National Toxicology Program
U.S. Department of Health and Human Services

Shift work at Night, Artificial Light at Night, and Circadian Disruption Workshop

Appendix B

Overview of experimental animal studies: Carcinogenicity

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Office of the Report on Carcinogens (ORoC)
Office of Health Assessment and Translation (OHAT)
Division of the National Toxicology Program
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Abstract

The effect of various alterations to light exposure on carcinogenesis were studied in numerous experimental animal studies. Table 1 provides an overview of information – type of exposure, carcinogenicity model, and biomarkers or other endpoints measured and relevant co-exposures from over 30 studies reporting on different types of exposure to light. Findings for the studies are not reported as the purpose of this abstract is to describe the available animal models. The exposure paradigms included constant light, constant dim light or contaminating light in the dark phase, intermittent light exposure in the dark phase, and shortening or lengthening photoperiods of light and dark; 12 hour light:dark days were considered to be the control groups. Three general model systems were used to assess the effect of light alteration on carcinogenicity: measuring the incidence or multiplicity of spontaneous tumors, tumor promotion of chemically initiated tumors, and tumor growth of human or rodent xenografts. Studies were conducted in both mice and rats and evaluated several types of tumors; of special interest are effects on the growth of human breast tumors as this is the cancer most studied in human studies. Many studies measured biomarkers of circadian rhythm and/or early markers of carcinogenicity such as nocturnal melatonin and its metabolite, 6-sulfatoxymelatonin, estrous cycle, serum sex hormones, clock gene expression, fatty acid uptake, linoleic acid tumor uptake and plasma levels, 13-HODE production, and clock gene expression.

Simulated shiftwork was also examined in mice and rats..Table 2 provides an overview of information – type of exposure, carcinogenicity model and biomarkers or other endpoints measure – from over 10 studies of the timing of light. These studies looked at the effect of shifts or light cycle inversion (periodically switching the light period with the dark period) and jet lag (periodic lengthening of the period of light to advance the time of day the light cycle starts and ends) on either (1) incidence of spontaneous neoplasm development in transgenic mice (2) promotion of chemically induced tumors and (3) tumor growth of several different types of implanted (xenograph) rodent tumors. In the xenograft studies, tumors were implanted after animals were subjected to changes in light timing in jet lag models although in one study tumor cells were inoculated after causing circadian disruption by subjecting the animals to different light and dark photoperiods (Wu et al. 1988). One study evaluated combined effects of light and meal timing on tumor bearing animals; meal timing (food restricted to the active, dark period) was used to entrain the circadian clock disrupted by chronic jet lag (Filipski et al. 2005). Similar to the light studies, biomarkers of circadian disruption (such as body temperature and locomotor activity, clock gene expression, serum corticosterone) or tumor development (gene expression) were also reported. Some studies also evaluated the effects of timing of light induced circadian disruption on immune effects and cancer.

Two studies evaluated effects of meal timing (not reported in Table 2) on tumor growth (osteosarcoma or pancreatic adenoma xenografts) in animals under standard light and dark environments; food was restricted to either the light or dark periods of the day. Control animals were given food *ad libitum.* These studies also measured biomarkers of circadian disruption.

Many studies on genetic (such as clock gene knock out rodents) or surgical models (pinealectomy, suprachiasmatic nuclei ablation) that evaluate the association between circadian disruption and carcinogenicity were identified but are not discussed because they are less relevant to exposures experienced by humans. Studies on blindness and physiological melatonin are also not reported although they may provide supporting information on mechanism of carcinogenicity.

Bibliography

1) Anderson LE, Morris JE, Sasser LB, Stevens RG. 2000. Effect of constant light on DMBA mammary tumorigenesis in rats. *Cancer Lett* 148(2): 121-126.

2) Anisimov VN, Baturin DA, Popovich IG, Zabezhinski MA, Manton KG, Semenchenko AV, Yashin AI. 2004. Effect of exposure to light-at-night on life span and spontaneous carcinogenesis in female CBA mice. *Int J Cancer* 111(4): 475-479.

3) Anisimov VN, Zhukova OV, Beniashvili DS, Bilanishvili VG, Menabde MZ. 1994. Light deprivation, electromagnetic fields and mammary carcinogenesis. *Adv Pineal Res* 7: 229-234.

