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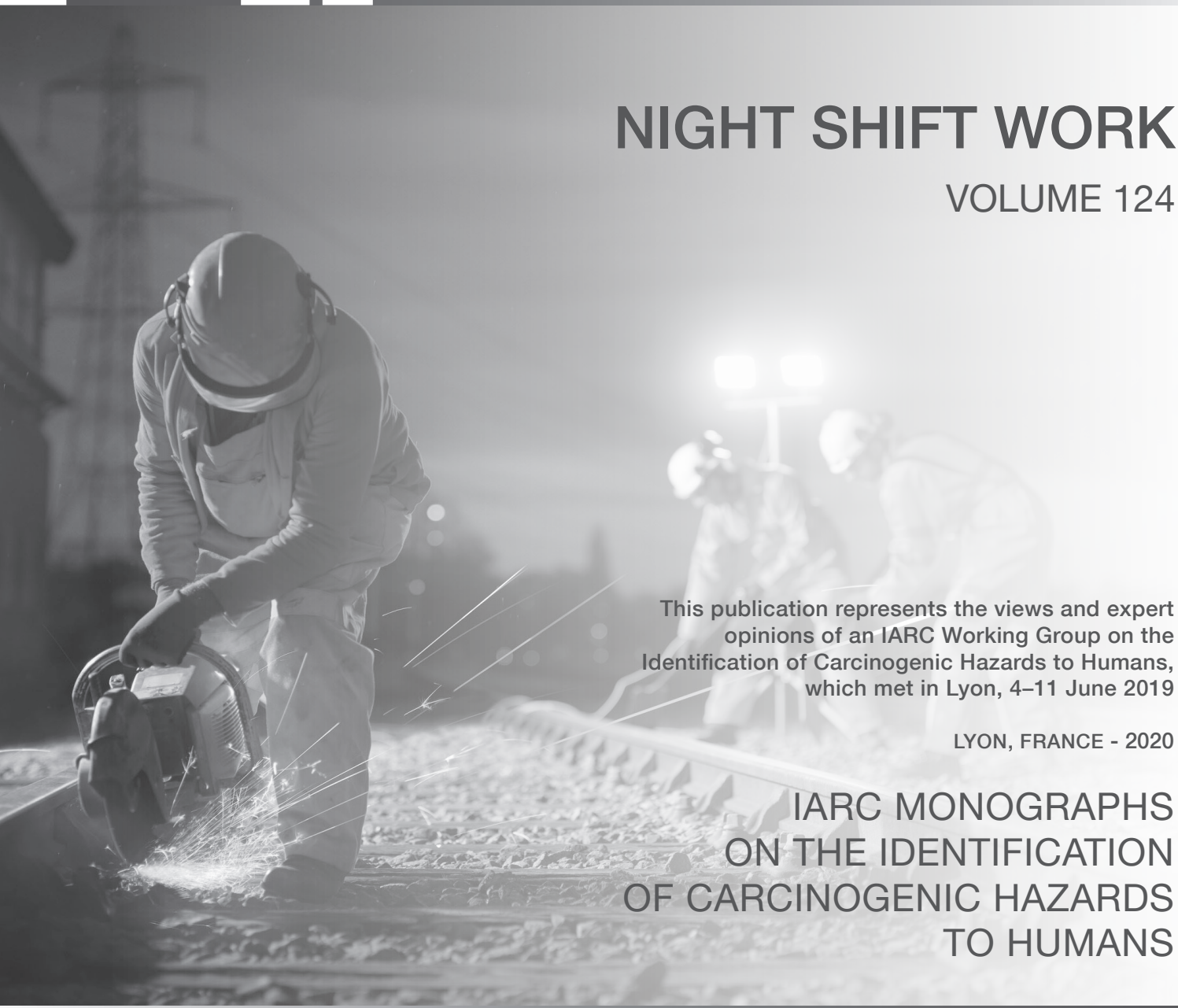

NIGHT SHIFT WORK

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IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

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3. CANCER IN EXPERIMENTAL ANIMALS

Experimental animal models in research facilities are generally kept in artificial light–dark (LD) schedules. The exposure of such animal models to alterations in the light–dark schedule can involve the following conditions: exposure to light for a variable period of time during the period of darkness or the natural night; repeated occurrences of exposure to light during the period of darkness or the natural night, and/or over multiple periods of darkness or natural nights, at any frequency; continuous light exposure; or shifts in the light–dark schedule, that is, the advance of light onset by 6–12 hours every 2–7 days. In rodent models of shifts in the light–dark schedule, exposure to light regularly occurs at the expected time of darkness.

In the studies of alterations in the light–dark schedule reviewed below, if circadian disruption was assessed and observed, it is briefly mentioned in the study description.

A few studies reviewed in the text that follows are not reported in Tables 3.1–3.5 because they were either inadequate for the evaluation of the carcinogenicity of alterations in the light–dark schedule in experimental animals (i.e. Bishehsari et al., 2016) or were described in insufficient detail to be tabulated (Jöchle, 1963; Joechle, 1964; van den Heiligenberg et al., 1999; Cos et al., 2006; Toth et al., 2017).

3.1 Shifts in the light–dark schedule

See also Table 3.1.

3.1.1 Mouse

Heterozygous PyMT oncogene female mice [FVB background, melatonin deficient] were exposed either to a constant schedule of 12 hours of light followed by 12 hours of darkness (LD12:12; $n = 12$ controls) or to shifts in the light–dark schedule ($n = 17$ exposed) from weaning (age, 3 weeks) until the age of 14 weeks. Exposed mice had their light–dark schedule inverted for three consecutive 24-hour periods every week. At the age of 14 weeks, 4–5 mice were killed by cervical dislocation every 4 hours over a 24-hour period. The mammary tumour mass was removed and weighed. The mammary tumour burden of exposed mice was significantly higher than that of controls (Kennaway, 2009). [The Working Group noted the limited experimental details.]

In a 70-week experiment to study chronic circadian rhythm disturbances, two groups of 25 female *Tp53*$^{R270H/+}$*WAPCre* mice prone to cancer of the breast [FVB background, melatonin deficient] (age, 8 weeks) were exposed either to a constant schedule of LD12:12 (control), or to a schedule of LD12:12 that was inverted at the end of every week by extending the light or dark phase to 24 hours (light exposure chamber, 141.5 lux, 345 μW/cm^2). The weekly 12-hour reversal of the light–dark schedule was not observed to disrupt the peripheral clock function in the liver. Mice were killed after tumour development, and the number of control and exposed mice bearing tumours was determined. In both groups, approximately 80% of the mice developed mammary

Table 3.1 Studies of carcinogenicity in experimental animals exposed to shifts in the light–dark schedule

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, latency, multiplicity, weight, or size of tumours	Significance	Comments
Full carcinogenicity Mouse, PyMT (F) 3 wk 11 wk Kennaway (2009)	Shifts in the LD schedule LD12:12 (control), LD12:12 schedule inverted for three consecutive 24-h periods per week (exposed) 12, 17 NR	*Mammary gland*: total tumours (gross examination) Weight (g): 6.0, 7.5*	*$P < 0.03$, one-tail test [not further specified]	Principal limitations: limited experimental details; no data on circadian disruption status Tumour weight read from graph
Full carcinogenicity Mouse, *p53*$^{R270H/+}$ *WAPCre* (F) 8 wk ~ 70 wk (read from graph) Van Dycke et al. (2015)	Shifts in the LD schedule LD12:12 (control), LD12:12 inverted 1×/wk by extending light or dark phase to 24 h (exposed) 25, 25 20, 21	*Mammary gland* All tumours Incidence: 18/20, 17/21 Latency (wk): 50.3, 42.6* Fibrosarcoma or carcinosarcoma Incidence: 12/20, 15/21 Carcinoma Incidence: 5/20, 1/21 Intraepithelial neoplasia Incidence: 1/20, 1/21 *Haematopoietic and lymphoid tissues*: lymphosarcoma Incidence: 3/20, 5/21	 NS *$P = 0.0127$, Kolmogorov–Smirnov NS NS NS NS	Mice were killed after tumour development or when moribund; strain with FVB background (melatonin deficient)
Full carcinogenicity Mouse, C57BL/6J (M, F) (combined) 4 wk 86 wk Kettner et al. (2015, 2016)	Shifts in the LD schedule LD12:12 (control) and weekly transfer between two rooms with LD12:12 conditions offset by 8 h (exposed) for each of wildtype; *Cry* mutant, *Per* mutant, *Alb*^{Cre}; *Bmal1*$^{fl/fl}$; *Car*$^{-/-}$; and *Fxr*$^{-/-}$ NR NR	*Liver*: hepatocellular carcinoma Incidence: 0/110, 7/80*; 7/60, 10/56; 10/80, 13/50**; 3/26, 5/43; 0/24, 0/25; 7/25, 19/31*** Multiplicity: 0, 2, 3, 6, 2.5, 6*, 23, 7, NR, NR, NR, NR Average size (cm): 0.00, 0.70, 0.59, 0.97*, 0.56, 1.08*, 0.12, 0.60*, NR, NR, NR, NR	 *$P = 0.007$ (Kaplan–Meier statistics), **[$P = 0.043$, Fisher one-tail exact test], ***[$P = 0.017$, Fisher two-tail exact test] *$P < 0.01$, Student *t*-test *$P < 0.001$, Student *t*-test	Percentage of survival, tumour multiplicity, and tumour size data were reported in graphic form; melatonin-deficient strain; approximately equal number of males and females of each genotype

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, latency, multiplicity, weight, or size of tumours	Significance	Comments
Full carcinogenicity Rat, inbred BN (F) 3 wk 147 wk Kort et al. (1986)	Shifts in the LD schedule LD12:12 (control), LD12:12 inverted 1×/wk by advancing by 12 h every Friday (exposed) 100, 100 30, 35	*Haematopoietic and lymphoid tissues*: histiocytic sarcoma Incidence: 1/100, 5/98	NS	Survival and body-weight data were reported in graphic form; melatonin-deficient strain

F, female; h, hour; LD, light–dark; LD12:12, 12 h of light followed by 12 h of darkness; M, male; NR, not reported; NS, not significant; wk, week.

tumours, including carcinomas and carcinosarcomas. The latency to mammary gland tumour development was reduced by 17% in the exposed mice compared with control mice (42.6 versus (vs) 50.3 weeks, respectively; Kolmogorov–Smirnov test, $P = 0.0127$). However, chronic weekly inversion of the light–dark schedule was not observed to affect the number of tumour-bearing mice or tumour type (mammary gland tumours, lymphosarcoma, or other tumours) (Van Dycke et al., 2015).

In the study by Kettner et al. (2015, 2016), groups of C57BL/6J (i) wildtype [melatonin deficient], (ii) *Cry1*$^{-/-}$;*Cry2*$^{-/-}$, (iii) *Per1*$^{-/-}$;*Per2*$^{-/-}$, (iv) *Alb*^{Cre};*Bmal1*$^{fl/fl}$, (v) *Car*$^{-/-}$, and (vi) *Fxr*$^{-/-}$ mice were fed standard mouse chow and water. From the age of 4 weeks until the age of 90 weeks, six groups of control mice were maintained at regular LD12:12 schedules, and six groups of mice were exposed to shifts in the light–dark schedule by being transferred once per week between two mouse rooms with LD12:12 schedules offset by 8 hours. Approximately equal numbers of male and female mice within each genotype were used. Mice were monitored twice per week, and moribund mice were killed for pathological analysis. At the end of the study, all tissues and organs were inspected, and abnormal tissues and tumours were processed for histological analysis. Circadian rhythm was disrupted in the exposed mice, and exposed wildtype and mutant mice showed significantly reduced survival compared with their respective control groups, with disease development including cancer. Compared with their respective controls, there was a significant increase in the incidence of hepatocellular carcinoma in exposed wildtype mice (7/80 exposed vs 0/110 controls, $P = 0.007$), in *Per1*$^{-/-}$;*Per2*$^{-/-}$ mice (13/50 exposed vs 10/80 controls [$P = 0.043$]), and in *Fxr*$^{-/-}$ mice (19/31 exposed vs 7/25 controls [$P = 0.017$]). Exposure to shifts in the light–dark schedule also increased the size of hepatocellular carcinomas in *Per* and *Cry* mutant mice and *Alb*^{cre}*Bmal1*$^{fl/fl}$ mice, and tumour multiplicity in *Per* mutant mice.

