

## Monitoring of Solar-UV Exposure among Schoolchildren in Five Japanese Cities Using Spore Dosimeter and UV-coloring Labels

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To monitor personal exposure to biologically effective solar-UV radiation, *Bacillus subtilis* spores on a membrane filter and UV-coloring labels were incorporated into a monitoring badge. The samples were covered with one of three types of filter sheet, dependent on the season, to reduce the amounts of exposure to measurable levels. Five fifth- or sixth-grade classes of primary schools, each consisting of 30–40 children, were chosen in northern (Sapporo), central (Tsukuba and Tokyo), and southern (Miyazaki and Naha) cities in Japan. In all four seasons, each child wore a badge on an upper arm for the entire waking hours, changing it daily, for a week. Upon collection of the badges, the survival of spores and the extent of coloration of the label were determined. The results were used to estimate the amount of daily exposure to biologically effective UV radiation, expressed as the value of spore inactivation dose. Unexpectedly, the average amounts of exposure were not directly correlated with the outdoor UV irradiance: in the two southern cities, despite high outdoor irradiance from spring to autumn, the average amounts of exposure were less than 3.1% of the average irradiance. Highly concentrated exposures occurred in two central cities on three days when extensive outdoor exercise took place. These results contradict the simple notion that childrens' exposure is in proportion to the outdoor UV irradiance, and support the view that the extent of solar-UV exposure is primarily determined by life-style rather than living location.

Key words: Solar-UV radiation — Spore dosimetry — Schoolchildren

Solar-UV radiation is one of the most ubiquitous genotoxic agents to which organisms on the earth are exposed. The grim possibility that this could be increased by the depletion of the stratospheric ozone layer due to anthropological release of chlorofluorocarbons has raised serious concern about medical, agricultural, and ecological consequences. The incidence of skin cancers is well correlated with the amount of UV irradiance, which in turn is primarily dependent on geography, and this dependence constitutes the basis of various models predicting the increase in skin cancer incidence as a consequence of ozone depletion.<sup>1–4)</sup> However, we have little knowledge about the extent of personal exposure to solar-UV radiation, and how it is related to environmental irradiance.

A major problem in quantifying the solar-UV exposure is how to measure it. Sunlight is polychromatic, and biologically harmful effects are mainly attributable to the shortest region of UV wavelengths. Therefore, to measure it with a broad-band UV detector is problematic, since the less damaging portions of the radiation are always more abundant. This problem can be circumvented by the use of a biological dosimeter that can automatically integrate the effectiveness spectrum over the entire UV wavelength range. For this purpose, the spore dosimeter that has been established and developed during the past decade by one

of us seems most suitable.<sup>5–7)</sup> We have fitted spore samples on a carrier into a badge similar to those used to monitor personal exposure to ionizing radiations.

As an auxiliary dosimeter, we utilized UV-induced coloring, since labels are commercially available that change in color from white to blue upon solar exposure. The amount of cumulative exposure can be estimated from the depth of the blue color with the use of a color meter. We have determined the spectral response of this label, and have established that the degree of coloration is positively correlated to the biological dose.

Another problem in developing the monitoring badge was that these dosimeters are too sensitive to measure daily exposure; for example, all the spores are inactivated and the label reaches color saturation in less than an hour on a sunny summer's day in Tokyo. Thus, we used a filter to reduce the amount of UV exposure. Among various materials examined, we found that a sheet of blue polyethylene is suitable, and we utilized sheets with three different thicknesses. Incorporating both types of dosimeters, we produced badges for the monitoring of solar-UV exposure among schoolchildren in five cities in Japan. As a first step, we chose schoolchildren because they belong to well-organized groups and could be instructed by class teachers. Eventually, we hope to obtain detailed indices of solar exposure according to life-style and residential location by using similar badges.

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MATERIALS AND METHODS

**Spore dosimeter** Spores of a UV-sensitive mutant strain of *Bacillus subtilis* TKJ6312 (*uvrA10 ssp-1*) were prepared as described.<sup>5</sup> In the original protocol, they were spotted on a membrane filter, and extracted by sonication. However, recovery by using sonication was laborious, and not suitable for the processing of a large number of samples. Therefore, the procedure of the sample preparation was modified as follows. The spores were suspended in a mixture of molten low-melting agarose (0.4%, SeaPlaque, FMC Bioproducts) and blue-dextran 2000 (0.04%). Four samples of 5  $\mu$ l of suspension, each carrying about  $10^5$  spores, were spotted onto a membrane filter (A010A025A, Advantec) which was placed in a 12-place manifold apparatus under a low vacuum. Each filter was cut into two, and each piece, containing two spore spots, was used in a badge as described below.

After the exposure, each piece was cut out, immersed in 1 ml of water, and heated at 75°C for 20 min with occasional vortexing to ensure full recovery. Survival was determined by pour-plating on CMM agar as described.<sup>5</sup> From the numbers of CFU of exposed (N) and control ( $N_0$ ) samples, the value of inactivation dose (ID) was calculated as follows:

$$ID = -\ln(N/N_0).$$

**UV-coloring label** White adhesive labels which color blue upon exposure to sunlight are commercially available (UVRI-LBL BLUE, Tomoegawa Seishi Co., Shizuoka) and are called 'UV-coloring labels' in this report. We determined the spectral response of these labels with the use of the Okazaki large spectrograph in identical settings to those employed to obtain the action spectrum for spore dosimetry.<sup>8,9)</sup>

Pieces of the UV-coloring label were exposed to monochromatic radiation at wavelengths of 254, 270, 290, 300, 313, 365, and 400 nm. The resultant coloration was measured using a color meter (CR321, Minolta Camera Co., Tokyo). As shown in Fig. 1A, the values of color difference ( $\Delta E$ ) increase dependent on the fluence. Shorter wavelengths were more effective than longer ones, though this wavelength dependence was modest. The difference of effectiveness between the shortest (254 nm) and the longest (400 nm) wavelengths was about 20-fold in contrast to the  $10^7$ -fold difference in effectiveness for spore inactivation. In order to quantitate this dependence, we chose a fixed value (20  $\Delta E$ ) of color difference, and the fluence required to produce this value was calculated from each regression relationship. The reciprocal of the fluence is a measure of the effectiveness of radiation at each wavelength. The results are plotted in Fig. 1B. For comparison, the action spectrum for the spore inactivation is also shown in the figure. Clearly, the UV-coloring label