4) Baturin DA, Alimova IN, Anisimov VN, Popovich IG, Zabezhinski MA, Provinciali M, Mancini R, Franceschi C. 2001. The effect of light regimen and melatonin on the development of spontaneous mammary tumors in HER-2/neu transgenic mice is related to a downregulation of HER-2/neu gene expression. *Neuro Endocrinol Lett* 22(6): 441-447.

5) Beniashvili DS, Benjamin S, Baturin DA, Anisimov VN. 2001. Effect of light/dark regimen on N-nitrosoethylurea-induced transplacental carcinogenesis in rats. *Cancer Lett* 163(1): 51-57.

6) Blask DE, Brainard GC, Dauchy RT, Hanifin JP, Davidson LK, Krause JA, Sauer LA, Rivera-Bermudez MA, Dubocovich ML, Jasser SA, Lynch DT, Rollag MD, Zalatan F. 2005. Melatonin-depleted blood from premenopausal women exposed to light at night stimulates growth of human breast cancer xenografts in nude rats. *Cancer Res* 65(23): 11174-11184.

7) Blask DE, Dauchy RT, Dauchy EM, Mao L, Hill SM, Greene MW, Belancio VP, Sauer LA, Davidson L. 2014. Light exposure at night disrupts host/cancer circadian regulatory dynamics: impact on the Warburg effect, lipid signaling and tumor growth prevention. *PLoS One* 9(8): e102776.

8) Blask DE, Dauchy RT, Sauer LA, Krause JA, Brainard GC. 2003. Growth and fatty acid metabolism of human breast cancer (MCF-7) xenografts in nude rats: impact of constant light-induced nocturnal melatonin suppression. *Breast Cancer Res Treat* 79(3): 313-320.

9) Bukalev AV, Vinogradov IA, Zabezhinskii MA, Semenchenko AV, Anisimov VN. 2012. [Morbidity and mortality rate from tumors and other causes in female rats under different light regime]. *Adv Gerontol* 25(4): 589-597.

10) Clausen OP, Iversen OH, Thorud E. 1984. Circadian variation in the susceptibility of mouse epidermis to tumour induction by methylnitrosourea. *Virchows Arch B Cell Pathol Incl Mol Pathol* 45(3): 325-329.

11) Cos S, Mediavilla D, Martinez-Campa C, Gonzalez A, Alonso-Gonzalez C, Sanchez-Barcelo EJ. 2006. Exposure to light-at-night increases the growth of DMBA-induced mammary adenocarcinomas in rats. *Cancer Lett* 235(2): 266-271.

12) Dauchy RT, Blask DE, Sauer LA, Brainard GC, Krause JA. 1999. Dim light during darkness stimulates tumor progression by enhancing tumor fatty acid uptake and metabolism. *Cancer Lett* 144(2): 131-136.

13) Dauchy RT, Dupepe LM, Ooms TG, Dauchy EM, Hill CR, Mao L, Belancio VP, Slakey LM, Hill SM, Blask DE. 2011. Eliminating animal facility light-at-night contamination and its effect on circadian regulation of rodent physiology, tumor growth, and metabolism: a challenge in the relocation of a cancer research laboratory. *J Am Assoc Lab Anim Sci* 50(3): 326-336.

14) Dauchy RT, Sauer LA, Blask DE, Vaughan GM. 1997. Light contamination during the dark phase in "photoperiodically controlled" animal rooms: effect on tumor growth and metabolism in rats. *Lab Anim Sci* 47(5): 511-518.

15) Dauchy RT, Xiang S, Mao L, Brimer S, Wren MA, Yuan L, Anbalagan M, Hauch A, Frasch T, Rowan BG, Blask DE, Hill SM. 2014. Circadian and melatonin disruption by exposure to light at night drives intrinsic resistance to tamoxifen therapy in breast cancer. *Cancer Res* 74(15): 4099-4110.

16) Filipski E, Delaunay F, King VM, Wu MW, Claustrat B, Grechez-Cassiau A, Guettier C, Hastings MH, Francis L. 2004. Effects of chronic jet lag on tumor progression in mice. *Cancer Res* 64(21): 7879-7885.

17) Filipski E, Innominato PF, Wu M, Li XM, Iacobelli S, Xian LJ, Levi F. 2005. Effects of light and food schedules on liver and tumor molecular clocks in mice. *J Natl Cancer Inst* 97(7): 507-517.