Toth et al. (2017) reported a study in which four groups of 38–40 male and female leukaemia-prone AKR/J mice [melatonin proficiency unclear] (age, 5 weeks) were exposed to a regular LD12:12 schedule or to shifts in the light–dark schedule throughout their lifespan. Mice were first stabilized for approximately 3 weeks on a regular LD12:12 schedule. Core temperature, locomotor activity, and running-wheel activity were monitored for 1 week during this period. Two groups of male and female mice were then switched to shifts in the light–dark schedule that were designed to mimic shift work. The dark (active) phase was extended by 8 hours on the first day of the simulated work week. This shift in the light–dark schedule was maintained for 5 days. On day 6 (the beginning of the simulated weekend), the onset of the dark (active) phase was advanced by 8 hours, thus returning to the control schedule. After 2 weekend-schedule days, the shift in the light–dark schedule began again. Mice remained on the shift in the light–dark schedule conditions for 4 weeks, and were then returned to a regular light–dark schedule for an additional 2 weeks. Room illumination conditions were 175 lux; within chambers at cage level with the chamber door closed, light intensity was approximately 5–10 lux during the dark phase (chamber internal lighting off) and 125–145 lux during the light phase (chamber internal lighting on). Median survival times for control females, exposed females, control males, and exposed males were 268, 251, 314.5, and 308 days, respectively. The combined analysis of all four groups revealed significant effects of sex, with male mice having a longer lifespan than female mice. The data were further analysed in subsets comprising mice that died before or after the median survival time for each group. [The Working Group noted that, although most AKR mice died from leukaemia or lymphoma, no data on tumour incidence or histopathological

verification of leukaemia or lymphoma were reported.]

3.1.2 Rat

In the study by Kort et al. (1986), a group of 100 inbred BN virgin female rats (age, 3 weeks) were exposed to shifts in the light–dark schedule over a long-term period. Every Friday, the automatic timer controlling the light for the exposed rats inverted the light–dark schedule by advancing the onset of the next light or dark period by 12 hours. Another group of 100 females exposed to a regular LD12:12 schedule served as controls. The experiment was terminated at age 150 weeks. There was no significant difference in survival between the exposed group and the control group. The mean body weight in the exposed group was significantly less than that in controls. Each organ system was examined in rats either found dead or moribund, or killed at age 150 weeks. Routine microscopic examination was performed on samples of more than 20 organs or tissues, and other organs were examined when suspected of neoplasms. The differences in tumour incidence were not significant for all organs or tissues (combined) or for any specific tissue or organ.

3.2 Shifts in the light–dark schedule with implant, transplant, graft, or modifying factors

See Table 3.2.

3.2.1 Shifts in the light–dark schedule with implant, transplant, or graft

(a) Mouse

The study by Li & Xu (1997) analysed the effect of shifts in the light–dark schedule on animal physiology and tumour progression using inbred male Kunming mice (age, 6 weeks) [assumed to be melatonin deficient]. Four groups of 10 mice were transplanted with either Ehrlich carcinoma or Sarcoma 180 and then maintained at regular LD12:12 schedules (control) or transferred between LD14:10 and LD10:14 schedules once every 3 days. Exposure to shifts in the light–dark schedule led to a significant increase in tumour growth rate by about 12% for the Sarcoma 180 model at 10 days, and to a significant reduction by 18% in survival time for the Ehrlich carcinoma model. The study also found that shifts in the light–dark schedule completely suppressed circadian rhythms of hypersensitivity reaction, neutrophil phagocytosis, leukocyte counts, and haemolysin. [The Working Group questioned the biological relevance of the small changes that were reported.]

In the study by Filipski et al. (2004), groups of B6D2F$_1$ male mice [melatonin proficient] (age, 6 weeks) were initially synchronized to a regular light–dark schedule (LD12:12) for 3 weeks. Mice were then maintained at LD12:12 (control), or else exposed to either an LD12:12 schedule that was advanced by 8 hours once every 2 days or to continuous light (LD24:0) (see also Table 3.5). Control mice displayed a coupled circadian rhythm of physical activity, body temperature, plasma corticosterone, and the expression of circadian genes *Per1* in the suprachiasmatic nuclei and *Per2* and *Nr1d1* in the liver. The circadian rhythm of these physiological parameters and gene expression patterns was completely abolished by the repeated advances in the light–dark schedule. Ten days after start of exposure, mice were inoculated with Glasgow osteosarcoma, and used to study the role of shifts in the light–dark schedule on tumour growth and survival in two experiments ("Experiment 2", n = 13 per group; "Experiment 3", n = 12–14 per group) and the effect of continuous light in a third experiment ("Experiment 4", n = 10 per group). Mice were killed 15 days after tumour inoculation. Compared with control mice, mice in the group exposed to shifts in the light–dark

Table 3.2 Studies of carcinogenicity in experimental animals exposed to shifts in the light–dark schedule, with implant, transplant, graft, or modifying factors

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, weight, or volume of tumours	Significance	Comments
Initiation–promotion (tested as promoter) Mouse, Kunming (M) 6 wk > 15 d for mice transplanted with Ehrlich carcinoma, 10 d for mice transplanted with Sarcoma 180 Li & Xu (1997)	Shifts in the LD schedule LD12:12 (control), transferred between LD14:10 and LD10:14 every 3 d (exposed) Transplantation of Ehrlich carcinoma or Sarcoma 180, method unclear 10, 10 NR	Sarcoma 180: total tumours Weight (g): 1.45, 1.62*	*$P < 0.05$, Student *t*-test	Principal strengths: two tumour models were used Principal limitations: size of tumours at the time of transplantation was unclear Statistical analyses of the effects on tumour growth were not convincing; survival (lifespan) reduced in Ehrlich carcinoma model by 18% ($P < 0.05$) (15 d for controls and 12.3 d for exposed); survival in Sarcoma 180 model, unclear; melatonin proficiency of strain unknown (assumed to be deficient)
Co-carcinogenicity Mouse, B6D2F$_1$ (M) 6 wk ≤ 2 wk after inoculation Filipski et al. (2004)	Shifts in the LD schedule LD12:12 (control), LD12:12 advanced by 8 h every 2 d (exposed); mice were initially synchronized to LD12:12 for 3 wk All mice inoculated subcutaneously with 3 mm^3 fragments of mouse Glasgow osteosarcoma in each flank 10 d after start of LD advances 13, 13 NR	Glasgow osteosarcoma: total tumours Weight at day 11 (mg): 647, 1330*	*$P = 0.001$, Student *t*-test	"Experiment 2": mice killed 15 d after tumour inoculation; accelerated tumour growth ($P < 0.001$, ANOVA test) and decrease in survival in exposed mice; melatonin-proficient strain
Co-carcinogenicity Mouse, B6D2F$_1$ (M) 6 wk ≤ 2 wk after inoculation Filipski et al. (2004)	Shifts in the LD schedule LD12:12 (control), LD12:12 advanced by 8 h every 2 d (exposed); mice were initially synchronized to LD12:12 for 3 wk All mice inoculated subcutaneously with 3 mm^3 fragments of mouse Glasgow osteosarcoma in each flank 10 d after start of LD advances 12, 14 NR	Glasgow osteosarcoma: total tumours Weight at day 11 (mg): 847, 1376*	*$P = 0.005$, Student *t*-test	"Experiment 3": mice killed 15 d after tumour inoculation; accelerated tumour growth ($P = 0.002$, ANOVA test) and decrease in survival in exposed mice; melatonin-proficient strain

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, weight, or volume of tumours	Significance	Comments
Co-carcinogenicity Mouse, B6D2F$_1$ (M) NR 25 d Filipski et al. (2005)	Shifts in the LD schedule LD12:12 (control), LD12:12 advanced by 8 h every 2 d (exposed) Subcutaneous implantation of 3 mm^3 Glasgow osteosarcoma 10 d after start of LD advances 13, 14 NR	Glasgow osteosarcoma: total tumours Weight after 12 d (mg): 1317 (95% CI, 1067–1567); 1997 (95% CI, 1458–2356)	NR Tumours grew significantly more quickly ($P = 0.04$, ANOVA test) in exposed vs control mice	Light conditions: fluorescent tube (spectrum, 4100 °K; light efficiency, 58–80 lumen/W); mean light intensity of 318 lux in the middle of each compartment and 129 lux at each side Mice killed 15 d after tumour implantation; melatonin-competent strain
Co-carcinogenicity Mouse, BALB/c-Foxn1^{nu} (F) NR ≤ 4 wk Kennaway (2009)	Shifts in the LD schedule LD12:12 (control), LD12:12 schedule inverted for three consecutive 24-h periods 1×/wk (exposed) Subcutaneous injection of 5 × 10^6 human breast cancer MCF-7 cells (in Matrigel® Becton Dickinson) 4 wk after start of LD alterations NR NR	Human breast cancer MCF-7: total tumours Volume (mm^3): after 2 wk, 150 (control), 110* (exposed); after 3 wk, 225 (control), 200 (exposed); after 4 wk, 370 (control), 275 (exposed)	*$P < 0.05$ (decrease) [test unspecified] NS	Principal limitations: limited experimental details Tumour volume reported in graphic form; melatonin-deficient strain
Co-carcinogenicity Mouse, C57BL/6 (M) 6 wk 22 d Wu et al. (2012)	Shifts in the LD schedule LD12:12 (control), light onset advanced by 8 h every 48 h (exposed) Subcutaneous injection of Lewis lung carcinoma cells (0.2 mL, 5 × 10^6/mL) into both flanks 10 d after start of LD advances 24, 24 24, 24	Lewis lung carcinoma: total tumours Volume (mm^3): 777 , 1238* *Lung*: metastases Incidence: 3/24, 10/24*	*$P = 0.026$, two independent-samples *t*-test *$P = 0.023$, χ^2 test	Melatonin-deficient strain

Table 3.2 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, weight, or volume of tumours	Significance	Comments
Co-carcinogenicity Rat, F344 (M) 50 d 6–8 wk Logan et al. (2012)	Shifts in the LD schedule LD12:12 (control), LD12:12 for 1–2 wk then advanced by 6 h every 2 d for a total of 10 advances (exposed) Injection of 1×10^5 MADB106 mammary tumour cells into jugular vein 20, 20 NR	*Lung*: tumours Incidence: 8/20, 16/20*	*$P < 0.0001$	Principal limitations: limited description of shifts in the LD schedule
Initiation–promotion (tested as promoter) Mouse, B6D2F$_1$ (M) 9–12 wk 10 mo Filipski et al. (2009)	Shifts in the LD schedule LD12:12 (control), period of light onset advanced by 8 h every 2 d for 10 mo (exposed) Daily intraperitoneal injection of 10 mg/kg bw DEN on days 1–11, then 7 mg/kg bw on days 21–33 and 41–46 (total dose, 243 mg/kg bw) NR NR	*Liver*: tumours Incidence: 11/13, 11/12 Multiplicity (range): 1–4, 1–6*	NS *$P = 0.028$ At least 2 tumours were observed in 77% of exposed mice ($P = 0.026$) compared with 33% of control mice	Principal limitations: small number of rats Liver tumours were hepatocellular carcinomas, cholangiocarcinomas, sarcomas, or mixed tumours with characteristics of both hepatocellular carcinoma and cholangiocarcinoma
Initiation–promotion (tested as promoter) Mouse, C57BL/6J (M) 2–3 mo 11 d Lee et al. (2019)	Shifts in the LD schedule LD12:12 (control), LD schedule advanced by 8 h every 2 d for 11 d (exposed) After LD12:12 conditions for 2 wk, all mice given subcutaneous injection in the right flank of 400 µg of 3-methylcholanthrene in peanut oil; after 30–60 d, mice separated into control and exposed groups 14, 14 14, 14	Total tumours [type unspecified] Relative tumour volume at 10 d: ~2.5 (control), ~4.8* (exposed) Relative tumour growth rate: ~0.15 (control), ~0.43** (exposed)	*$P < 0.001$, two-way ANOVA, and Bonferroni multiple comparisons test; normalized data derived from three independent experiments ** $P < 0.0001$, two-tail Student *t*-test	Tumour volume and tumour growth-rate data provided in graphic form; melatonin-deficient strain

bw, body weight; d, day; DEN, diethylnitrosamine; F, female; h, hour; LD, light–dark; LD10:14, 10 hours of light followed by 14 hours of darkness; LD12:12, 12 hours of light followed by 12 hours of darkness; LD14:10, 14 hours of light followed by 10 hours of darkness; M, male; mo, month; NR, not reported; NS, not significant; vs, versus; wk, week.

schedule displayed significantly accelerated tumour growth and decreased survival time in both experiments. Exposure to continuous light in the third experiment was not observed to have any effect on tumour growth or survival.