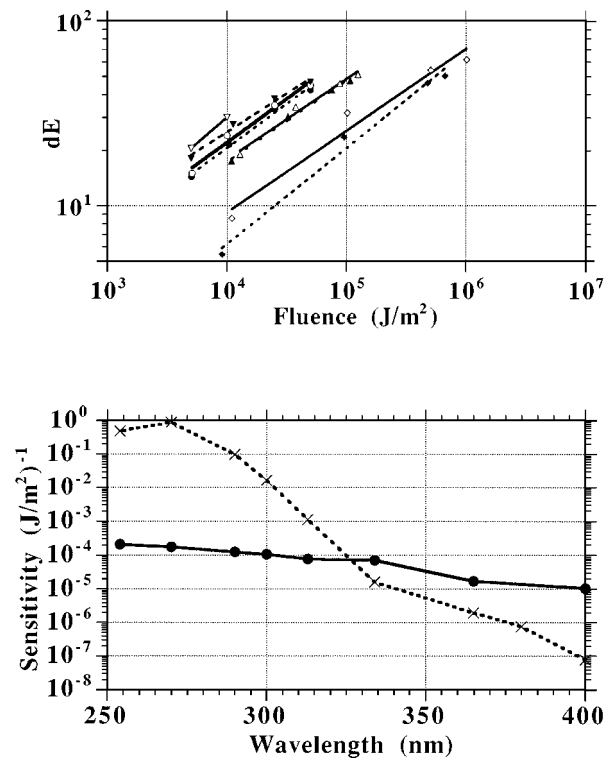


Fig. 1. Response spectrum of the UV-coloring label. Upper panel: The color difference ( $\Delta E$ ) against the fluence of monochromatic UV radiation at wavelengths of 254 ( $\nabla$ ), 270 ( $\blacktriangledown$ ), 290 ( $\circ$ ), 300 ( $\bullet$ ), 313 ( $\triangle$ ), 334 ( $\blacktriangle$ ), 365 ( $\diamond$ ), and 400 ( $\blacklozenge$ ) nm. Lower panel: The effectiveness spectrum ( $\bullet$ ) is shown as the inverse of the fluence required to produce a fixed value ( $\Delta E=20$ ) of the color difference. For comparison, the inactivation action spectrum for the spores is shown ( $\times$ ).

alone can not measure the biological effectiveness, but it can serve as an indication of the exposure through experimental correlations with spore dosimetry, as shown below.

**Spectral absorption of filter sheet** To utilize spore dosimetry and the coloring label for daily exposure monitoring, we considered that the effective dose should be reduced to less than one-tenth. A suitable filter should exhibit uniform absorption in the whole range of solar UV radiation. Among various materials examined, blue polyethylene used for collecting garbage in the laboratory turned out to be suitable, though the original sample with a thickness of 0.04 mm reduced the transmission only to a half. We ordered additional types of sheets with a thickness of 0.07 mm and 0.1 mm. The three types of sheets were designated BPS04, BPS07, and BPS10 (Umeya Sangyo, Tokyo).

The optical properties of BPS04, BPS07, and BPS10 were determined by the use of the Okazaki large spectrograph. As shown in Fig. 2, each filter reduced the

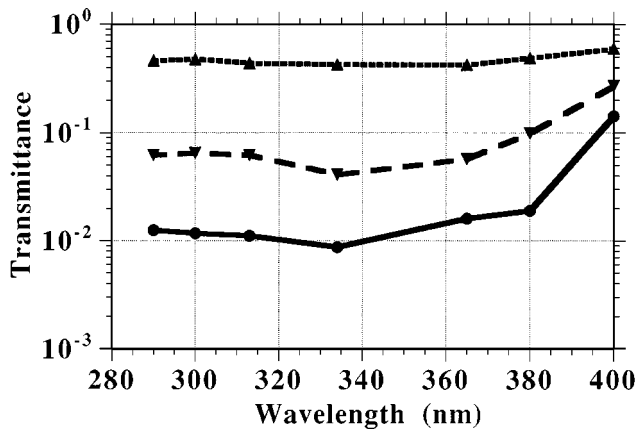


Fig. 2. Spectral transmission of the three filtering sheets, BPS04 (▲), BPS07 (▼), and BPS10 (●).

transmission uniformly in the crucial range of UV wavelengths between 280 nm and 334 nm. While the thinnest sheet, BPS04, exhibited relatively flat transmission over the wavelength range up to 400 nm, the thicker two exhibited notable increases in transmittance at longer UV wavelength than 365 nm.

In field experiments on spore dosimetry carried out in Tokyo, BPS04, BPS07, and BPS10 filters reduced the biological effectiveness of solar radiation 2.08, 14.0, and 57.7 times, respectively. We applied these reduction factors to estimate the inactivation dose in this report.

**Construction of the badge** In the final design (Fig. 3), each badge contains pieces of UV-coloring label and membrane filter carrying two spots of  $10^5$  spores. One spot is masked and serves as a control. A filter sheet of BPS04, BPS07, or BPS10, according to the season, is used to cover the other spore spot and the label. Another piece of UV-coloring label with different types of filters to allow the estimation of UVA (>320 nm) exposure is also included, and an analysis of the results obtained from this portion of the label will be presented elsewhere. Except for the regions over the spore spot and the UV-coloring label, the badge was covered with cardboard and a plastic case. The cardboard carries instructions for the children and an identification number. The badges are each placed in a black envelope marked with the day on which it is to be opened and used. A set of seven badges, a pencil, and a small notebook to be used for recording outdoor activities during the week, are included in a box.

After collection of the badges, color difference of UV-coloring labels was determined using a color meter. Most of the samples exhibited small values of color difference. We performed the assay of spore survival only for those samples exhibiting significant coloration.

In this report, we use the term “exposure” to indicate the amount of personal exposure estimated from the badge worn on an upper arm, whereas the term “irradiance” is used to indicate the amount of global UV irradiance estimated from a horizontally placed badge exposed to unobstructed sunlight.

## RESULTS

**Outline of exposure monitoring** One class of 30–40 schoolchildren in the fifth-grade (10–11 years old at the start of this project) of primary schools in each of five cities, Sapporo (43.0°N, 141.2°E), Tsukuba (36.1°N, 140.0°E), Tokyo (35.4°N, 139.5°E), Miyazaki (31.6°N, 131.3°E), and Naha (26.1°N, 127.4°E), participated in this study. We chose these four cities outside Tokyo since routine spectral measurements with Brewer spectrophotometers have been performed in them by the Meteorological Agency.<sup>10</sup> Tokyo was added because a continuous monitoring study with spore dosimetry has been carried out there by one of us.<sup>7,11</sup> After instruction by a class teacher, each child was given a set of seven badges, one of which was to be taken out each morning and hooked onto the shirt at an upper arm for the entire day.

