the UVA. More data on UVC carcinogenesis are needed to clarify the differences with UVB carcinogenesis. Only after understanding the nature of these differences might we be able to make a meaningful extension of the action spectrum into the UVC.

The premise that radiant energies of different wavelengths can be weighted and added to arrive at an effective UV dose is obviously fundamental to the derivation of the SCUP action spectrum. There are

reports, however, that UVA radiation may counteract the carcinogenicity of UVB radiation (21, 30, 31). Thus far these effects have been found to be weak and often statistatically insignificant and therefore quantitatively negligible. Moreover, the mere fact that a single action spectrum can adequately predict the carcinogenicity of UVA and UVB sources inherently justifies its derivation.

For the present data set various UV sources have been used to induce skin tumors in SKH:HR1 hairless mice: ranging from low UV exposures (50 J/m^2/day) from sources with UVB radiation to excessive UV exposures (240,000 J/m^2/day) from sources with mainly longwave UVA radiation. The time lapses until 50% of the animals bore tumors in different experiments varied from 2 months to 2 years. A proper spectral weighting with the SCUP action spectrum focuses these scattered data points (Fig. 1) on a common dose-time relationship (Fig. 4). Evidently, such an action spectrum is operationally useful for a definition of the carcinogenic UV dose. It should be stressed, however, that it is an operationally defined action spectrum that does not yield direct and unambiguous information on the photochemical reactions leading to tumor formation. The overall shape of the SCUP action spectrum below 340 nm roughly resembles what one would expect with UV-induced mutations, but the shoulder above 340 nm appears somewhat high. The definition of the action spectrum above 340 nm is not very accurate, and an extension into the visible may be necessary, but we lack sufficient data to quantify the carcinogenicity of wavelengths larger than 390 nm (see Fig. 3). As UV carcinogenesis is probably an intricate, dynamical multistep process, the interpretation of the present action spectrum is by no means straightforward. The poor results we obtained thus far with action spectra constructed from data on mutagenesis and epidermal transmission appear to underline the complexity of the spectral response.

ACKNOWLEDGMENTS

Charles Meulemans, M.Sc., of Philips Lighting, Inc., has played a crucial role in providing the Dutch group with the various UV sources. The Dutch National Cancer Foundation and the Ministry of Housing, Physical Planning and the Environment funded the experiments of the Dutch group.

APPENDIX A: MATHEMATICAL DETAILS ON THE COMPUTATION OF THE ACTION SPECTRUM

We define the expected equivalent dose, $<E_i> = f W_i$, in Equation C as

$$<E_i> = e^c \int S_i(\lambda)\, A(\lambda)\, d\lambda \qquad \text{(E)}$$

and we rewrite Equation C as

$$\chi^2 = \sum_i X_i^2 \qquad \text{(F)}$$

with

$$X_i = \frac{\ln(<E_i>/E_i)}{e_i}$$

Next we take $c = 0$ for the Utrecht data, and c is unknown and to be determined for the Philadelphia data. $A(\lambda)$ is defined as in Equation D, and its coefficients a_j need to be determined. Note that A(λ) in Equation E is no longer dimensionless: it has been multiplied by the f value for the Utrecht data, and e^c equals the match factor between the Philadelphia and Utrecht data.

If we have $N - 1$ coefficients a_j to define $A(\lambda)$, then c is the Nth parameter, a_N, to be determined. These N parameters are found by minimizing the χ^2 for the data at hand. This is done by an algorithm (the Fortran subroutine LMDER1 from the MINPACK package contained in the mathematical library ACCULIB) which searches the minimum of a "sum of squares" (Equation F) along its gradient for the N parameters that need to be optimized. The gradient is determined from the first derivatives:

$$\frac{\partial \chi^2}{\partial a_j} = \sum_{i=2}^{14} 2X_i \mathrm{JC}_{ij}, \qquad \text{for } j < N \qquad \text{(G)}$$

and

$$\frac{\partial \chi^2}{\partial a_N} = \sum_{i=10}^{14} 2X_i \mathrm{JC}_{iN} \qquad \text{(H)}$$