Filipski et al. (2005) conducted a study in which B6D2F$_1$ male mice [melatonin competent] (age, 6–8 weeks) were maintained in compartments lit with a fluorescent tube, with a light spectrum of 4100 °K and a light efficiency of 58–80 lumen/W (mean light intensity of 318 lux in the middle of each compartment and 129 lux at each side). The mice were synchronized to standard lighting conditions of LD12:12 for 2–3 weeks, and were then either maintained at this lighting regimen (n = 13, control group) or were exposed to shifts in the light–dark schedule by 8 hours every 2 days (n = 14, exposed group) for 10 days. All control and exposed mice were then given a subcutaneous implantation of a 3 mm^3 fragment of Glasgow osteosarcoma in both flanks, and tumour weight was measured daily. At 15 days after tumour implantation, all control and exposed mice were killed. The body weights of the control and exposed mice were observed to increase by similar amounts. Shifts in the light–dark schedule were observed to severely alter the circadian rhythms in the expression of clock genes in the liver and tumours of mice bearing Glasgow osteosarcoma. Tumours grew significantly more quickly in exposed mice than in control mice. On day 12, before tissue sampling mean tumour weight was 1317 mg (95% confidence interval, CI, 1067–1567) in control mice and 1997 mg (95% CI, 1458–2356) in exposed mice.

Kennaway (2009) reported a study in which BALB/c-Foxn1^{nu} mice [melatonin deficient, age and number not reported, assumed to be females] were exposed to shifts in the light–dark schedule for 4 weeks before being subcutaneously injected with human breast cancer MCF-7 cells (5×10^6; in Matrigel® Becton Dickinson). Exposed mice had their LD12:12 schedule inverted for three consecutive 24-hour periods by delaying the onset of the period of darkness by 12 hours once every week. Controls were kept at a regular LD12:12 schedule throughout the experimental period. Tumour volume was measured at 2, 3, and 4 weeks after the injection, during which time the exposed group were maintained under conditions of shifts in the light–dark schedule. There was a significant difference [statistical test unspecified] in tumour volume after 2 weeks, with a lower volume in the exposed mice. A trend towards lower tumour volumes persisted in the exposed mice, but no significant differences were observed throughout the remainder of the experiment. [The Working Group noted the limited experimental details.]

Wu et al. (2012) reported a study in which 48 male C57BL/6 [melatonin deficient] mice (age, 4 weeks) were initially synchronized to a regular light–dark schedule (LD12:12). After 2 weeks, the mice were either maintained at LD12:12 (controls, n = 24) or else exposed to advances in light onset by 8 hours every 48 hours (exposed, n = 24). After 10 days of exposure, all mice were subcutaneously injected with Lewis lung carcinoma cells (0.2 mL, 5×10^6 per mL) in both flanks. There was no significant difference in body weight between the control and exposed groups. Circadian rhythm was disrupted in the group exposed to shifts in the light–dark schedule. Tumours were palpable in 10 out of 24 control mice and 21 out of 24 exposed mice (significant increase; P = 0.0025, χ^2 test) 10 days after the injection. The tumours grew significantly faster (P = 0.004, ANOVA test) in the exposed group compared with the controls; mean tumour volume was significantly higher in the exposed group (1238 mm^3 vs 777 mm^3) 22 days after tumour inoculation. All of the samples, including the lung and tumour tissues, were examined histopathologically; it was reported that 10 out of 24 exposed mice had lung metastases compared with only 3 out of 24 control mice (P = 0.023).

(b) Rat

Logan et al. (2012) reported a study in which groups of 20 male Fischer 344 rats (age, 50 days) were either maintained at regular light–dark schedules of LD12:12 (control group) or exposed to LD12:12 for 1–2 weeks followed by a 6-hour advance in the light–dark schedule every 2 days for a total of 10 advances (exposed group). All rats were then injected with MADB106 mammary tumour cells (1×10^5 cells) into the jugular vein and kept for 6–8 weeks under a regular LD12:12 cycle to determine tumour frequency or prevalence. Circadian rhythm was disrupted in the exposed group, and it was observed that shifts in the light–dark schedule significantly ($P < 0.0001$) increased the incidence of lung tumours.

3.2.2 Shifts in the light–dark schedule with modifying factors

Mouse

Filipski et al. (2009) investigated the role of shifts in the light–dark schedule in the promotion of hepatocarcinogenesis induced by diethylnitrosamine (DEN) in 44 male B6D2F$_1$ mice [melatonin competent] (age, 6–8 weeks). The mice were initially synchronized to standard lighting conditions of 12 hours of light and 12 hours of darkness (LD12:12), with food and water ad libitum, for 3 weeks. The mice were then given daily intraperitoneal injections of DEN at 10 mg/kg body weight (bw) from day 1 to 11, and then at 7 mg/kg bw from day 21 to 33 and from day 41 to 46 (total dose, 243 mg/kg bw). They were then randomized to LD12:12 (control group) or to LD12:12 advanced by 8 hours every 2 days (exposed group) [the number of mice at the start per group was not reported]. DEN was observed to disrupt the circadian rhythm in both control and exposed groups of mice. Exposure to shifts in the light–dark schedule was also observed to disrupt the circadian rhythm, but partial recovery was observed after 5 months. Mice were killed after 10 months, and liver, lung, and kidneys were examined for macroscopic and microscopic neoplastic lesions. Microscopic examination showed that liver tumours were found in 11 out of 12 (92%) exposed mice compared with 11 out of 13 (85%) control mice. Liver tumour multiplicity was significantly ($P = 0.028$) higher in exposed mice (range, 1–6) than in control mice (range, 1–4). At least two liver tumours were observed in 77% of exposed mice ($P = 0.026$) compared with 33% of the control mice. In exposed mice, up to four different tumour types were observed in the same liver (i.e. hepatocellular carcinomas, cholangiocarcinomas, sarcomas, or mixed tumours with characteristics of both hepatocellular carcinoma and cholangiocarcinoma); four exposed mice had two different types of liver cancer and one had all four types of liver cancer. In control mice, a single histological type of tumour per liver [not further specified] was observed. [The Working Group noted the small number of mice, the lack of data on survival, and the fact that the carcinogen doses used in this study were very high, as indicated by the very high incidence of hepatocellular carcinoma in both groups.]

Bishehsari et al. (2016) reported a study to determine the effects of shifts in the light–dark schedule on alcohol-associated colon carcinogenesis in TS4Cre × APC^{lox468} mice (age, 4 weeks) [sex not reported]. Mice were given a diet initially supplemented by 3% ethanol, increased to 15% over 2 weeks, then maintained at 15% for another 2 weeks. Mice were then either maintained at a regular LD12:12 schedule (controls, $n = 3$) or exposed to weekly reversals of the light–dark schedule (exposed, $n = 5$). The study was terminated after 8 weeks of exposure to ethanol with or without exposure to shifts in the light–dark schedule. Mice exposed to both ethanol and shifts in the light–dark schedule developed a greater number of colon polyps and carcinoma in situ, and demonstrated an increased incidence of advanced adenoma, than control mice. [The Working Group noted that this model had not

been used previously to identify possible carcinogens. More importantly, group sizes used in the study (n = 3–5 mice per group) were far smaller than those required to support a statistically robust evaluation. The study was considered inadequate for the evaluation.]

In the experiment by Lee et al. (2019), male C57BL/6J mice [melatonin deficient] (age, 2–3 months) were kept under standard lighting conditions of LD12:12 with food and water available ad libitum. After acclimation for 2 weeks, mice were given subcutaneous injections of 400 µg of 3-methylcholanthrene in peanut oil along the right flank. After 30–60 days, mice were separated into two groups of 14 control mice and 14 mice exposed to shifts in the light–dark schedule. The control group was maintained at the LD12:12 lighting schedule, and the exposed group was subject to repeated 8-hour advances in the LD12:12 schedule every 2 days for 11 days. Circadian rhythm was disrupted in the exposed group. Tumour growth was measured with a digital calliper 3 times per week, and mice were killed when the tumour exceeded 20 mm in diameter. After 6, 8, and 10 days, the relative tumour volumes in the exposed group were significantly ($P < 0.001$) increased compared with those in the control group (data were obtained from three independent experiments). The relative tumour growth rate calculated from linear regression was also significantly ($P < 0.0001$) increased in the exposed mice compared with the controls. [The Working Group noted that no data were provided for tumour histopathology, and that tumour volume and tumour growth rate data were provided in graphic form.]

3.3 Extreme changes in photoperiod

See Table 3.3.

3.3.1 Excluded publication

One publication that was excluded from this review (Khan et al., 2018) studied the effects of artificial light at night on the circadian expression patterns of clock and clock-controlled genes, including several genes involved in ovarian carcinogenesis, in female zebrafish. The authors reported on the appearance of thecoma and granulosa cell tumours after exposure to continuous light for 1 year, but no quantitative data were provided (e.g. tumour incidence).

3.3.2 Main characteristics and review of the relevant studies

The aims of the long-term studies discussed here were to investigate the dynamics of tumour incidence and their histopathological characterization in mice and rats exposed to continuous light, compared with a regular light–dark schedule (LD12:12), a natural light–dark schedule involving large seasonal changes in photoperiod, or continuous darkness.

The experimental rodent models studied included known melatonin-proficient species and strains such as female CBA mice (Anisimov et al., 2004) and female Wistar rats (Bukalev et al., 2013), and melatonin-deficient mouse strains such as female FVB HER-2/neu mice (Baturin et al., 2001) or female 129/Sv mice (Popovich et al., 2013). Male and female LIO rats [Wistar-derived] have also been used, although their melatonin proficiency does not seem to be documented (Vinogradova et al., 2009, 2010). Age at the start of continuous illumination ranged from 25 days to 14 months, with most studies starting at age 1–5 months.

Light intensity in the groups exposed to continuous light ranged from 750 lux (Vinogradova et al., 2009, 2010; Bukalev et al., 2013) to about 2500 lux (Anisimov et al., 2004; Popovich et al., 2013). Light intensity was not always consistent between the continuous-light and the artificial

Table 3.3 Studies of carcinogenicity in experimental animals exposed to extreme changes in photoperiod

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, or number of tumours	Significance	Comments
Full carcinogenicity Mouse, CBA (F) 2 mo ≤ 971 d Anisimov et al. (2004)	Changes in photoperiod LD12:12 (control), LD24:0 50, 50 15, 0	*All sites*: tumours Incidence: 4/50, 15/50* Number: 5 (malignant, 3), 22 (malignant, 19) *Lung* Adenoma Incidence: 1/50, 1/50 Adenocarcinoma Incidence: 1/50, 7/50* *Liver*: hepatocellular carcinoma Incidence: 0/50, 4/50 *Haematopoietic and lymphoid tissues*: malignant lymphoma Incidence: 0/50, 6/50* *Mammary gland*: adenocarcinoma Incidence: 1/50, 2/50	 *$P < 0.001$, Fisher exact test NR NS *$P < 0.05$, Fisher exact test NS *$P < 0.02$, Fisher exact test NS	Principal strengths: melatonin-proficient strain; large groups (50 per group); full histopathology Light intensity: 300 lux in control group and 2500 lux in continuous-light group
Full carcinogenicity Mouse, FVB HER-2/neu (F) 2 mo ≤ 45 wk Baturin et al. (2001)	Changes in photoperiod LD12:12, LD24:0 30, 25 20% at 45 wk, 20% at 45 wk	*Mammary gland*: adenocarcinoma Incidence: 23/30 (76.7%), 19/25 (76.0%) Multiplicity: 3.3 (SD, 0.4), 5.0 (SD, 0.5)*	 NS * $P < 0.02$, Student *t*-test	Melatonin-deficient strain
Full carcinogenicity Mouse, 129/Sv (F) 5 mo Lifetime Popovich et al. (2013)	Changes in photoperiod LD12:12 (control), LD24:0 46, 46 22 (47.8%) at 800 d, 13 (28.3%) at 800 d*	*All organs*: all tumours Incidence: 39/45 (86.7%), 35/43 (81.4%) Number: 39, 38 *Uterus* Haemangioma Number: 6, 12 Sarcoma Number: 30, 20	 NS NS NS NS	Principal strengths: full histopathology Melatonin-deficient strain; cumulative incidence curves over time for LD24:0 vs LD12:12: $P = 0.0055$ for tumour-bearing mice and $P = 0.0183$ for fatal-tumour-bearing mice (χ^2 test); *survival: $P < 0.05$, Fisher exact test Light intensity: 70 lux in the LD12:12 group and 2600 lux in the LD24:0 group