The first series of exposure monitorings was performed for a week starting from December, 1994 in Sapporo, Tsukuba, Miyazaki, and Naha. In Tokyo, the start of the experiment was delayed, so the winter monitoring was performed in December, 1995. Spring, summer, and autumn monitorings were performed in April and May, July, and October in 1995, respectively. Due to individual circumstances at each school, the monitoring schedules did not exactly coincide at the five localities. We collected about 1,200 samples from each season’s monitoring, and measured the color difference ( $\Delta E$ ) of the UV-coloring label. Most of the samples showed little change in coloration. We considered that a value of color difference ( $\Delta E$ ) below 15 was insignificant, since no inactivation of spores was seen in such samples (surviving fractions were scattered within the error-range of the colony-formation assay, i.e., 0.8 and 1.2). Therefore, we carried out the spore survival assay for spore dosimetry only for samples where the labels showed significant coloration.

Some of the colorations of the UV-coloring label appeared spurious; the color was dark, but purplish, and did not match the blue color due to solar exposure. These were considered to have been wetted in showers or by accident. Data from such labels were discarded.

**Estimation of ID from color difference of UV-coloring labels** The thinnest filter (BPS04), which we used for the monitoring in winter, reduces the transmittance to only about a half, so it was necessary to use a thicker filter for other seasons. We used BPS10 for the spring and summer monitoring, but it proved to be too opaque under most

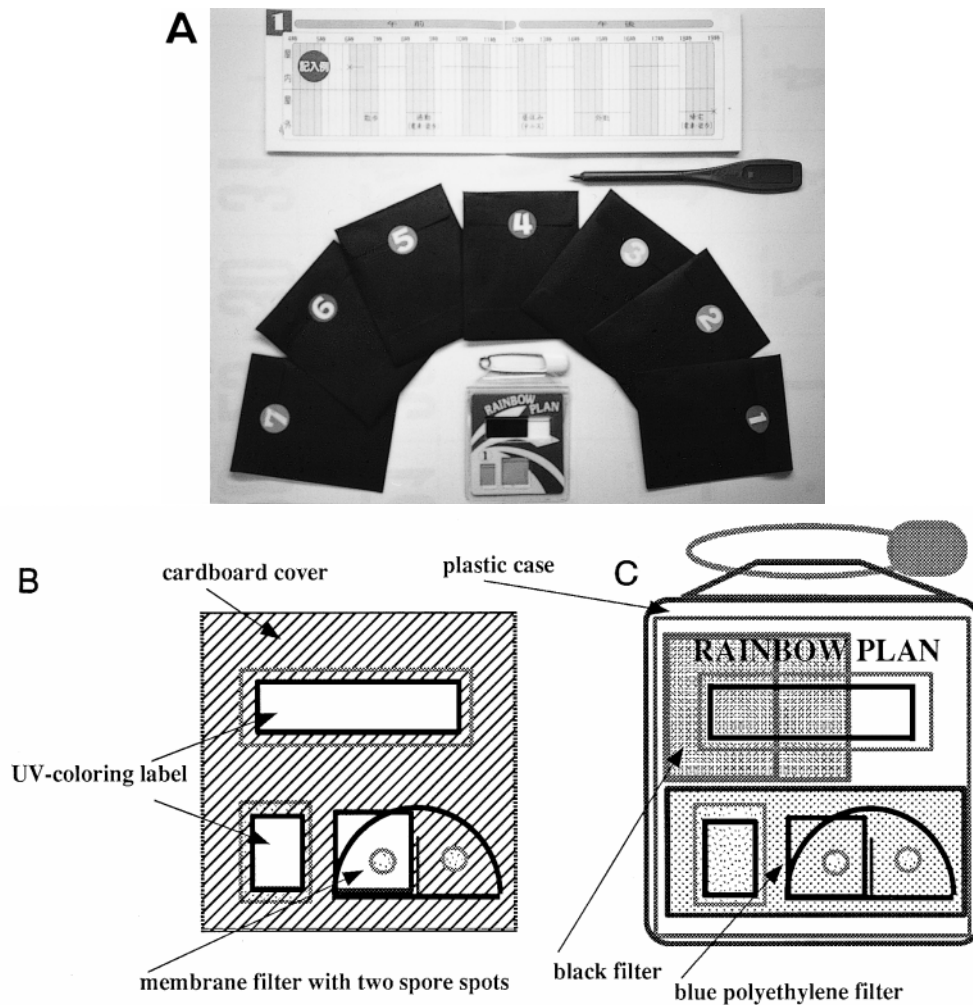


Fig. 3. A set of seven monitoring badges and a notebook to record outside activities (A). An inside (B) and outside (C) view of each badge is shown.

conditions, and only 136 samples among 1,171 in the spring monitoring exhibited significant coloration ( $>15 \Delta E$ ). Therefore, we used filter sheet BPS07 with intermediate thickness for the autumn monitoring. In retrospect, we think that the use of BPS07 for all seasons would have been preferable to ensure consistency of the data.

The plots in Fig. 4 show the regression relationships between the values of color difference and spore inactivation using the three different sheets. For each set, the values were regressed to a power function as shown in this figure. From these regression relationships, we estimated the ID values. These values were multiplied by the appropriate transmission factors of the three types of filters. The statistics of the ID values for each monitoring day are summarized in Table I. In this table, after the locality and

the date, the global insolation (Global INS in  $\text{MJ}/\text{m}^2$ ) from the record of each local observatory for the day is shown.

From the summer, three badges at each site were exposed to unobstructed sunlight for whole days during the monitoring. The average values correspond to daily global UV irradiance and represent the maximum solar-UV exposure on each day at each site. The data of global irradiance were missing for the winter and spring, but we tried to estimate them from the regression relationships obtained between the amount of global insolation and daily ID at each site as developed previously for the monitoring study in Tokyo.<sup>7)</sup> Estimated values of daily ID representing the solar-UV irradiance for the day are shown in the table. The numbers refer to the recovered samples

excluding those considered spoiled. For the remaining valid samples, the mean ID and the standard deviation (SD) were derived. The maximum ID (Max ID) values from each daily monitoring are shown in the last column.