18) Filipski E, Levi F. 2009. Circadian disruption in experimental cancer processes. *Integr Cancer Ther* 8(4): 298-302.

19) Filipski E, Li XM, Levi F. 2006. Disruption of circadian coordination and malignant growth. *Cancer Causes Control* 17(4): 509-514.

20) Filipski E, Subramanian P, Carriere J, Guettier C, Barbason H, Levi F. 2009. Circadian disruption accelerates liver carcinogenesis in mice. *Mutat Res* 680(1-2): 95-105.

21) Hamilton T. 1969. Influence of environmental light and melatonin upon mammary tumour induction. *Br J Surg* 56(10): 764-766.

22) Isobe Y, Fukamachi K, Hida H, Tsuda H, Nishino H. 2008. Diethylnitrosamine-induced hepatic lesions are greater in rats maintained under a light-dark cycle than under constant light, related to the locomotor activity rhythm. *Asian Pac J Cancer Prev* 9(4): 619-624.

23) Kothari L. 1988. Effect of melatonin on the mammary gland morphology, DNA synthesis, hormone profiles and incidence of mammry cancer in rats. In *The Pineal Gland and Cancer*. Gupta D, Attanasio A, Reiter RJ, eds. London: Brain Research and Promotion. pp. 210-219.

24) Kothari LS. 1987. Influence of chronic melatonin on 9,10-dimethyl-1,2-benzanthracene-induced mammary tumors in female Holtzman rats exposed to continuous light. *Oncology* 44(1): 64-66.

25) Kothari LS, Shah PN, Mhatre MC. 1982. Effect of continuous light on the incidence of 9,10-dimethyl-1,2-benzanthracene induced mammary tumors in female Holtzman rats. *Cancer Lett* 16(3): 313-317.

26) Kothari LS, Shah PN, Mhatre MC. 1984. Pineal ablation in varying photoperiods and the incidence of 9,10-dimethyl-1,2-benzanthracene induced mammary cancer in rats. *Cancer Lett* 22(1): 99-102.

27) Lang R, Hintner H, Hermann A, Brandstaetter R. 2003. Photoperiod modulates melanoma growth in C57BL/6 mice. *Exp Dermatol* 12(4): 510-513.

28) Li JC, Xu F. 1997. Influences of light-dark shifting on the immune system, tumor growth and life span of rats, mice and fruit flies as well as on the counteraction of melatonin. *Biol Signals* 6(2): 77-89.

29) Li XM, Delaunay F, Dulong S, Claustrat B, Zampera S, Fujii Y, Teboul M, Beau J, Levi F. 2010. Cancer inhibition through circadian reprogramming of tumor transcriptome with meal timing. *Cancer Res* 70(8): 3351-3360.

30) Logan RW, Zhang C, Murugan S, O'Connell S, Levitt D, Rosenwasser AM, Sarkar DK. 2012. Chronic shift-lag alters the circadian clock of NK cells and promotes lung cancer growth in rats. *J Immunol* 188(6): 2583-2591.

31) Mao L, Dauchy RT, Blask DE, Slakey LM, Xiang S, Yuan L, Dauchy EM, Shan B, Brainard GC, Hanifin JP, Frasch T, Duplessis TT, Hill SM. 2012. Circadian gating of epithelial-to-mesenchymal transition in breast cancer cells via melatonin-regulation of GSK3beta. *Mol Endocrinol* 26(11): 1808-1820.

32) Nakajima H, Narama I, Matsuura T, Nomura T. 1994. Enhancement of tumor growth under short light/dark cycle in mouse lung. *Cancer Lett* 78(1-3): 127-131.

33) Nelson RJ, Blom JM. 1994. Photoperiodic effects on tumor development and immune function. *J Biol Rhythms* 9(3-4): 233-249.

34) Otalora BB, Madrid JA, Alvarez N, Vicente V, Rol MA. 2008. Effects of exogenous melatonin and circadian synchronization on tumor progression in melanoma-bearing C57BL6 mice. *J Pineal Res* 44(3): 307-315.

35) Perissin L, Zorzet S, Rapozzi V, Giraldi T. 1989. Effects of different light cycles and stress on urinary excretion of melatonin and tumor spread in mice bearing Lewis lung carcinoma. *Pharmacol Res* 21 Suppl 1: 131-132.