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, or number of tumours	Significance	Comments
Full carcinogenicity Rat, LIO (M) 25 d Lifetime Vinogradova et al. (2009); see also Bukalev et al. (2012)	Changes in photoperiod LD12:12, NL, LD24:0, LD0:24 57, 50, 50, 51 NR	*Testes*: leydigoma Number: 7, 6, 4, 6 *All organs* All tumours Incidence: 17/57 (29.8%), 11/50 (22.0%), 13/50 (26.0%), 11/51 (21.6%) Multiplicity: 1.35, 1.18, 1.08, 1.36 Number: 23, 13, 14, 15 Malignant tumours Incidence: 7/57 (12.3%), 6/50 (12.0%), 10/50 (20.0%), 5/51 (9.8%) Multiplicity: 1.35, 1.18, 1.08, 1.36 Number: 9, 6, 10, 5 *Haematopoietic and lymphoid tissues*: malignant lymphoma or leukaemia Number: 3, 4, 6, 3	 NS NS NR NR NS NR NR NR	Principal strengths: full histopathology Accelerated tumour development in LD24:0 group compared with LD12:12 group In the NL group, photoperiod ranged from 4.5 h of light in winter to 24 h in summer
Full carcinogenicity Rat, LIO (F) 25 d Lifetime Vinogradova et al. (2009)	Changes in photoperiod LD12:12, NL, LD24:0, LD0:24 40, 48, 54, 61 NR	*Testes*: leydigoma Number: 7, 6, 4, 6 *All organs* All tumours Incidence: 21/40 (52.5%), 34/48 (70.8%)*, 24/54 (44.4%), 15/61 (24.6%)** Multiplicity: 1.38, 1.41, 1.63, 1.07 Number: 29, 48, 39, 16	 NS *$P < 0.05$ (increase) vs LD12:12, Fisher exact test [presumably] **$P < 0.001$ (decrease) vs LD12:12, Fisher exact test [presumably] NR NR	Principal strengths: full histopathology Accelerated tumour development in LD24:0 group compared with LD12:12 group In the NL group, photoperiod ranged from 4.5 h of light in winter to 24 h in summer

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, or number of tumours	Significance	Comments
Vinogradova et al. (2009) (cont.)		Malignant tumours		
		Incidence: 5/40 (12.5%), 7/48 (14.6%), 7/54 (13.0%), 3/61 (4.9%)	NS	
		Multiplicity: 1.35, 1.18, 1.08, 1.36	NR	
		Number: 5, 9, 7, 3	NR	
		Mammary gland		
		Benign tumours (fibroma or fibroadenoma)		
		Incidence: 14/40, 27/48*, 18/54, 5/61**	*$P < 0.05$ (increase) vs LD12:12 **$P < 0.01$ (decrease) vs LD12:12	
		Number: 15, 30, 21, 5	NR	
		Adenocarcinoma		
		Number: 0, 0, 1, 0	NR	
Full carcinogenicity Rat, LIO (M) 1 mo Lifetime Vinogradova et al. (2010); see also Lotosh et al. (2013)	Changes in photoperiod LD12:12, LD24:0 43, 34 Mean lifespan: 766 d (SD, 25.4), 744 d (SD, 28)	*All organs*		Principal strengths: full histopathology
		All tumours		
		Incidence: 15/43 (34.9%), 12/34 (35.3%)	NS	
		Multiplicity: 1.4, 1.08	NR	
		Number: 21, 13	NR	
		Malignant tumours		
		Incidence: 8/43 (18.6%), 10/34 (29.4%)	NS	
		Number: 8, 10	NR	
		Haematopoietic and lymphoid tissues: malignant lymphoma or leukaemia		
		Number: 3, 6	NR	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, or number of tumours	Significance	Comments
Full carcinogenicity Rat, LIO (M) 14 mo Lifetime Vinogradova et al. (2010); see also Lotosh et al. (2013)	Changes in photoperiod LD12:12, LD24:0 43, 90 Mean lifespan: 766 d (SD, 25.4), 818 d (SD, 18.1)	*All organs* All tumours Incidence: 15/43 (34.9%), 26/90 (28.9%) Multiplicity: 1.40, 1.31 Number: 21, 34 Malignant tumours Incidence: 8/43 (18.6%), 9/90 (10.0%) Number: 8, 9 *Testes*: leydigoma Number: 7, 16 *Soft tissue* Benign tumours (angiofibroma, fibroma, or chondroma) Number: 0, 3 Sarcoma Number: 0, 4	 NS NR NR NS NR NR NR NR	Principal strengths: full histopathology
Full carcinogenicity Rat, LIO (F) 1 mo Lifetime Vinogradova et al. (2010)	Changes in photoperiod LD12:12, LD24:0 30, 36 Mean lifespan: 844 d (SD, 33.6), 658 d (SD, 22.8)	*All organs* All tumours Incidence: 17/30 (56.7%), 20/36 (55.6%) Multiplicity: 1.47, 1.75 Number: 25, 35 Malignant tumours Incidence: 5/30 (16.7%), 5/36 (13.9%) Number: 5, 5 *Mammary gland* Benign tumours (fibroma or fibroadenoma) Incidence: 12/30, 16/36 Number: 13, 19	 NS NR NR NS NR NS NR	Principal strengths: full histopathology

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, or number of tumours	Significance	Comments
Vinogradova et al. (2010) (cont.)		Adenocarcinoma		
		Number: 0, 1	NR	
		Uterus		
		Benign tumours (polyp, fibroma, or fibromyoma)		
		Number: 3, 6	NR	
		Adenocarcinoma		
		Number: 0, 1	NR	
Full carcinogenicity Rat, LIO (F) 14 mo Lifetime Vinogradova et al. (2010)	Changes in photoperiod LD12:12, LD24:0 30, 71 Mean lifespan: 844 d (SD, 33.6), 811 d (SD, 20.0)	*All organs*		Principal strengths: full histopathology
		All tumours		
		Incidence: 17/30 (56.7%), 30/71 (45.3%)	NS	
		Multiplicity: 1.47, 1.37	NR	
		Number: 25, 41	NR	
		Malignant tumours		
		Incidence: 5/30 (16.7%), 11/71 (15.5%)	NS	
		Number: 5, 11	NR	
		Mammary gland: benign tumours (fibroma or fibroadenoma)		
		Incidence: 12/30, 19/71	NS	
		Number: 13, 27	NR	
		Haematopoietic and lymphoid tissues: malignant lymphoma or leukaemia		
		Number: 3, 7	NR	

Table 3.3 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, or number of tumours	Significance	Comments
Full carcinogenicity Rat, Wistar (F) 25 d Lifetime Bukalev et al. (2013)	Changes in photoperiod LD12:12, LD24:0, LD0:24, NL 40, 54, 61, 48 At 24 mo: 25 (62.5%), 6 (11.1%), 23 (37.7%), 15 (31.2%)	*All organs* Benign tumours (mainly fibroma or fibroadenoma of mammary gland) Incidence: 24/40 (60.0%), 32/54 (59.3%), 13/61 (21.3%)*, 39/48 (81.3%)* Malignant tumours Incidence: 5/40 (12.5%), 7/54 (13.0%), 3/61 (4.9%)*, 9/48 (18.8%)	*$P < 0.05$ (χ^2 test), LD0:24 (decrease) or NL (increase) vs LD12:12 * $P < 0.05$ (χ^2 test), LD0:24 vs LD12:12 (decrease)	Principal strengths: large lifetime study with a 5.6-fold decrease in the 2-yr survival rate for LD0:24 vs LD12:12 Principal limitations: many other diseases, more frequent in LD24:0 and NL rats Benign or malignant tumour incidences similar for LD24:0 and LD12:12 groups; LD0:24 protective against all pathologies including benign or malignant tumours; in the natural light (NL) group, photoperiod ranged from 4.2 h of light in winter to 24 h in summer; L, 750 lux at cage level

d, day; F, female; h, hour; LD, light-dark; LD0:24, continuous darkness; LD12:12, 12 h of light followed by 12 h of darkness; LD24:0, continuous light; M, male; mo, month; NL, natural light; NR, not reported; NS, not significant; SD, standard deviation; vs, versus; wk, week; yr, year.

or natural light–dark schedules. Light intensity was not specified in three studies (Jöchle, 1963; Joechle, 1964; Baturin et al., 2001).

(a) Mouse

Groups of 30 female C3H/HeJ mice [melatonin proficient] (age, 100 days), which have an inherited autosomal recessive retinal degeneration, were exposed to either a regular light–dark schedule (LD12:12) or to continuous light (LD24:0) for 400 days (Jöchle, 1963). Exposure to continuous light (LD24:0) was observed to prolong estrus phase duration by 60–80% compared with exposure to a schedule of LD12:12. Exposure to continuous light also delayed the appearance of spontaneous mammary tumours compared with exposure to a regular light–dark schedule: at age 351–400, 401–450, and 451–499 days, 2 (LD24:0) versus 6 (LD12:12), 6 versus 7, and 0 versus 4 tumours appeared, respectively. At age 500 days, there was a cumulative incidence of 11 mammary tumours out of 30 mice in the group exposed to continuous light compared with 24 mammary tumours out of 30 mice in the group exposed to a schedule of LD12:12. The mice exposed to continuous light had a prolonged survival compared with the mice exposed to LD12:12 (Jöchle, 1963; see also Joechle, 1964). [The Working Group noted the very limited information given regarding the experimental methods, including the number of mice at the start. No histopathology or statistical comparisons were provided.]

In a subsequent article (Joechle, 1964), female C3H/A mice [melatonin proficient] were subject to the same experimental conditions as described above for Jöchle (1963). Those exposed to LD24:0 displayed minor disturbances of the estrus cycle, with a maximum of a 24 hour increase in the duration of estrus compared with the mice exposed to LD12:12. There was an earlier occurrence of mammary tumours in the group exposed to LD24:0 compared with the group exposed to LD12:12, with 16 (LD24:0) versus 4 (LD12:12) tumours observed on days 151–300, and a higher cumulative number of tumours of 21 versus 12 at the age of 450 days. Mice exposed to continuous light also had a shorter lifespan than mice exposed to LD12:12. [The Working Group noted that limited information was provided regarding the experimental methods, including the number of mice at start. No histopathology or statistical comparisons were reported.]

A well-designed study (Anisimov et al., 2004) clearly demonstrated large and statistically significant differences in tumour incidence between groups of female CBA mice [melatonin proficient] (age, 2 months) exposed to either LD12:12 (controls, $n = 50$) or LD24:0 ($n = 50$) for their lifetime. Light intensity at the bottom of the cages was 300 lux for the control group and 2500 lux for the group exposed to continuous light. An earlier first tumour was observed in the group exposed to LD24:0 (312 days) compared with the group exposed to LD12:12 (610 days). Compared with the group exposed to LD12:12, the group exposed to LD24:0 demonstrated an increased number of mice that developed tumours (15 out of 50 vs 4 out of 50, $P < 0.001$), an increased number of total tumours (22 vs 5), and an increased number of malignant tumours (19 vs 3). Tissue or organ sites with significant increases in tumour incidence in the group exposed to LD24:0 were the lung (adenocarcinoma, 7 out of 50 vs 1 out of 50) and haematopoietic and lymphoid tissues (malignant lymphoma, 6 out of 50 vs 0 out of 50). A non-statistically significant increase in the incidence of tumours was seen in the liver (4 out of 50 vs 0 out of 50). Mice exposed to LD24:0 had a faster mortality rate after the age of 900–1050 days compared with mice exposed to LD12:12. [The Working Group noted the adequate power and statistical significance of most comparisons, but also noted the fact that the light intensity exposure differed between the groups.]

Baturin et al. (2001) reported a study in which 104 homozygous FVB HER-2/neu female transgenic mice [melatonin deficient] (age, 2 months)

were randomly allocated to one of four groups; two groups were exposed to LD12:12 and two groups were exposed to LD24:0. For each exposure schedule, one group remained untreated and the other was given access (at night) to melatonin dissolved in tap water. Estrus cycle was reported to be unaltered in the mice from any group [data not shown]. In groups that did not receive melatonin, 23 out of 30 mice (76.7%) in the LD12:12 group and 19 out of 25 mice (76.0%) in the LD24:0 group developed one or more tumours of the mammary gland, all of which were classified as mammary adenocarcinomas. The number of mice that developed lung metastases was also similar between these two groups. However, the mammary tumour multiplicity was significantly increased in the LD24:0 group, and the percentage of mice with four or more mammary tumours increased to 60% compared with 33% for the LD12:12 group ($P < 0.05$). [The Working Group noted that no statistical comparison between survival curves was provided.]