**Statistics of daily exposure** There are 140 data of average daily exposure in the five cities (rows in Table I). With regard to the mean values of ID for each class, most were less than 5 ID, and were considered to represent negligible exposure. There are 8 day-classes exceeding 5 ID. Among them, 5 were less than 6 ID and these were observed in Miyazaki. The remaining three exceeded 10

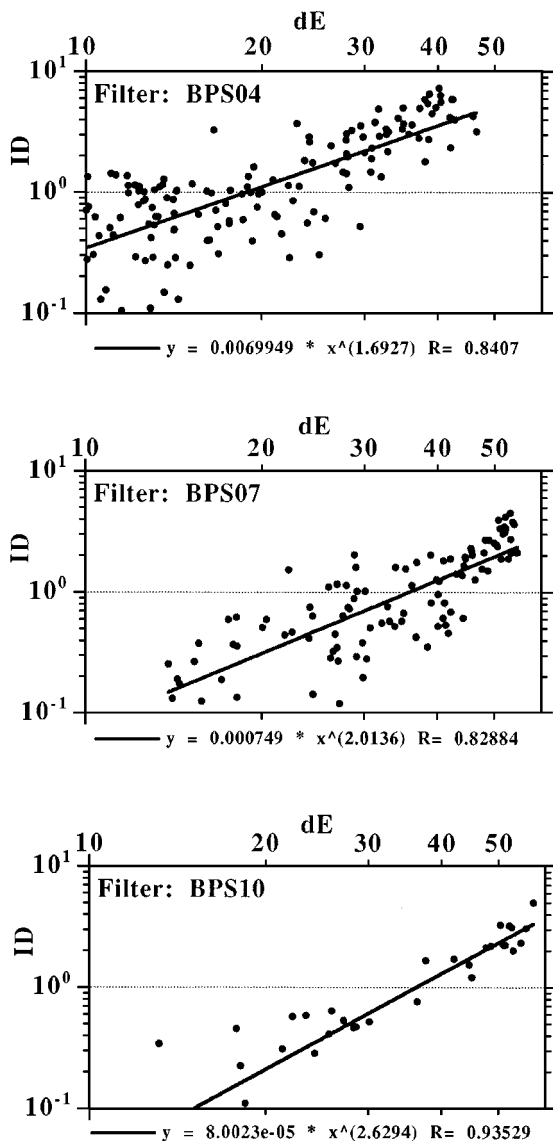


Fig. 4. Correlations between the values of color difference (dE) and ID with a BPS04, BPS07, or BPS10 filter. The power-function regression is shown below each panel.

ID; 21.5 (Tokyo, July 13), 19.2 (Tokyo, April 27), and 15.2 (Tsukuba, April 24). Global insolation and total irradiance were also high on these three days. The high exposures seem to have been due to outdoor school activities which took place in good weather, as discussed below.

In order to investigate individual cases of high exposure, daily data for each season at the five localities were binned in intervals of 5 ID, and the results are shown in Fig. 5. Note that the ordinate is logarithmic, and the bottom line shows one sample.

In the winter, all the values except one were less than 10 ID. The means were less than 1.0 for most of the data in Sapporo, Tsukuba, and Tokyo, reflecting the low UV irradiance. In Sapporo, 5 samples exhibiting more than 4.0 ID were from 3 children taking a skiing vacation. In Miyazaki, the mean ID values were relatively large on three days (Friday, Sunday, and Monday), when the outside irradiance was also high. In Naha, the mean values were less than 2 ID, but there were some individuals exposed more than 5 ID; the maximum exposure was 12.5 ID on Friday from a child involved in outdoor sport for 450 min.

In the spring, 89 data exceeded 10 ID, and among them 86 were below 30 ID. The exposure was more or less uniform for all the sites, with two exceptional cases. One was April 27 in Tokyo and the other was April 24 in Tsukuba. On these days, most samples showed high levels of exposure, reflecting whole-day outdoor activity (an excursion in Tokyo and a sports day in Tsukuba). Except for these two occasions, the rest of the samples are scattered, with the values depending mostly on the weather. Two data exceeding 40 ID occurred on the same excursion

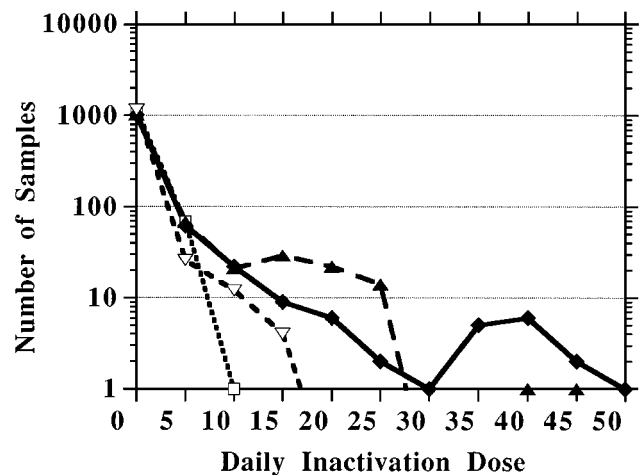


Fig. 5. The individual daily values of ID in winter ( $\square$ ), spring ( $\blacktriangle$ ), summer ( $\blacklozenge$ ), and autumn ( $\nabla$ ), are binned in units of 5, and the numbers of cases within each bin are shown.