36) Popovich IG, Zabezhinski MA, Panchenko AV, Piskunova TS, Semenchenko AV, Tyndyk ML, Yurova MN, Anisimov VN. 2013. Exposure to light at night accelerates aging and spontaneous uterine carcinogenesis in female 129/Sv mice. *Cell Cycle* 12(11): 1785-1790.

37) Schwimmer H, Metzer A, Pilosof Y, Szyf M, Machnes ZM, Fares F, Harel O, Haim A. 2014. Light at night and melatonin have opposite effects on breast cancer tumors in mice assessed by growth rates and global DNA methylation. *Chronobiol Int* 31(1): 144-150.

38) Shah PN, Mhatre MC, Kothari LS. 1984. Effect of melatonin on mammary carcinogenesis in intact and pinealectomized rats in varying photoperiods. *Cancer Res* 44(8): 3403-3407.

39) Travlos GS, Wilson RE, Murrell JA, Chignell CF, Boorman GA. 2001. The effect of short intermittent light exposures on the melatonin circadian rhythm and NMU-induced breast cancer in female F344/N rats. *Toxicol Pathol* 29(1): 126-136.

40) van den Heiligenberg S, Depres-Brummer P, Barbason H, Claustrat B, Reynes M, Levi F. 1999. The tumor promoting effect of constant light exposure on diethylnitrosamine-induced hepatocarcinogenesis in rats. *Life Sci* 64(26): 2523-2534.

41) Van Dycke KC, Rodenburg W, van Oostrom CT, van Kerkhof LW, Pennings JL, Roenneberg T, van Steeg H, van der Horst GT. 2015. Chronically Alternating Light Cycles Increase Breast Cancer Risk in Mice. *Curr Biol* 25(14): 1932-1937.

42) Vinogradova IA, Anisimov VN, Bukalev AV, Ilyukha VA, Khizhkin EA, Lotosh TA, Semenchenko AV, Zabezhinski MA. 2010. Circadian disruption induced by light-at-night accelerates aging and promotes tumorigenesis in young but not in old rats. *Aging (Albany NY)* 2(2): 82-92.

43) Vinogradova IA, Anisimov VN, Bukalev AV, Semenchenko AV, Zabezhinski MA. 2009. Circadian disruption induced by light-at-night accelerates aging and promotes tumorigenesis in rats. *Aging (Albany NY)* 1(10): 855-865.

44) Waldrop RD, Saydjari R, Rubin NH, Rayford PL, Townsend CM, Jr., Thompson JC. 1989. Photoperiod influences the growth of colon cancer in mice. *Life Sci* 45(8): 737-744.

45) Wu J, Sanchez de la Pena S, Halberg F, Cornelissen G, Wetterberg L, Halberg E, Lakatua D, Bingham C, Harvey J, Bazin H, et al. 1988. Chronosynergistic effects of lighting schedule-shift and cefodizime on plasmacytoma growth and host survival time. *Chronobiologia* 15(1-2): 105-128.

46) Wu M, Zeng J, Chen Y, Zeng Z, Zhang J, Cai Y, Ye Y, Fu L, Xian L, Chen Z. 2012. Experimental chronic jet lag promotes growth and lung metastasis of Lewis lung carcinoma in C57BL/6 mice. *Oncol Rep* 27(5): 1417-1428.

47) Wu MW, Li XM, Xian LJ, Levi F. 2004. Effects of meal timing on tumor progression in mice. *Life Sci* 75(10): 1181-1193.

48) Yasuniwa Y, Izumi H, Wang K-Y, Shimajiri S, Sasaguri Y, Kawai K, Kasai H, Shimada T, Miyake K, Kashiwagi E, Hirano G, Kidani A, Akiyama M, Han B, Wu Y, Ieiri I, Higuchi S, Kohno K. 2010. Circadian Disruption Accelerates Tumor Growth and Angio/Stromagenesis through a Wnt Signaling Pathway. *PLoS One* 5(12): e15330.