Popovich et al. (2013) reported a lifetime study in which 92 female 129/Sv mice [melatonin deficient] (age, 2 months) were randomly allocated to two groups and exposed to either LD12:12 or LD24:0 up to age 20 months. Light exposure at the bottom of the cages was 70 lux for the group exposed to LD12:12 and 2600 lux for the group exposed to LD24:0. Estrus cycle length was significantly prolonged in the group exposed to LD24:0 and the proportion of mice with irregular estrus cycles reached 68.4% in this group compared with less than 20% in the group exposed to LD12:12. Survival at 800 days was significantly reduced in the group exposed to LD24:0 (28.3%) compared with the group exposed to LD12:12 (47.8%). All mice were autopsied, and all tumours found were examined microscopically. The first tumour in the LD24:0 group occurred 2 months earlier than in the LD12:12 group. There was no statistically significant difference in the incidence of total tumours (39 out of 45 in the LD12:12 group vs 35 out of 43 in the LD24:0 group). The uteri of the majority of the mice were enlarged at autopsy. There was a non-significant increase in the number of uterine haemangiomas in the group exposed to LD24:0, and a non-significant increase in the number of sarcomas of the uterus in the group exposed to LD12:12.

(b) Rat

Vinogradova et al. (2009) (see also Bukalev et al., 2012) reported a study in which 208 male and 203 female LIO rats [Wistar-derived, melatonin status not reported] (age, 25 days) were allocated to one of four groups exposed to light–dark schedules of either: LD12:12 (57 males, 40 females); natural light, in which photoperiod ranged from 4.5 hours of light in winter to 24 hours in summer (50 males, 48 females); LD24:0 (50 males, 54 females), that is, continuous light; or LD0:24 (51 males, 61 females), that is, continuous darkness. Light intensity was: 750 lux for the groups exposed to LD12:12 and LD24:0; varied over the range 50–1000 lux in the group exposed to conditions of natural light, according to the time of day and weather [equivalent to latitude ~62°]; and was less than 0.5 lux (with a dim red light for service) in the group exposed to LD0:24. Compared with the group exposed to LD12:12, the first tumours were detected 156 days earlier among males and 21 days earlier among females in the group exposed to LD24:0 (continuous light), and 324 days later among males and 21 days earlier among females in the group exposed to LD0:24 (continuous darkness). The accelerated tumour development in the group exposed to LD24:0 did not translate into an increased incidence of tumours. Over the 1200 days of the study, total tumour incidence in males was 13 out of 50 (26%) in the LD24:0 group versus 17 out of 57 (29.8%) in the LD12:12 group. In females, total tumour incidence was 24 of 54 (44.4%) in the LD24:0 group versus 21 out of 40 (52.5%) in the LD12:12 group. Differences in total tumour incidence and in the

incidence of tumours in specific organ or tissue sites were not statistically significant. The mean survival was shorter in female rats exposed to LD24:0 compared with those exposed to LD12:12. The mean survival of cancer-bearing female rats was significantly reduced in the LD24:0 group compared with the LD12:12 group. In males, exposure to either natural light or LD0:24 significantly influenced tumour incidence, tumour multiplicity, number of malignant tumours, and mean survival. However, in females, exposure to natural light significantly increased total tumour incidence compared with exposure to LD12:12 (70.8% vs 52.5%; $P < 0.05$) and significantly ($P < 0.01$) shortened the mean lifespan of both tumour-bearing and cancer-bearing rats. Exposure to continuous darkness (LD0:24) significantly reduced the incidence of total tumours compared with exposure to LD12:12 (24.6% vs 52.5%; $P < 0.001$) in female rats. Compared with exposure to LD12:12, the incidence of benign mammary tumours (fibroma or fibroadenoma) was significantly increased ($P < 0.05$) by exposure to natural light and significantly decreased ($P < 0.01$) by exposure to LD0:24. [The Working Group noted that the time of year when rats in the group exposed to natural light entered the study was not reported.]

Vinogradova et al. (2010) (see also Lotosh et al., 2013) reported a study in which 267 male and 135 female outbred LIO rats (age, 1 month) were randomly allocated to one of two groups and exposed to a light–dark schedule of either LD12:12 or LD24:0. At the age of 14 months, 34 male and 36 females remained alive in the group exposed to LD24:0. At this stage, 90 male rats and 71 female rats from the group exposed to LD12:12 were reallocated to the group exposed to LD24:0, leaving 43 male and 30 female rats exposed to LD12:12. Compared with rats exposed to LD12:12 from the age of 1 month, mean lifespan was significantly shortened in female rats (but not in male rats) exposed to continuous light from the age of 1 month ($P < 0.01$); no significant differences in mean lifespan were seen in male or female rats exposed to continuous light from the age of 14 months. No statistically significant differences were found in comparisons of total tumour incidence and organ- or tissue-specific tumour incidence by sex.

Bukalev et al. (2013) reported a lifetime study of a total of 203 female Wistar rats (age, 25 days) that were exposed to a light–dark schedule of either LD12:12 (control), LD24:0, LD0:24, or natural light. In the group exposed to natural light, photoperiod [equivalent to latitude ~62°] ranged from 4.2 hours of light in winter to 24 hours in summer. The 2-year survival was reduced in the groups exposed to LD24:0 (11.1%), LD0:24 (37.7%), and natural light (31.2%), compared with the group exposed to LD12:12 (62.5%); however, no statistical analysis was provided. Tumour incidence was similar among rats exposed to LD12:12 (benign, 60.0%; malignant, 12.5%) or LD24:0 (benign, 59.3%; malignant, 13.0%). Tumour incidence in the group exposed to LD0:24 (continuous darkness) was significantly lower (benign, 21.3%; malignant, 4.9%), and exposure to natural light significantly increased the incidence of benign tumours (benign, 81.3%; malignant, 18.8%). In all groups, benign tumours were mostly fibromas or fibroadenomas of the mammary gland; malignant tumours were all observed to have originated in the haematopoietic system, breast, uterus, or kidney. [The Working Group noted that the time of year when the group exposed to natural light entered the study was not reported.]

3.4 Extreme changes in photoperiod with modifying factors

See also Table 3.4.

Table 3.4 Studies of carcinogenicity in experimental animals exposed to extreme changes in photoperiod with modifying factors

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, number, or surface area of tumours	Significance	Comments
Co-carcinogenicity Rat, Outbred (F) 5–6 mo ≤ 23 wk Khaetski (1965)	Changes in photoperiod Natural illumination + DMBA (control), LD24:0 + DMBA Intravenous injections of DMBA (1.5 mg) 5× at 10-d intervals (for ~7 wk) beginning 7 wk after start of experiment NR NR	*Ovary*: granulosa cell tumour Incidence: 0/15, 4/17 *Mammary gland*: tumours Incidence: 6/15, 5/17	 [NS] [NS]	Principal limitations: limited details on exposure design; no data on the time of light switching on and off; small number of rats; only one sex; no statistics reported Continuous light from 300-W electric lamp fixed 1.5 m high; all surviving rats killed 16 wk after the last DMBA injection; earliest mammary tumours detected after the 4th and 13th week in the control and continuous-light groups, respectively
Initiation–promotion (tested as promoter) Rat, Outbred (F) 5–6 mo ≤ 12 wk Khaetski (1965)	Changes in photoperiod Natural illumination + DMBA (control), LD24:0 + DMBA Intravenous injections of DMBA (1.5 mg) 6× at 10-d intervals (for ~9 wk) NR NR	*Mammary gland*: all tumours Incidence: 10/14, 12/14 Multiplicity: 1.7, 3.1 Surface area (cm^2): 2.6, 8.7	 [NS] NR NR	Principal limitations: limited details on exposure design; no data on the time of light switching on and off; no statistics reported; small number of rats; only one sex Continuous light from 300-W electric lamp fixed 1.5 m high; rats exposed for additional 12 wk to either natural light or constant light 4 wk after the last DMBA injection, then killed
Co-carcinogenicity Rat, Sprague-Dawley (F) 43 d 8 mo Hamilton (1969)	Changes in photoperiod LD12:12, LD24:0 30 mg DMBA by gavage at age 50 d 26, 21 NR	*Mammary gland* All tumours Incidence: 15/26 (58%), 20/21 (95%)* Multiplicity: 1.39, 2.71 Number: 36, 57 Adenocarcinoma Multiplicity: 0.62, 0.19 Number: 16, 4* Fibroadenoma Multiplicity: 0.77, 2.52 Number: 20, 53*	 *[$P = 0.006$, two-tail Fisher exact test] NR NR NR *$P < 0.001$ (decrease) NR *$P < 0.001$ (increase)	Principal strengths: relatively long observation period

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, number, or surface area of tumours	Significance	Comments
Initiation–promotion (tested as promoter) Rat, Sprague-Dawley (F) 58 d ~20 wk Aubert et al. (1980)	Changes in photoperiod Sham LD12:12, sham LD24:0, PX LD12:12, PX LD24:0 25 mg DMBA by gavage 2 d after either sham surgery or PX 25, 25, 25, 25 NR	*Mammary gland*: all tumours No significant difference in tumour incidence		Principal limitations: no microscopic evaluation of tissues; no statistical analysis reported Continuous light increased mammary tumour latency in sham-operated rats (n = 21; 77.3 d, P < 0.02) compared with sham LD12:12 controls (n = 20, 64.8 d)
Co-carcinogenicity Rat, Holtzman (F) 1 d 6 mo Kothari et al. (1982)	Changes in photoperiod LD10:14 (control), LD24:0 20 mg DMBA by gavage at age 55 d 25, 47 NR	*Mammary gland* All tumours Incidence: 17/25 [68.0%], 45/47 (95.7%)* Multiplicity: 1.12, 2.18 Adenocarcinoma Incidence: 15/25 (60.0%), 45/47 (95.7%)* Multiplicity: [0.88], [2.16] Number: 15, 97 Fibroadenoma Incidence: 2/25, 1/47 Multiplicity: [0.12], [0.02] Number: 2, 1	 *[P = 0.0024, two-tail Fisher exact test] NR *[P = 0.0002, two-tail Fisher exact test] NR NR [NS] NR NR	Principal strengths: relatively long observation period Principal limitations: data were not clearly presented throughout the manuscript Statistical analysis was not reported
Co-carcinogenicity Rat, Holtzman (F) 1 d 6 mo Kothari et al. (1984)	Changes in photoperiod LD10:14 (control), LD24:0 20 mg DMBA by gavage at age 55 d 25, 60 NR	*Mammary gland* All tumours Incidence: 17/25 [68.0%], 58/60 (96.7%)* Multiplicity: 1.13, 2.26 Number: 19, 131 Adenocarcinoma Incidence: 15/25 (60.0%), 57/60 (95.0%)*	 *[P = 0.0007, two-tail Fisher exact test] NR NR *[P = 0.0002, two-tail Fisher exact test]	Principal strengths: relatively long observation period Principal limitations: data were not clearly presented throughout the manuscript Statistical analysis was not reported; controls appear to be as for Kothari et al. (1982) (same data) or else Kothari et al. (1984) is a follow-up study

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, number, or surface area of tumours	Significance	Comments
Co-carcinogenicity Rat, Holtzman (F) 1 d 6 mo Kothari et al. (1984)	Changes in photoperiod LD10:14 (control), LD24:0 PX at birth and 20 mg DMBA by gavage at age 55 d 23, 29 NR	*Mammary gland* All tumours Incidence: 15/23 (65.2%), 26/29 (90.0%)* Multiplicity: 1.20, 2.65 Number: 18, 43 Adenocarcinoma Incidence: 14/23 (60.9%), 24/29 (82.8%)	 *[$P = 0.04$, two-tail Fisher exact test] NR NR [NS]	Principal strengths: relatively long observation period Principal limitations: data were not clearly presented throughout the manuscript Statistical analysis was not reported
Initiation–promotion (tested as initiator) Rat, Holtzman (F) 1 d 27 wk Subramanian & Kothari (1991)	Changes in photoperiod Intact + LD10:14 (control), intact + LD24:0, PX + LD10:14 (control), PX + LD24:0 10 mg DMBA by gavage 1× at age 55 d 20, 20, 20, 20 NR	*Mammary gland*: carcinoma Incidence: 70.0%, 80.0%, 87.5%, 90.0% Multiplicity: 1.4 ± 0.2, 1.4 ± 0.3, 1.4 ± 0.2, 1.4 ± 0.2	 NS NS	Principal strengths: relatively long observation period
Co-carcinogenicity Rat, Sprague-Dawley (F) 26 d 13 wk Anderson et al. (2000)	Changes in photoperiod LD8:16 (control), LD24:0 8 mg DMBA by gavage at age 52 d 50, 50 NR	*Mammary gland*: all tumours Incidence: 19/50, 8/50* Multiplicity: 2.6, 1.1	 *$P < 0.05$ (decrease) NR	Principal limitations: no histopathological examination
Co-carcinogenicity Rat, NR (F) 1 mo At least ≤ 390 d Anisimov et al. (1994)	Changes in photoperiod LD12:12, LD24:0, LD0:24 Intravenous injections of MNU at 50 mg/kg bw 1×/wk for 3 wk from age 6 wk 30, 50, 50 NR	*Mammary gland* All tumours Incidence: 12/22 (55%), 32/35 (91%)*, 6/38 (16%)** Adenocarcinoma Incidence: 7/22 (31%), 20/35 (57%)*, 1/38 (3%)**	 *$P < 0.05$ (increase) **$P < 0.05$ (decrease) *$P < 0.05$ (increase) **$P < 0.05$ (decrease)	Principal limitations: number of animals at start not reported; no information on survival or body weight; only one sex used; limited details on exposure design The effective number of animals (denominator) is the number of animals alive at appearance of first mammary gland tumour; see also Anisimov et al. (1996)