Table I. Summary of Daily Exposure Monitoring

Site	Date	Global INS <sup>a)</sup>	Global ID <sup>b)</sup>	Number	Mean ID <sup>c)</sup>	SD <sup>d)</sup>	Max ID <sup>d)</sup>
Sapporo	19941220	5.53	2.70	29	0.63	0.37	1.28
Sapporo	19941221	6.34	3.33	30	0.80	0.37	1.73
Sapporo	19941222	3.40	1.28	30	0.43	0.23	0.95
Sapporo	19941223	6.43	3.40	30	0.59	1.60	7.02
Sapporo	19941224	6.80	3.71	30	0.53	1.34	6.28
Sapporo	19941225	1.95	0.54	30	0.09	0.16	0.92
Sapporo	19941226	8.05	4.81	30	0.64	1.22	5.05
Tsukuba	19941220	5.68	8.56	36	0.47	0.36	1.29
Tsukuba	19941221	8.57	16.12	36	0.66	0.45	2.18
Tsukuba	19941222	10.65	22.52	34	0.89	0.82	3.38
Tsukuba	19941223	9.66	19.38	35	0.71	0.97	4.12
Tsukuba	19941224	9.85	19.97	36	1.01	0.95	4.11
Tsukuba	19941225	8.46	15.80	36	0.48	0.64	2.75
Tsukuba	19941226	7.16	12.22	36	0.45	0.76	3.27
Tokyo	19951214	8.90	13.11	35	1.19	0.65	2.94
Tokyo	19951215	8.20	11.56	36	0.76	0.54	1.90
Tokyo	19951216	9.80	15.21	36	0.64	0.59	2.88
Tokyo	19951217	10.80	17.66	36	0.54	1.06	5.62
Tokyo	19951218	11.10	18.42	36	0.87	0.49	2.07
Tokyo	19951219	3.80	3.54	36	0.37	0.16	0.74
Tokyo	19951220	6.60	8.28	36	0.48	0.34	1.31
Miyazaki	19941215	2.98	5.27	37	1.01	1.25	8.13
Miyazaki	19941216	12.47	47.66	37	4.78	1.53	8.35
Miyazaki	19941217	8.25	25.24	37	1.25	1.01	4.21
Miyazaki	19941218	12.14	45.73	36	4.12	3.12	9.64
Miyazaki	19941219	12.78	49.49	37	5.14	1.71	8.25
Miyazaki	19941220	4.30	9.26	37	0.42	0.36	1.69
Miyazaki	19941221	2.46	3.92	37	0.40	0.23	0.86
Naha	19941222	10.64	34.12	37	1.23	0.86	4.06
Naha	19941223	14.28	53.65	37	1.86	2.54	12.51
Naha	19941224	8.00	22.00	37	0.89	0.81	3.25
Naha	19941225	8.52	24.24	35	0.69	0.72	2.49
Naha	19941226	7.26	18.95	35	0.61	0.70	2.90
Naha	19941227	11.80	40.00	36	1.54	1.97	7.52
Naha	19941228	8.13	22.55	37	0.76	0.80	2.77
Sapporo	19950425	22.31	23.06	32	2.98	1.95	7.37
Sapporo	19950426	5.01	2.32	32	0.19	0.10	0.48
Sapporo	19950427	15.47	13.13	32	1.32	0.90	3.71
Sapporo	19950428	21.48	21.76	30	4.36	2.87	13.74
Sapporo	19950429	22.36	23.14	30	2.57	4.42	18.88
Sapporo	19950430	12.30	9.23	31	1.27	2.86	15.21
Sapporo	19950501	23.31	24.67	30	3.48	3.73	13.44
Tsukuba	19950424	25.29	85.18	29	15.25	6.29	27.15
Tsukuba	19950425	4.47	5.92	28	0.13	0.03	0.21
Tsukuba	19950426	11.49	25.30	30	0.62	0.40	1.58
Tsukuba	19950427	23.36	75.39	29	2.03	1.45	5.10
Tsukuba	19950428	13.65	32.98	30	1.02	0.72	2.87
Tsukuba	19950429	12.13	27.51	28	1.36	2.22	11.60
Tsukuba	19950430	8.28	15.29	28	1.39	2.73	11.34
Tokyo	19950425	2.30	1.64	37	0.45	0.17	0.75
Tokyo	19950426	11.07	18.34	37	0.66	0.60	2.88
Tokyo	19950427	21.64	51.45	37	19.19	10.48	45.37
Tokyo	19950428	10.98	18.11	37	0.99	0.50	1.96
Tokyo	19950429	19.51	43.86	36	2.71	5.90	27.73
Tokyo	19950430	7.96	11.04	34	0.35	0.39	1.66
Tokyo	19950501	4.49	4.58	38	0.21	0.12	0.66
Miyazaki	19950509	27.49	160.81	37	2.27	1.51	5.51
Miyazaki	19950510	22.99	122.14	35	2.63	1.86	7.91
Miyazaki	19950511	25.60	144.12	37	3.09	1.97	8.14
Miyazaki	19950512	27.36	159.64	36	2.73	2.33	9.24
Miyazaki	19950513	25.42	142.56	29	5.92	7.08	27.60
Miyazaki	19950514	14.71	61.45	25	2.88	5.59	20.30
Miyazaki	19950515	6.96	19.43	33	0.33	0.18	0.78
Naha	19950426	8.09	22.38	38	0.71	0.55	2.79
Naha	19950427	8.67	24.90	37	1.12	0.70	3.81
Naha	19950428	25.08	127.61	38	3.44	4.84	16.91
Naha	19950429	20.04	90.36	37	4.62	8.54	27.17
Naha	19950430	12.04	41.26	38	1.93	4.30	22.01
Naha	19950501	20.57	94.06	38	1.12	2.52	15.75
Naha	19950502	19.35	85.62	35	2.56	1.63	5.52
Sapporo	19950713	16.42	16.15	29	1.67	0.77	3.31
Sapporo	19950714	5.96	23.49	28	0.45	0.33	1.70
Sapporo	19950715	22.09	31.26	28	3.09	2.45	9.82
Sapporo	19950716	16.67	24.23	23	0.96	0.89	2.70

Table I. (Continued)