where the summation in Equation G includes all UV sources (Table 1) except the TUV lamp ($i = 1$), and the summation in Equation H only includes the xenon arc data from Philadelphia ($i = 10 \ldots 14$; Table 1). JC stands for the Jacobian,

$$\mathrm{JC}_{ij} = (e_i <E_i>)^{-1} \frac{\partial <E_i>}{\partial a_j}$$

which equals $1/e_i$ if $j = N$. If $j < N$ then

$$\frac{\partial <E_i>}{\partial a_j} = (e^c) \int L_j(\lambda)\, S_i(\lambda)\, A(\lambda)\, d\lambda \qquad \text{(I)}$$

with

$$L_j(\lambda) = \prod_k \frac{\lambda - \lambda_k}{\lambda_j - \lambda_k}, \qquad k = 1 \ldots N - 1 \text{ except } k = j$$

The first derivatives in Equations G and H become equal to zero in the minimum of χ^2. The variance, var_j, in the jth parameter is estimated from the corresponding diagonal element of the covariance matrix, CV:

$$\mathrm{var}_j = \frac{\mathrm{CV}_{jj}\, \chi^2}{\mathrm{df}} \qquad \text{(J)}$$

with df = 13 – N. The error in the jth parameter equals the square root of var_j.

The covariance matrix is the inverse of the Hessian

$$H_{ij} = \frac{\partial^2 \chi^2}{\partial a_i \partial a_j} \qquad \text{(K)}$$

For i and $j < N$ we get

$$H_{ij} = \sum_{k=2}^{14} 2\left\{1 - \ln\left(\frac{<E_k>}{E_k}\right)\right\} \mathrm{JC}_{ki}\mathrm{JC}_{kj} + \frac{2\chi_k}{e_k E_k} \cdot \frac{\partial^2 E_k^2}{\partial a_i \partial a_j} \qquad \text{(L)}$$

As $<E_k> \approx E_k$ for a good fit to the data, it follows that Equation L becomes

$$H_{ij} = \sum_{k=2}^{14} 2\mathrm{JC}_{ki}\mathrm{JC}_{kj} \qquad \text{(M)}$$

With either i or j or both i and j equal to N Equation K becomes

$$H_{ij} = \sum_{k=10}^{14} 2\mathrm{JC}_{ki}\mathrm{JC}_{kj} \qquad \text{(N)}$$

Note that the Hessian is symmetrical, *i.e.*, $H_{ij} = H_{ji}$. The values of X_i and JC_{ij} are computed through Fortran routines provided by the user and values of H_{ij} by the minimization algorithm.

The value of f, Equation C, is equal to the maximum value, $A(\lambda_{max})$, of the so-obtained action spectrum. Division by f normalizes this action spectrum to 1 in its maximum.

APPENDIX B: SENSITIVITY ANALYSIS

To test how well the final solution for $A(\lambda)$ is determined locally at a certain wavelength we can distort $A(\lambda)$ at that wavelength and assess the effect of this local distortion on the fit to the data. If a large distortion does not significantly alter the quality of the fit, then $A(\lambda)$ is obviously not very well defined at that wavelength.

To this end we can take a segment of Δ (in nm) width centered around a certain wavelength out of $A(\lambda)$, and multiply $A(\lambda)$ by a factor of e^{ϵ} locally at that segment, with $x = d$ or $x = -d$. This will yield $\chi^2(d)$ or $\chi^2(-d)$, respectively. Through the points $[d,\chi^2(d)]$, $[0,\chi^2(0)]$ and $[-d,\chi^2(-d)]$ runs a parabola from which we can estimate the positive and negative excursions, x_+ and x_-, which will increase χ^2 with 1:

$$x_+ = \frac{d}{C}\{B + (2C + B^2)^{1/2}\} \quad \text{(O)}$$

$$x_- = \frac{d}{C}\{B - (2C + B^2)^{1/2}\} \quad \text{(P)}$$

with $B = \chi^2(d) - \chi^2(-d)$, and $C = 2\,[\chi^2(d) - 2\chi^2(0) + \chi^2(-d)]$. In our calculations we took $\Delta = 5$ nm and $d = 0.02$. The displacements x_+ and x_- are inversely proportional to Δ.

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