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, number, or surface area of tumours	Significance	Comments
Initiation–promotion (tested as promoter) Rat, F344/N (F) 50 d 26 wk Travlos et al. (2001)	Changes in photoperiod LD12:12, LD12:12 + intermittent light at night (5× 1-min exposures), LD12:12 + PX Intraperitoneal injection of MNU at 50 mg/kg bw at age 50 d 40, 40, 40 34, 34, 31	*Mammary gland* All tumours Incidence: 70%, 70%, 78% Multiplicity: 2.18, 1.89, 2.39 Number: 61, 53, 74	 NS NS NS	Principal limitations: the high dose of MNU may have precluded the identification of a carcinogenic response
Co-carcinogenicity Rat, Wistar (M+F) (combined) Gestation day 1 Lifetime Beniashvili et al. (2001)	Changes in photoperiod LD12:12 (control), LD24:0, LD0:24 Maternal exposure to ENU (80 mg/kg bw) on gestation day 18 or 19 61, 34, 40 NR	*All tumours* (in male offspring) Incidence: 26%, 85%*, 13%** *Peripheral nervous system* (in male offspring): all tumours Incidence: 20%, 62%*, 10% *Kidney* (in male offspring): all tumours (all mesenchymal) Incidence: 2%, 21%*, 3% *All tumours* (in female offspring) Incidence: 32%, 70%*, 11%** *Peripheral nervous system* (in female offspring): all tumours Incidence: 28%, 54%*, 7%** *Kidney* (in female offspring): all tumours (all mesenchymal) Incidence: 2%, 9%, 5%	 *$P < 0.01$ (increase) **$P < 0.01$ (decrease) *$P < 0.01$ (increase) *$P < 0.01$ (increase) *$P < 0.01$ (increase) **$P < 0.01$ (decrease) *$P < 0.01$ (increase) **$P < 0.01$ (decrease) NS	All groups of male and female offspring housed under LD12:12 schedule after weaning at age 1 mo

Table 3.4 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Incidence, multiplicity, number, or surface area of tumours	Significance	Comments
Initiation–promotion (tested as promoter) Rat, Outbred (M) NR ≤ 20 wk Panchenko et al. (2008)	Changes in photoperiod LD12:12 + DMH (control), LD24:0 + DMH Subcutaneous injection of DMH (21 mg/kg bw) 5× at 1-wk intervals NR NR	*Colon*: carcinoma Incidence: 17/19, 17/19 Multiplicity: 1.5 ± 0.2, 1.8 ± 0.2 *Colon (ascending)*: carcinoma Incidence: 11/19, 16/19*	 [NS] NS *$P < 0.05$	Principal limitations: DMH doses may have been too high to permit identification of tumour-promoting effect; no data on circadian disruption status

bw, body weight; d, day; DMBA, 7,12-dimethylbenz[*a*]anthracene; DMH, 1,2-dimethylhydrazine; ENU, *N*-ethyl-*N*-nitrosourea; F, female; LD, light-dark; LD0:24, continuous darkness; LD8:16, 8 hours of light followed by 16 hours of darkness; LD10:14, 10 hours of light followed by 14 hours of darkness; LD12:12, 12 hours of light followed by 12 hours of darkness; LD24:0, continuous light; M, male; min, minute; MNU, *N*-methyl-*N*-nitrosourea; mo, month; NR, not reported; NS, not significant; PX, pinealectomy/pinealectomized; wk, week.

Rat

(a) 7,12-Dimethylbenz[a]anthracene

In the first experiment of a study (Khaetski, 1965), groups of female outbred non-parous rats [number at start and strain not reported] (age, 5–6 months) were exposed to one of the following light–dark schedules: Group 1, constant light (LD24:0) using a 300 W electric lamp fixed 1.5 m high; Group 2, "natural" illumination [not further specified]; and Group 3, 2–3 hours of light followed by 21–22 hours of darkness. Beginning at 7 weeks from the start of the experiment, all rats were given five intravenous injections of 7,12-dimethylbenz[*a*]anthracene (DMBA) at 1.5 mg at 10-day intervals. The rats were killed when the tumours reached a diameter of 10 mm. Rats without tumours were killed 16 weeks after the last DMBA injection. In Group 1 (LD24:0), 4 out of 17 rats developed ovarian granulosa cell tumours, 2 out of 17 developed malignant mammary tumours, and 3 out of 17 developed benign mammary tumours [no significant increase in any incidence compared with Group 2]. In Group 2 ("natural" illumination), 6 out of 15 rats developed mammary tumours [histopathology not further specified] and no ovarian tumours were observed. In Group 3, 9 out of 19 rats developed malignant mammary tumours and 5 out of 19 developed benign mammary tumours. [The Working Group noted the inconsistency in data reported: in table 3 it was stated that 79% of rats of Group 3 had mammary tumours, that is, 15 out of 19 rats, but 14 out of 19 were reported.] The earliest mammary tumours were detected in groups 1, 2, and 3 at weeks 13, 4, and 4, respectively. [The Working Group noted that the number of rats per group was small.]

In a second experiment from the same study, female outbred rats [number at start and strain not reported] (age, 5–6 months) were given six intravenous injections of DMBA at 1.5 mg at 10-day intervals. At 4 weeks after the last injection, rats were subdivided into two groups: Group 1, exposed to continuous light (LD24:0); and Group 2, exposed to "natural" illumination [not further specified]. All surviving rats were killed 16 weeks after the last injection of the carcinogen. Mammary tumours [histopathology not further specified] developed in 12 out of 14 rats exposed to LD24:0 and in 10 out of 14 rats exposed to natural light [no significant difference]. In Groups 1 and 2 the multiplicity of mammary tumours was 3.1 and 1.7 per rat, and the surface area of tumours was 8.7 and 2.6 cm^2, respectively [no statistical analysis was reported] (Khaetski, 1965). [The Working Group noted that the number of rats per group was small.]

Hamilton (1969) reported a study in which 47 female Sprague-Dawley rats (age, 43 days) were exposed to either LD12:12 ($n = 26$, controls) or to LD24:0 ($n = 21$, continuous light), and were given a single oral dose of 30 mg DMBA by gavage at the age of 50 days and observed for 8 months. Compared with DMBA-treated control rats, DMBA-treated rats exposed to LD24:0 demonstrated a higher incidence and number of mammary tumours. Although the total incidence of mammary tumours was significantly increased in rats exposed to LD24:0 (20 out of 21 rats, 95%) compared with rats exposed to LD12:12 (15 out of 26 rats, 58%), rats exposed to LD12:12 developed significantly more mammary gland adenocarcinomas (16 vs 4, $P < 0.001$) than rats exposed to LD24:0. Most (53 out of 57) of the mammary tumours in the group exposed to LD24:0 were benign lesions (fibroadenomas); the large number of fibroadenomas seen in this group was responsible for the reported increase in the incidence of mammary tumours and total tumour number. [The Working Group noted that because rats exposed to continuous light developed significantly fewer mammary cancers than rats exposed to LD12:12, the implications of the results of this study are unclear.]

Aubert et al. (1980) reported a study in which two groups of 50 female Sprague-Dawley rats

(age, 58 days) were exposed to DMBA (25 mg) by gavage 2 days after either pinealectomy or sham surgery. Each group was then divided into another two groups, and exposed to either LD12:12 or LD24:0 (continuous light) and observed for approximately 20 weeks. In rats that had undergone sham surgery, mammary tumour latency was significantly increased in those exposed to LD24:0 compared with those exposed to LD12:12; this effect was abolished by pinealectomy. However, no significant difference in tumour incidence was seen between the groups.

Kothari et al. (1982) performed a study in which pregnant female Holtzman rats were exposed to continuous light, beginning on day 10–12 of gestation and continuing until parturition. At parturition, one group of dams and pups was maintained under continuous light (LD24:0), and the other group of dams and pups was transferred to a room maintained at a LD10:14 schedule. On day 21 after parturition, female offspring from both groups were weaned and dams were removed from the study. At the age of 55 days, 25 female rats exposed to LD10:14 and 47 female rats exposed to LD24:0 were given DMBA (20 mg) by gavage and observed for mammary tumour development for 6 months. Exposure to LD24:0 increased both mammary tumour incidence [$P = 0.0024$] and mammary tumour multiplicity [statistics not reported] compared with exposure to LD10:14. Almost all tumours were diagnosed as adenocarcinomas; there was therefore a significant increase in the incidence of mammary adenocarcinomas. The mean tumour latency period was significantly shorter in rats exposed to DMBA plus LD24:0 compared with rats exposed to DMBA plus LD10:14.

In a follow-on study, Kothari et al. (1984) reported a study in which pregnant female Holtzman rats were exposed to continuous light, beginning on day 10–12 of gestation and continuing until parturition. At 1–2 days after parturition, 52 female pups underwent pinealectomy and 85 female pups were left intact. Both intact and pinealectomized pups were then assigned to one of two groups exposed to either LD24:0 or LD10:14. At 21 days after parturition, female offspring from all groups were weaned and dams were removed from the study. At the age of 55 days, female rats were given DMBA (20 mg) by gavage and observed for mammary tumour development for 6 months. In intact female rats, exposure to LD24:0 increased both mammary tumour incidence [$P = 0.0007$] and mammary tumour multiplicity [statistics not reported] compared with rats exposed to LD10:14. Almost all mammary tumours identified in the study were diagnosed as adenocarcinomas; there was therefore a significant increase in the incidence of mammary adenocarcinomas. The mean tumour latency period was shorter in rats exposed to DMBA plus LD24:0 compared with rats exposed to DMBA plus LD10:14. [The Working Group noted that tumour responses in intact rats were virtually identical to those reported in Kothari et al. (1982). On this basis, it appears that the data reported in Kothari et al. (1982) may have been used again in Kothari et al. (1984).] In pinealectomized rats, exposure to LD24:0 increased both mammary tumour incidence [$P = 0.04$] and mammary tumour multiplicity [statistics not reported] compared with rats exposed to LD14:10. As was the case for intact rats, almost all mammary tumours were diagnosed as adenocarcinomas; however, the incidence of this tumour type was not significantly increased. As observed for intact rats, the mean tumour latency period was shorter in rats exposed to DMBA plus LD24:0 compared with rats exposed to DMBA plus LD10:14.

[The Working Group noted that mammary tumour data presented in an article by Shah et al. (1984) and recapitulated in Mhatre et al. (1984) are essentially identical to those presented in Kothari et al. (1982, 1984). Because the data

presented in these articles do not appear to be unique, they do not merit further discussion.]

The study by Subramanian & Kothari (1991) examined the role of continuous light on the incidence of DMBA-induced mammary tumours in intact or neonatally pinealectomized female Holtzman rats. Groups of 20 intact and pinealectomized rats were reared under light–dark schedules of either LD10:14 or LD24:0. Intact and pinealectomized female rats were given DMBA (10 mg) by gavage at the age of 55 days, and monitored for mammary tumour appearance and multiplicity for 27 weeks. Continuous light (LD24:0) did not significantly increase tumour incidence or multiplicity in either intact or pinealectomized rats compared with their respective LD10:14 control groups.

Anderson et al. (2000) performed a study in which groups of 50 virgin female Sprague-Dawley rats (age, 26 days) were exposed to either constant light (LD24:0) or a light–dark schedule of 8 hours of light followed by 16 hours of darkness (LD8:16) until termination of the study. At the age of 52 days, rats were given DMBA (8 mg) by gavage and monitored for mammary tumour development until terminal necropsy 13 weeks later. Rats exposed to constant light demonstrated a significant decrease in the incidence of mammary tumours compared with rats exposed to LD8:16. Mammary tumour incidence was 16% in rats in the constant light group versus 38% in rats in the LD8:16 group. Mean mammary tumour multiplicity was 1.1 tumour per rat in the group exposed to continuous light versus 2.6 tumours per rat in the group exposed to LD8:16. [The Working Group noted that a weakness of the study design was that tumour incidence was based on palpation and gross pathology only; tumours were not evaluated microscopically to confirm malignancy.]