Site	Date	Global INS <sup>a)</sup>	Global ID <sup>b)</sup>	Number	Mean ID <sup>c)</sup>	SD <sup>d)</sup>	Max ID <sup>d)</sup>
Sapporo	19950718	29.43	31.48	29	3.56	2.60	10.52
Sapporo	19950719	26.89	14.56	29	2.91	1.23	5.19
Tsukuba	19950714	10.24	100.11	30	2.85	1.66	5.60
Tsukuba	19950715	23.14	102.96	29	4.02	4.89	18.79
Tsukuba	19950716	7.09	18.54	30	0.44	0.70	2.65
Tsukuba	19950717	6.73	10.16	31	0.69	0.77	4.00
Tsukuba	19950718	7.23	24.26	29	1.04	1.02	4.56
Tsukuba	19950719	14.40	35.74	31	0.84	0.87	3.43
Tsukuba	19950720	7.37	9.40	29	0.49	0.69	3.85
Tokyo	19950712	8.00	9.02	36	1.17	1.10	4.40
Tokyo	19950713	18.30	64.05	36	21.46	16.29	50.07
Tokyo	19950714	11.50	30.34	32	0.86	0.97	3.66
Tokyo	19950715	19.70	27.38	35	1.72	2.11	9.44
Tokyo	19950716	8.20	4.04	25	0.76	1.95	9.66
Tokyo	19950717	8.60	9.87	34	0.34	0.20	1.00
Tokyo	19950718	4.40	4.21	31	0.20	0.09	0.42
Miyazaki	19950711	18.21	142.12	35	2.48	2.11	10.14
Miyazaki	19950712	22.16	170.62	36	2.35	1.50	5.74
Miyazaki	19950713	27.73	193.60	37	4.97	8.39	38.62
Miyazaki	19950714	27.17	179.30	35	3.99	3.69	16.07
Miyazaki	19950715	26.37	145.35	34	5.37	8.64	46.54
Miyazaki	19950716	25.61	154.89	24	4.75	6.51	21.85
Miyazaki	19950717	12.11	85.60	36	1.10	0.88	4.65
Naha	19950704	23.40	138.46	36	1.08	1.13	5.16
Naha	19950705	23.20	151.20	36	2.72	3.07	11.59
Naha	19950706	25.90	119.09	37	1.94	3.33	13.09
Naha	19950707	25.10	125.72	35	3.29	3.61	15.12
Naha	19950708	23.10	157.25	36	1.36	4.11	24.77
Naha	19950709	6.70	20.31	37	0.69	1.39	6.73
Naha	19950710	13.90	58.59	36	0.80	0.68	3.24
Sapporo	19951007	13.99	6.04	31	1.05	1.58	8.03
Sapporo	19951008	5.34	2.90	31	0.27	0.50	2.86
Sapporo	19951009	13.42	11.75	33	2.65	1.53	7.72
Sapporo	19951010	15.67	15.56	31	1.29	2.01	9.28
Sapporo	19951011	16.21	17.86	32	1.09	0.59	2.45
Sapporo	19951012	15.66	16.23	32	1.25	0.77	3.12
Sapporo	19951013	12.30	5.25	31	2.12	1.23	4.67
Tsukuba	19951013	15.31	21.88	30	2.03	1.30	4.26
Tsukuba	19951014	15.60	20.08	32	1.37	1.72	6.81
Tsukuba	19951015	16.22	21.70	31	2.12	3.26	13.30
Tsukuba	19951016	7.49	9.12	32	2.06	0.97	4.06
Tsukuba	19951017	15.70	22.91	32	0.95	1.24	4.26
Tsukuba	19951018	14.79	18.61	32	0.47	0.41	2.24
Tsukuba	19951019	7.26	8.26	31	0.30	0.21	0.94
Tokyo	19951013	12.00	20.77	37	1.15	0.70	3.91
Tokyo	19951014	15.20	29.88	36	1.15	1.52	6.19
Tokyo	19951015	15.90	32.02	37	1.76	2.23	8.56
Tokyo	19951016	7.80	9.72	37	0.34	0.17	0.95
Tokyo	19951017	16.30	26.26	37	1.85	0.73	3.52
Tokyo	19951018	12.50	20.10	37	0.49	0.29	1.56
Tokyo	19951019	8.90	14.38	36	1.14	0.37	1.89
Miyazaki	19951012	18.95	29.97	36	1.71	1.44	7.84
Miyazaki	19951013	19.32	31.42	36	2.07	1.22	4.53
Miyazaki	19951014	12.79	18.43	37	2.76	3.73	14.13
Miyazaki	19951015	13.16	14.57	36	2.10	2.94	13.49
Miyazaki	19951016	15.62	30.20	36	1.11	0.96	3.60
Miyazaki	19951017	16.65	29.09	33	0.67	0.40	1.68
Miyazaki	19951018	13.69	28.30	34	0.63	0.63	3.37
Naha	19951018	11.50	8.66	35	0.57	0.41	1.71
Naha	19951019	4.10	3.41	35	0.31	0.18	0.89
Naha	19951020	13.70	17.15	36	0.99	1.30	7.72
Naha	19951021	16.90	32.72	37	2.43	4.27	17.68
Naha	19951022	12.30	26.50	37	3.03	4.85	18.40
Naha	19951023	17.30	25.40	34	0.44	0.29	1.23
Naha	19951024	10.40	9.88	36	0.50	0.36	1.60

a) Daily global insolation (MJ/m<sup>2</sup>).

b) Daily global irradiance expressed as the value of ID.

c) Average daily exposure with standard deviation (SD) expressed as the value of ID.

d) Maximum value of ID.

sion day in Tokyo. On the same day, 19 individuals in the class of 37 were exposed more than 20 ID.

In the summer, the average and individual exposures were variable and scattered, being dependent on variable weather and outdoor activities. There is one exceptional case in Tokyo on July 13, exhibiting the mean ID value of 21.5 and the maximum individual exposure of 50.1 ID. This was due to the outdoor curriculum in the school, i.e., swimming, during which clothes with the badge were exposed to the sun at the poolside, followed by outside physical education. Behavioral records indicated that on average the children were involved in outdoor activities for 3 h on this particular day. Thus, this exceptional exposure occurred due to the coincidence of extensive outdoor activity and fine weather. Among 22 samples exceeding 20 ID, 16 occurred on this day in Tokyo. The remaining 5 were from Miyazaki on three days for 4 individuals, and one was from Naha on Sunday. In all these cases, more than 3 h of outdoor activity was reported, except for one case showing 32.8 ID despite only 90 min of recorded outdoor activity.

In the autumn, the exposure was uniformly low; only 17 data exceeded 10 ID, and all were less than 20 ID. Among the former, four occurred in Naha on Saturday and Sunday, four in Miyazaki on Friday and Saturday, and two in Tokyo on Sunday.

**Statistics of weekly exposure** Seven daily data were summed for each individual to obtain weekly statistics. Since only those data with 7 valid data points were used for the statistics, the numbers of available sets were reduced (total 560 person-weeks). The weekly exposure statistics are shown in Fig. 6.

In the winter, the mean value in Miyazaki (17.4 ID) was the highest, followed by Naha (7.0 ID), Tokyo (4.9 ID), Tsukuba (4.8 ID) and the lowest in Sapporo (3.3 ID). High average exposure in Miyazaki was mainly due to the sum of the exposure on three days; two weekdays when outdoor sport took place, and one Sunday. Three upper outlier data in Sapporo are for children involved in skiing.

In the spring, the highest mean was in Tokyo (24.3 ID), followed by Tsukuba (23.0 ID), Miyazaki (17.4 ID), Sapporo (15.9 ID), and Naha (15.2 ID). High values in Tokyo and Tsukuba were caused mostly by one weekday as described above, i.e., 79% and 66% were accounted for by exposure on a single day in Tokyo and Tsukuba, respectively. In the summer, the mean exposure was highest in Tokyo (25.5 ID), followed by Miyazaki (24.9 ID), Sapporo (13.1 ID), Naha (12.3 ID), and Tsukuba (10.6 ID). As in spring, the high value in Tokyo was mostly (84%) due to exposure on one weekday. In contrast, the major daily exposures in Miyazaki were distributed on three days from July 13 to 16 (Sunday). There were 3 individuals exhibiting more than 50 ID in Miyazaki, the maximum weekly exposure being 91.0 ID.

In the autumn, the amounts of weekly exposure were evenly distributed, and the range of the mean exposure at the five localities was small. The highest exposure was at Miyazaki (10.8 ID), followed by Tsukuba (9.0 ID), Sapporo (9.1 ID), Tokyo (7.8 ID), and Naha (7.6 ID). Several individuals were exposed to more than 20 ID, but none exceeded 30 ID.

## DISCUSSION

This is, to our knowledge, the first attempt to employ biodosimetry to monitor solar-UV exposure among human populations. The action spectrum for spore inactivation closely parallels the spectra for DNA-damaging activity in various systems.<sup>9, 12, 13</sup> The value of ID obtained by measuring the survival of UV-sensitive spores is a direct index of exposure to biologically effective UV, integrating the effectiveness of the entire range of UV wavelengths. Furthermore, this is one of the most sensitive dosimeters to changes of the irradiance spectrum at the shortest region of wavelengths, such as those that might be increased following ozone depletion.

Since the majority of samples received small doses as judged from the end point of spore inactivation, it was necessary to employ an auxiliary dosimeter that gave results positively correlated with the biological effectiveness. In this regard, the UV-coloring label was useful; it is highly sensitive, changing coloration with small amounts of exposure. Also, it has a long shelf-life without significant background coloration (<2  $\Delta E$  assayed more than one year after sample preparation). The values of color difference ( $\Delta E$ ) could be correlated with the values of ID by applying simple power functions. One disadvantage of the label was that it exhibited abnormal coloration when wetted. In suspicious cases, careful comparisons with the data obtained from the spore dosimetry were necessary to determine if the coloration was entirely due to solar exposure.

The use of filter sheets was necessary to reduce irradiance to measurable ranges for the spore dosimeter and the UV-coloring label. The filter also functions to protect samples from direct environmental and artificial assaults. Although the three types of filters made of blue polyethylene worked as expected, possible complications due to the employment of filters should be borne in mind. Though all three types were made of the same dye and solvent, the correlation factors between the values of ID and  $\Delta E$  were distinctly different: a given value of  $\Delta E$  corresponded to a smaller value of ID when the thicker filter sheet was used. This implies that the transmission at the longer UV or visible wavelengths contributed significantly to the coloration. At this point, we think BPS07 is most suitable for the monitoring of daily exposure in Japan.



We could group the extent of the daily individual exposures into three levels, low ( $<20$  ID), moderate ( $20 < \text{ID} < 40$ ), and high ( $>40$  ID). At the height of solar elevation under a clear sky in Tokyo, rates of global irradiance are about 0.35 ID/min. Thus, a one-hour exposure could produce about 20 ID. In the present 4,711 samples, cases of moderate exposure amounted to 50 (1.1% of total), and those of high exposure to 11 (0.25% of total). All of these cases occurred in spring and summer, and none of them occurred in Sapporo. Two days in Tokyo

and one day in Tsukuba, in which the cases are concentrated, correspond to days of outdoor school activities, while the others in Miyazaki and Naha correspond to individual outdoor activities.

In discussing the results of weekly exposure, it is helpful to look at the ratios of the average exposure to the global irradiance as shown in Fig. 7. Total global irradiance was low in Sapporo in all seasons. However, the exposure was relatively constant, the ratios of the exposure and the irradiance being between 16.5% (winter) and 9.8% (sum-

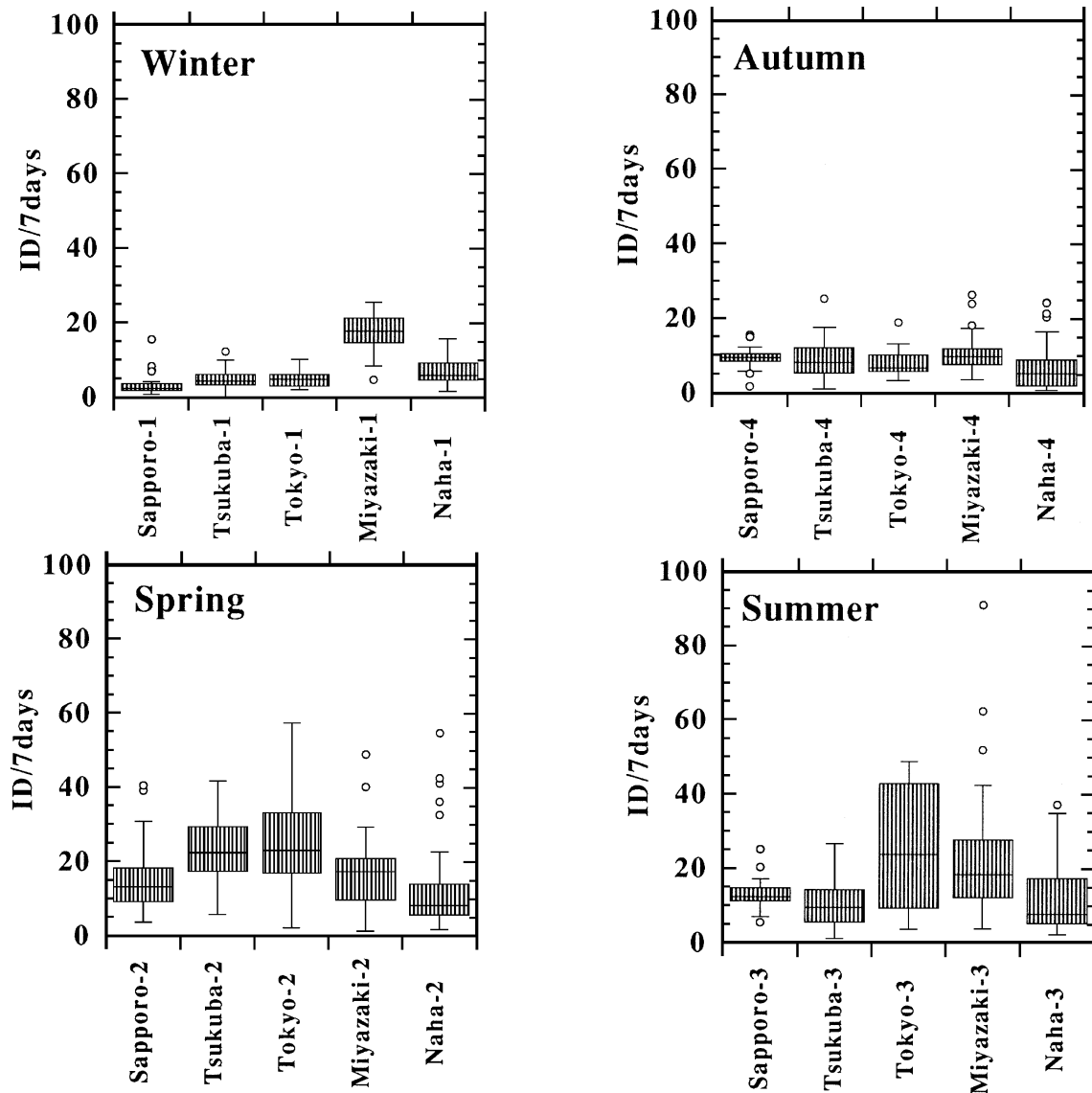


Fig. 6. Weekly exposure expressed as the total values of ID for a week. Each box is drawn with the upper and lower quartile from the mean (marked), and the bars represent  $\pm 1.5$  of the interquartile distance. The circles are outliers.