(b) N-*methyl*-N-*nitrosourea*

Anisimov et al. (1994) (see also Anisimov et al., 1996) reported a study to determine the effects of light–dark schedules on the induction of mammary cancers in female rats [strain not reported] (age, 1 month). Rats were allocated to one of three groups, and exposed to either LD12:12 (n = 30), continuous light (LD24:0; n = 50), or continuous darkness (LD0:24, n = 50). After 2 weeks of exposure, all rats were given the first of three intravenous injections of *N*-methyl-*N*-nitrosourea (MNU) at 50 mg/kg bw at 1-week intervals. Compared with a mammary tumour incidence of 55% (12 out of 22) in the MNU-treated LD12:12 group, 91% (32 out of 35) of rats in the LD24:0 group developed mammary tumours. By contrast, mammary tumour incidence in the LD0:24 group was 16% (6 out of 38). The same pattern was observed if tumour data were limited to mammary gland adenocarcinoma: compared with a 31% mammary adenocarcinoma incidence (7 out of 22) in the LD12:12 group, rats in the LD24:0 group had a 57% incidence of mammary adenocarcinomas (20 out of 35) and rats in the LD0:24 group had a 3% incidence of mammary adenocarcinoma (1 out of 38). Each of these differences from the LD12:12 group was statistically significant ($P < 0.05$). [The Working Group noted the lack of details provided on the duration of the experiment (at least up to 390 days) and exposure design, and that the high dose of MNU may have had an impact on survival and tumour incidence.]

In a later study performed by Travlos et al. (2001) using the same carcinogen-induced mammary cancer model, intact or pinealectomized female Fischer 344/N rats (age, ~50 days) were given a single intraperitoneal injection of MNU at 50 mg/kg bw. Two groups of MNU-treated rats, one intact (n = 40) and the other pinealectomized (n = 40), were then exposed to a light–dark schedule of LD12:12. A third group of intact rats (n = 40) was also

exposed to LD12:12 with five intermittent exposures to 1 minute of light at 2 hour intervals, every night. All rats were observed for 26 weeks after treatment with MNU. A strong mammary cancer response was seen in all groups. Both exposure to intermittent light at night and pinealectomy did not have any statistically significant effect on mammary cancer incidence or multiplicity in this study. [The Working Group noted that the high dose of MNU may have precluded the identification of a carcinogenic response.]

(c) N-*ethyl*-N-*nitrosourea*

Beniashvili et al. (2001) performed a study in which groups of 24 pregnant female Wistar-derived outbred rats were exposed to either a daily schedule of 12 hours of light followed by 12 hours of darkness (LD12:12), constant light (LD24:0), or continuous darkness (LD0:24). The different light exposure regimens were initiated on the first day of gestation, and were continued throughout gestation and for 1 month after delivery. Each pregnant rat was given a single intravenous injection of *N*-ethyl-*N*-nitrosourea at 80 mg/kg bw on day 18 or 19 of gestation. At 1 month after delivery, offspring (males and females) from all groups were exposed to LD12:12 until their natural death. Compared with offspring exposed to LD12:12 during gestation and the early postpartum period, offspring exposed to LD24:0 demonstrated significant increases in the incidence of total tumours in males and females, in peripheral nervous system tumours in males and females, and in kidney tumours in males. All kidney tumours were mesenchymal tumours. In contrast, compared with offspring exposed to LD12:12 during these periods, offspring exposed to LD0:24 demonstrated a significantly lower total tumour incidence in males and females, and a significantly lower incidence of tumours in the peripheral nervous system in females.

(d) *Diethylnitrosamine*

To investigate the possible promoting activity of light at night on hepatocarcinogenesis induced in rats by DEN, a total of 65 male Wistar rats were given drinking-water containing DEN at a dose of 10 mg/kg bw every day for 6 weeks and then randomized into three experimental groups: DEN only (20 rats; negative control); DEN plus phenobarbital (30 mg per rat per day for 4 weeks; 22 rats, positive control), a promoter of tumours of the liver; or DEN plus continuous light (LD24:0) (23 rats). All rats were observed until death (≤ 5 months in all groups) (van den Heiligenberg et al., 1999). At 3 months after the start of the experiment, laparotomic evaluations demonstrated no [statistically significant] differences between groups in terms of total incidence of gross lesions on the surface of the liver (72% in rats treated with DEN only vs 89% in rats treated with DEN plus phenobarbital and 95% in rats treated with DEN plus continuous light). In contrast, when compared with the DEN-only group, groups treated with either DEN plus phenobarbital or DEN plus continuous light demonstrated statistically significant increases in the percentage of rats with six or more grossly visible nodules on the surface of the liver and in the percentage of rats with a largest nodule of size at least 3 mm. [The Working Group noted that these data were presented only as bar graphs and not as precise numerical data for lesion incidence.] At the time of death, all rats demonstrated grossly detectable nodules on the surface of the liver (van den Heiligenberg et al., 1999). [The Working Group noted that this study was weakened by the high dose of DEN that may have precluded the identification of the carcinogenic response, and by the fact that the total number of lesions throughout the hepatic parenchyma was not quantified in each rat.]

(e) 1,2-Dimethylhydrazine

Male outbred LIO Wistar-derived rats (white rats from the Rappolovo breeding nursery, Russian Federation) were subdivided into five groups (Panchenko et al., 2008). Four groups were given five subcutaneous injections of 1,2-dimethylhydrazine (DMH) at a dose of 21 mg/kg bw at 1 week intervals from the first day of the experiment, and exposed to: Group 1, LD12:12 (L, 250 lux; D, 0.5 lux); Group 2, LD24:0; Group 3, LD24:0 plus melatonin at a concentration of 20 mg/L in the drinking-water during "nighttime", 6 times per week, from the first injection of DMH and for 20 weeks after the last DMH injection; and Group 4, LD0:24, with the exception of a 15-minute exposure to red light to clean the rats' cages (5 lux, 3 times per week). Group 5 consisted of 5 intact rats that were not given DMH (control) and were exposed to a light–dark schedule of LD12:12 (L, 250 lux). Rats were killed 20 weeks after the last DMH injection. The incidence of colon carcinoma was 17 out of 19 (89%), 17 out of 19 (89%), 11 out of 19 (58%; $P < 0.01$, decrease), and 12 out of 19 (63%) in Groups 1–4, respectively. However, the incidence of ascending colon carcinoma was 11 out of 19 (58%), 16 out of 19 (84%; $P < 0.05$, increase), 9 out of 19 (47%), and 9 out of 19 (47%) in groups 1–4, respectively. In conclusion, exposure to constant light may have promoted DMH-induced colon carcinogenesis in rats (Panchenko et al., 2008). [The Working Group noted that histopathology was performed on the ascending and descending colon and the rectum. The Working Group also noted the absence of data on circadian disruption, and that the DMH dose may have been too high to permit identification of a tumour-promoting effect.]

(f) Supporting studies

Cos et al. (2006) reported that light at night increased the growth rate of DMBA-induced mammary tumours, although the effect on the incidence or multiplicity of DMBA-induced mammary tumours in rats was not evaluated. In this study, alteration in the light–dark schedule was initiated when palpable mammary tumours were present. Female Sprague-Dawley rats that had been treated with 20 mg DMBA were assigned to groups when their first palpable mammary tumour reached 1 cm in diameter. Groups of 16 tumour-bearing rats were exposed to either: LD12:12 (L, 300 lux); constant light (300 lux); LD12:12 with a 30-minute period of normal light (300 lux) at the midpoint of the dark cycle; or LD12:12 with normal light (300 lux) during the period of light and dim light (0.21 lux) during the period of darkness. After 12 weeks, compared with rats in the first group (LD12:12), mean tumour surface area was increased ($P < 0.05$) in all three groups exposed to light at night. [The Working Group noted that mean tumour surface areas were provided in graphic form.]

3.5 Extreme changes in photoperiod with implant, transplant, or graft

See also Table 3.5.

3.5.1 Mouse

In the study by Otálora et al. (2008), groups of 10–21 C57BL/6 male mice [age, not reported; body weight, ~22 g; melatonin deficient] were given a subcutaneous injection of murine B16 melanoma cells to study the effects of exposure to continuous light (LD24:0) on tumour growth and circadian rhythm of core body temperature. Exposure to LD24:0 accelerated tumour progression (malignancy scored semiquantitatively; ANOVA test, $P < 0.05$) and abolished the circadian rhythm of core body temperature. [The Working Group noted that statistical analyses were inappropriate, reducing the suitability of the study for further evaluation.]

Table 3.5 Studies of carcinogenicity in experimental animals exposed to extreme changes in photoperiod with implant, transplant, or graft

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Growth rates, malignancy, weight, or volume of tumours	Significance	Comments
Co-carcinogenicity Mouse, B6D2F$_1$ (M) 6 wk ≤ 2 wk after inoculation Filipski et al. (2004)	Changes in photoperiod LD12:12 (control), LD24:0; mice were initially synchronized to LD12:12 for 3 wk Subcutaneous inoculation with 3 mm^3 fragments of mouse Glasgow osteosarcoma in each flank at age 9 wk 10, 10 NR	*Glasgow osteosarcoma*: total tumours Weight at 13 d (mg): 1700, 1550	NS	Principal limitations: tumour weight read from graph "Experiment 4": no effect on tumour growth and survival; melatonin-proficient strain
Co-carcinogenicity Mouse, C57BL/6 (M) NR 21 d after inoculation Otálora et al. (2008)	Changes in photoperiod LD12:12 (control with loggers), LD24:0 (with loggers) Subcutaneous inoculation with 0.5 × 10^6 murine B16 melanoma cells in left flank 10 d after exposure 12, 21 9, 11	*B16 murine melanoma*: total tumours Malignancy score: 3.25, 3.62*	*Light at night significantly increased rate of tumour progression (malignancy score: $P < 0.05$, ANOVA test)	Principal strengths: studied the role of light at night on tumour growth Principal limitations: statistical analyses considered inappropriate by the Working Group Melatonin-deficient strain
Initiation–promotion (tested as promoter) Mouse, BALB/c (M) 8 wk ≤ 21 d (PC3) or ≤ 17 d (HeLa) after inoculation Yasuniwa et al. (2010)	Changes in photoperiod HeLa + LD12:12 (control), HeLa + LD24:0, PC3 + LD12:12 (control), PC3 + LD24:0 Subcutaneous injection of 1 × 10^6 HeLa (human cervical cancer) or 1 × 10^6 PC3 (human prostate cancer) cells at two dorsal sites per mouse 16, 16, 8, 8 NR	*Human cervical adenocarcinoma (HeLa cells) or prostatic small cell carcinoma (PC3 cells)*: total tumours Volume (mm^3): at 17 d, 1100, 1600*; at 21 d, 350, 700*	*Tumours displayed increased volume in mice exposed to LD24:0 compared with mice exposed to LD12:12 ($P < 0.01$ for both models)	Melatonin-deficient strain; tumour volume read from graph

Table 3.5 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Growth rates, malignancy, weight, or volume of tumours	Significance	Comments
Initiation–promotion (tested as promoter) Rat, RNU (F) NR ≤ 72 d after inoculation Blask et al. (2003)	Changes in photoperiod LD12:12 (control), LD24:0 Subcutaneous inoculation with 3 mm^3 tumour tissue (MCF-7 human breast cancer tissue xenograft, 10^7 cells) blocks to inguinal region 3, 4 NR	*Human MCF-7 breast carcinoma*: total tumours Weight at 55 d (g): 2.8, 6.1*	MCF-7 tumour xenografts grew twice as fast in rats exposed to LD24:0 than in those exposed to LD12:12 ($P < 0.05$); *tumour volume significantly increased by more than 2-fold	Principal strengths: measured endogenous melatonin level under both LD24:0 and LD12:12 cycles Principal limitations: very small number of animals per group Rats were exposed to either LD12:12 or LD24:0 for 40 d after inoculation; tumour volume read from graph
Co-carcinogenicity Rat, Buffalo (M) NR ≤ 25 d after inoculation Blask et al. (2005)	Changes in photoperiod Six groups all exposed to light for 12-h period, then light at either 0.0 (i.e. LD12:12), 0.02, 0.05, 0.06, 0.08, or 345 (i.e. LD24:0) μW/cm^2 for 12-h period Subcutaneous inoculation with 3 mm^3 tumour block 2 wk after exposure 6, 6 NR	*Rat hepatocarcinoma 7288CTC*: total tumours Weight (g): LD12:12, 1.5 at 13 d; LD24:0, 8 at 8 d	Tumour grafts displayed increased rate of growth in rats exposed to light at night at 0.05, 0.06, 0.08, or 345 μW/cm^2 ($P < 0.05$) compared with those in rats exposed to 0.0 (i.e. LD12:12) and 0.02 μW/cm^2	Principal strengths: tested the impact of multiple types of light-at-night conditions on tumour growth; used two graft models Principal limitations: small number of animals per group Tumour weight read from graph
Co-carcinogenicity Rat, RNU (F) NR ≤ 35 d after inoculation Blask et al. (2005)	Changes in photoperiod Six groups exposed to light for 12-h period then light at either 0.0 (i.e. LD12:12), 0.02, 0.05, 0.06, 0.08, or 345 (i.e. LD24:0) μW/cm^2 for 12-h period Subcutaneous inoculation with 3 mm^3 tumour xenograft block 2 wk after exposure 6, 6 NR	*Human MCF-7 breast carcinoma*: total tumours Weight at 17 d (g): LD12:12, 1.8; LD24:0, 5.8	Tumour xenografts displayed increased rate of growth in rats exposed to light at night at 0.05, 0.06, 0.08, or 345 μW/cm^2 ($P < 0.05$) compared with those in rats exposed to 0.00 (i.e. LD12:12) and 0.02 μW/cm^2	Principal strengths: tested the impact of multiple types of light-at-night conditions on tumour growth; used two graft models Principal limitations: small number of animals per group Tumour weight read from graph

Table 3.5 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Growth rates, malignancy, weight, or volume of tumours	Significance	Comments
Co-carcinogenicity Rat, RNU (F) NR 30 d after inoculation Blask et al. (2014)	Changes in photoperiod LD12:12 (control), LD12:12 with dim light during the dark phase (0.08 μW/cm²) Subcutaneous inoculation with 3 mm³ human MCF-7 breast cancer 6 wk after exposure 6, 6 NR	*Human MCF-7 breast carcinoma*: total tumours Weight at 19 d (g): 2.4, 6.0	Tumours in rats exposed to light at night (0.08 μW/cm²) displayed a significant accelerated growth rate compared with those in rats exposed to LD12:12 (control) ($P < 0.01$)	Principal limitations: no survival or body-weight data; short duration of exposure; small number of animals used in tumour growth analysis Tumour weight read from graph
Co-carcinogenicity Rat, Buffalo (M) 5 wk ≤ 25 d after inoculation Dauchy et al. (1997)	Changes in photoperiod LD12:12 (control), LD12:12 with 0.08 μW/cm² light contamination during the dark phase, LD24:0 (345 μW/cm²) Subcutaneous injection of 3 mm³ rat hepatocarcinoma 7288CTC at age 12 wk 6, 6, 6 NR	*Morris hepatoma (rat hepatocarcinoma) 7288CTC*: total tumours Weight at 15 d (g): 2.2, 5, 10.4 Growth rate (g/d): 0.72 ± 0.09, 1.30 ± 0.15*, 1.48 ± 0.17*	Tumour growth rate displayed a positive relationship with the intensity of light at night; rats exposed to LD24:0 displayed the fastest rate (*$P < 0.001$) of tumour growth, followed by rats exposed to LD12:12 with light contamination (*$P < 0.001$); rats exposed to LD12:12 (control) demonstrated the lowest tumour growth rate	Principal strengths: compared the effect of two types of light-at-night conditions on the rate of tumour growth Principal limitations: small number of animals per group Tumour weight read from graph
Co-carcinogenicity Rat, RNU (F) NR NR, > 15 d Wu et al. (2011)	Changes in photoperiod LD12:12 (control), LD12:12 with light contamination (0.08 μW/cm²) Subcutaneous inoculation of 3 mm³ tumour tissue block after 2 wk of exposure 36, 36 NR	*Human MCF-7 SR⁻ breast cancer xenograft*: total tumours Latency to onset (d): 15, 11 Growth rate (g/d): 0.26 ± 0.04, 0.56 ± 0.03*	*$P < 0.05$, ANOVA test	Principal strengths: adequate number of animals used in the study Principal limitations: the approach of euthanizing animals for tumour isolation was unclear

Table 3.5 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Agent tested Exposure schedule No. of animals at start No. of surviving animals	Growth rates, malignancy, weight, or volume of tumours	Significance	Comments
Full carcinogenicity Rat, Wistar (M) NR 13 d after inoculation Guerrero-Vargas et al. (2017)	Changes in photoperiod LD12:12 (control), LD24:0 Subcutaneous inoculation with 5×10^6 rat C6 glioma cells after 5 wk of exposure 7, 7 NR	*Rat glioma C6 cell line*: total tumours Volume at 11 d (mm^3): 400, 750 Volume at 13 d (mm^3): 250, 650 Weight at 13 d (g): 0.5, 1.2*	*Tumours in rats exposed to LD24:0 grew faster ($P = 0.0240$) and larger in terms of weight ($P < 0.05$) and volume ($P < 0.01$) compared with those in rats exposed to LD12:12 (control)	Tumour volume and weight read from graph

d, day; F, female; h, hour; LD, light-dark; LD12:12, 12 h of light followed by 12 h of darkness; LD24:0, continuous light; M, male; NR, not reported; NS, not significant; wk, week.

Yasuniwa et al. (2010) studied HeLa (human cervical cancer cell) and PC3 (human prostate cancer cell) xenograft models using four groups of male BALB/c nude mice [melatonin deficient] (age, 8 weeks). Mice were given a subcutaneous injection of 1×10^6 HeLa (two groups, $n = 16$ per group) or PC3 (two groups, $n = 8$ per group) cells and then exposed to light–dark schedules of either LD12:12 (control) or LD24:0. Compared with the groups exposed to LD12:12, tumours in the LD24:0 groups (after 21 days for PC3 cells and after 17 days for HeLa cells) demonstrated a significantly increased volume ($P < 0.01$) and also significantly increased tumour micro-vessels and stroma.

3.5.2 Rat

Blask et al. (2003) studied the effects of exposure to continuous light on the growth of MCF-7 human breast cancer xenografts (10^7 cells) and plasma melatonin in immunodeficient female Rowett nude (RNU) rats [age, not reported]. Beginning 40 days after inoculation, rats were exposed to light–dark schedules of either LD12:12 or LD24:0 (continuous light). Compared with rats exposed to LD12:12 ($n = 3$), tumour growth rate was significantly increased ($P < 0.05$) and tumour weight at 55 days was significantly increased ($P < 0.05$) by more than 2-fold in rats exposed to LD24:0 ($n = 4$). Exposure to LD24:0 also completely suppressed the circadian rhythm of plasma melatonin. [The Working Group noted the very small group sizes.]

In the study by Blask et al. (2005), the effects of various intensities of light at night were investigated in groups of male Buffalo rats [age not reported, adult] bearing the rat hepatocarcinoma 7288CTC syngeneic graft and in groups of female RNU rats [age not reported, adult] bearing the MCF-7 breast cancer xenograft. Starting 2 weeks before tumour implantation, and continuing for up to 35 days after implantation, rats were exposed to a schedule of 12 hours of light and 12 hours of darkness, with six different intensities of light during the 12-hour period of darkness ($n = 6$ per group per light intensity): 0.00 (i.e. LD12:12, controls), 0.02, 0.05, 0.06, 0.08, or 345 (i.e. LD24:0 or constant light) μW/cm^2. In both models, rats exposed to light at 0.05, 0.06, 0.08, or 345 μW/cm^2 demonstrated significantly ($P < 0.05$) faster tumour growth rates compared with those exposed to light at 0.00 (controls) and 0.02 μW/cm^2. In male Buffalo rats, mean tumour weights were about 1.5 g at 13 days in those exposed to LD12:12 and about 8 g at 8 days in those exposed to LD24:0 [estimated by the Working Group from graphical data]. In female RNU rats, mean tumour weights at 17 days were about 1.8 g in those exposed to LD12:12 and about 5.8 g in those exposed to LD24:0 [estimated by the Working Group from graphical data]. There was also a dose-dependent suppression of plasma nocturnal melatonin levels in both rat models. [The Working Group noted the small number of rats.]

The study by Blask et al. (2014) used the same female RNU rat [age not reported, adult] MCF-7 human breast cancer xenograft model and approaches as described in the previous studies (Blask et al., 2003, 2005) to investigate the effect of exposure to dim light (0.08 μW/cm^2) during the period of darkness on tumour growth. Exposure to LD12:12 (controls) or to a schedule of 12 hours of light and 12 hours of dim light during the period of darkness was started 6 weeks before tumour implantation and continued for up to 30 days after implantation. Compared with rats maintained at LD12:12, rats exposed to dim light during the period of darkness ($n = 6$ rats per group) demonstrated a dramatically accelerated rate of tumour growth ($P < 0.01$) during the 30-day observation period. Dim light during the period of darkness also completely suppressed host serum melatonin and disrupted the circadian-regulated rhythm of the host–cancer balance. [The Working Group noted the small number of rats.]

The study by Dauchy et al. (1997) used the male Buffalo rat hepatocarcinoma 7288CTC syngeneic graft model to study the effect of dim light during the period of darkness on tumour growth. In this study, rats (age, 5 weeks) were exposed to either LD12:12 (controls), 12 hours of light and 12 hours of light contamination at 0.2 lux or 0.08 μW/cm², or LD24:0 schedules (n = 6 per group). Tumour growth rate displayed a positive relationship with the intensity of light at night. Compared with LD12:12 controls, rats exposed to LD24:0 displayed the fastest rate of tumour growth followed by rats exposed to light contamination during the period of darkness ($P < 0.001$). In addition, exposure to either LD24:0 or light contamination during the period of darkness was observed to completely suppress the plasma level of melatonin, and exposure to LD24:0 (but not to light contamination during the period of darkness) was observed to disrupt the circadian rhythm of plasma lipids in the hosts. [The Working Group noted the small number of rats.]

The study by Wu et al. (2011) used the same female RNU rat MCF-7 human breast cancer xenograft model described in Blask et al. (2005, 2014) to study the effects of dim light contamination (0.8 μW/cm²) on tumour growth and latency to onset; rats [age not reported, adult] were divided into two groups (n = 36 per group) and exposed to either LD12:12 (control) or to light contamination during the 12-hour period of darkness, starting 2 weeks before implantation. Tumour growth in rats exposed to light contamination during the period of darkness was significantly increased ($P < 0.05$) compared with LD12:12 controls (0.56 g per day vs 0.26 g per day) (Wu et al., 2011). [The Working Group noted that the duration of the study was not reported.]

Guerrero-Vargas et al. (2017) studied the effect of exposure to continuous light on the growth of rat C6 glioma grafts implanted in two groups of seven male Wistar rats. Exposure to continuous light (LD24:0) was observed to significantly accelerate tumour growth and significantly increase tumour volume and weight compared with exposure to LD12:12. Continuous light also abolished the circadian rhythm of body temperature, immune function, energy homeostasis, and metabolism. [The Working Group noted the small number of rats.]

3.6 Evidence synthesis for cancer in experimental animals

The evaluation of the carcinogenicity of alterations in the light–dark schedule in experimental animals was primarily based on the well-designed lifetime carcinogenicity studies reported by Anisimov et al. (2004) and Kettner et al. (2016). The Kettner et al. (2016) article reported a series of independent studies in which male and female mice of three strains (one wildtype and two genetically engineered) were exposed to shifts in the light–dark schedule in the form of repeated 8-hour advances until age 90 weeks. Compared with control mice of each strain exposed to a regular light–dark schedule of LD12:12, exposure to shifts in the light–dark schedule was observed to significantly increase the incidence of hepatocellular carcinoma in all three strains. The study by Anisimov et al. (2004) compared tumour incidence and latency in wildtype female mice exposed for life to either a light–dark schedule of LD12:12 (control) or to continuous light (LD24:0). Statistically significant increases in the incidence of lung adenocarcinoma, malignant lymphoma, and total tumours were observed in mice exposed to LD24:0. The positive results reported in a few other studies in rodents exposed to shifts in the light–dark schedule or to continuous light, and in many studies using carcinogen-induced or transplantable tumour models, support the carcinogenicity of alterations in the light–dark schedule demonstrated in the lifetime carcinogenicity

evaluations of Kettner et al. (2016) and Anisimov et al. (2004).

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