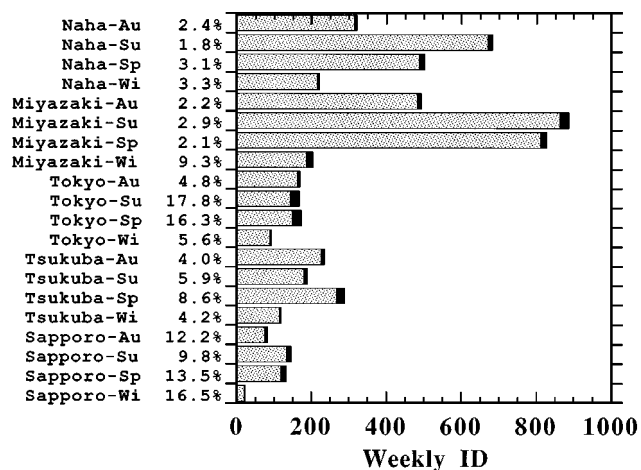


Fig. 7. Weekly global irradiance (dotted columns) and average exposure (black columns). The name of the city and the seasons (Wi, Sp, Su, and Au stand for winter, spring, summer, and autumn, respectively) are followed by the ratio of the average exposure to the total global irradiance expressed as a percentage.

mer). In Tsukuba and Tokyo, the ratios exceeded 10% in three weeks in spring and summer, and the ratios in other seasons were about 5%. In Miyazaki and Naha, the ratios were less than 5% except in winter, when it was 9.3% in Miyazaki. The week with the lowest ratio was 1.8% in summer in Naha. It is interesting to note that, in these two southern cities, the extent of exposure is relatively uniform in spite of the large seasonal changes of the outdoor irradiance.

All three days exhibiting mean ID values of more than 15 involved scheduled outdoor activities of the school. This indicates that those activities are the most important factor regarding the children's exposure. It is likely that such activities are avoided in both Miyazaki and Naha during the hot season, so that such concentrated exposure did not occur there. Thus, those schoolchildren who participated in this study were not exposed to solar-UV radi-

ation in proportion to the amount of the outdoor irradiance. Their main sources of exposure are outdoor school activities, either scheduled events or sports-club activities. When these outdoor exertions took place in good weather during months of high irradiance, the majority of children in the class were exposed to significant amounts of UV. Individual exposures occurred sporadically for those children involved in intensive sports training.

It is interesting that most of the schoolchildren in the two southern cities seem to behave in an apparently rational manner, avoiding excessive exposure from spring to autumn, while attaining reasonable exposure in winter. Perhaps these children are encouraged to undertake outdoor activities in winter, and discouraged in other seasons, except in the evenings. It is likely that school curricula and schedules are arranged to favor such behavior. We are not certain whether this is a traditional way of living in these localities or a recent practice resulting from environmental awareness, but it is possible that both of these factors play some role. It is clear that the major determinant of solar-UV exposure of schoolchildren is their life-style, including both social activities and individual behaviors, and their exposures are not quantitatively correlated to the outdoor UV irradiance, which is geologically and meteorologically determined.

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REFERENCES

- 1) "IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 55, Solar and Ultraviolet Radiation" (1992). IARC, Lyon.
- 2) De Grujil, F. R., van der Meer, J. B. and van der Leun, J. C. Dose-time dependency of tumor formation by chronic UV exposure. *Photochem. Photobiol.*, **37**, 53-62 (1983).
- 3) Rundel, R. D. and Nachtwey, D. S. Projections of increased non-melanoma skin cancer incidence due to ozone depletion. *Photochem. Photobiol.*, **38**, 571-591 (1983).
- 4) Henriksen, T., Dahlback, A., Larsen, S. H. H. and Moan, J. Ultraviolet-radiation and skin cancer. Effect of an ozone layer depletion. *Photochem. Photobiol.*, **51**, 579-582 (1990).
- 5) Munakata, N. Killing and mutagenic action of sunlight upon *Bacillus subtilis* spores: a dosimetric system. *Mutat. Res.*, **82**, 263-268 (1981).
- 6) Munakata, N. Genotoxic action of sunlight upon *Bacillus subtilis* spores: monitoring studies at Tokyo, Japan. *J. Radiat. Res.*, **30**, 338-351 (1989).
- 7) Munakata, N. Biologically-effective dose of solar ultraviolet radiation estimated by spore dosimetry in Tokyo since

1980. *Photochem. Photobiol.*, **58**, 386–392 (1993).
- 8) Watanabe, M., Furuya, M., Miyoshi, Y., Inoue, Y., Iwahashi, I. and Matsumoto, K. Design and performance of the Okazaki large spectrograph for photobiological research. *Photochem. Photobiol.*, **36**, 491–498 (1982).
- 9) Munakata, N., Morohoshi, F., Hieda, K., Suzuki, K., Furusawa, Y., Shimura, H. and Ito, T. Experimental correspondence between spore dosimetry and spectral photometry of solar ultraviolet radiation. *Photochem. Photobiol.*, **63**, 74–78 (1996).
- 10) Ito, T. UV-B observation network in the Japan meteorological agency. In “Frontiers of Photobiology,” ed. A. Shima, M. Ichihashi, Y. Fujiwara and H. Takebe, pp. 515–518 (1993). Elsevier, Amsterdam.
- 11) Munakata, N. Continued increase in biologically effective dose of solar UV radiation determined by spore dosimetry from 1980 to 1993 in Tokyo. *J. Photochem. Photobiol. B*, **31**, 63–68 (1995).
- 12) Setlow, R. B. The wavelengths in sunlight effective in producing skin cancer: a theoretical analysis. *Proc. Natl. Acad. Sci. USA*, **71**, 3363–3366 (1974).
- 13) Tyrrell, R. M. Solar dosimetry with repair-deficient bacterial spores: action spectra, photoproduct measurements and a comparison with other biological systems. *Photochem. Photobiol.*, **27**, 571–579 (1978).