Table 1. Alternating light model studies

	LL vs. LD or DD	Dim light vs. LL and/or LD	Intermittent light in dark phase	Altered LD (short/long days, natural light[a])
Spontaneous tumors				
Lung, leukemia, and liver	*Tumors*: incidence, multiplicity *Biomarkers*: estrous cycle			
Mammary gland	*Tumors*[b]: incidence, multiplicity *Biomarkers*: HER2/Nu expression *Co-exp*[c]: melatonin			
Uterus	*Tumors*: incidence *Biomarkers and other endpoints*: estrous cycle, acceleration of aging			
Tumor NOS				*Tumors*: onset *Biomarkers* and other endpoints: serum hormones, serum cholesterol and lipoproteins, SOD activity; homeostatic stability; lifespan *Co-exp*[c]*:* melatonin
Initiation promotion				
DMBA – mammary tumor	*Tumors*: incidence, onset *Biomarkers*: DNA synthesis, nocturnal 6-sulfatoxymelatonin, estrous cycle, plasma estradiol, plasma prolactin *Co-exp*[c]: melatonin	*Tumors*: growth *Biomarkers and other endpoints*: nocturnal 6-sulfatoxymelatonin, serum estradiol, survival		*Tumors*: incidence *Biomarkers*: immune function *Co-exp*[c]: melatonin

	LL vs. LD or DD	Dim light vs. LL and/or LD	Intermittent light in dark phase	Altered LD (short/long days, natural light[a])
DMH – colon cancer	*Tumors*: growth, aberrant crypt foci incidence *Biomarker*: apoptosis, serum melatonin, Cox-2 expression *Co-exp*[c]: melatonin			
DEN liver nodules	*Tumor*: liver nodule incidence; GST-pi^{+} lesion incidence			
Urethane – lung tumors				*Tumors*: incidence, growth
NMU – mammary tumor	*Tumors*: incidence, onset		*Tumors*: incidence, multiplicity, size *Biomarker*: nocturnal serum melatonin *Co-exp*[c]: melatonin	
Xenografts/tumor growth				
Human MCF breast tumors	*Tumors*: growth *Biomarkers*: nocturnal melatonin, FA uptake, LA uptake, 13-HODE	*Tumors*[d]: growth, onset *Biomarkers*: Warberg effect, DNA synthesis, apoptosis, proliferation markers, DNA damage, nocturnal melatonin, FA uptake, LA uptake, 13-HODE, serum corticosterone, serum estradiol, GSK3β phosphorylation, Erk1/2 phosphorylation, protein expression *Co exp*[c]: melatonin		
Mouse mammary cell lines or tumors			*Tumors*: growth *Biomarkers*: 6-sulfatoxymelatonin, DNA	*Tumors*: growth *Biomarkers*: 6-sulfatoxymelatonin, DNA

	LL vs. LD or DD	Dim light vs. LL and/or LD	Intermittent light in dark phase	Altered LD (short/long days, natural light[a])
			methylation *Co exp*[c]: melatonin	methylation *Co exp*[c]: melatonin
Melanoma (murine)	*Tumors*: growth *Biomarkers*: body temperature *Co-exp*[c]: melatonin			
Human Hela (epidermoid)	*Tumors*: growth *Biomarkers*: oxidative stress, WNT10 and whole gene expression			
Rat hepatoma		*Tumors*: growth *Biomarkers*: nocturnal melatonin, plasma lipids, FA uptake, LA uptake, 13-HODE; DNA damage, proliferation markers; Erk1/2 phosphorylation, protein expression		
Mouse lung carcinoma	*Tumors*: metastasis *Biomarkers*: nocturnal melatonin			*Tumors*: metastasis *Biomarkers*: nocturnal melatonin
Mouse colon adenocarcinoma cells				*Tumors*: growth, multiplicity
Mouse osteosarcoma	*Tumors*: growth *Biomarkers and other endpoints*: body temperature, locomotor activity, survival			

LL = constant light; LD = light:dark (usually 12 hour cycle) , which is the control group; DD = constant dark.
FA = fatty acids; LA = linoleic acids; HODE = 13-hydroxyoctadecadienoic acid.
[a] Transgenic animal model.
[b] Coexposure of different light exposures with melatonin.

[c]Natural light from Northern Russia.
[d]One study exposed animals to increase light intensity in the dark phase, ranging form dim to bright light

Table 2. Shiftwork model studies involving timing of light exposure

	Altered LD (light cycle inverted)	Jet lag (advancing time on light cycle)	Jet lag and meal timing
Spontaneous tumors			
Mammary gland	*Tumors*[a]: onset *Biomarkers*: serum corticosterone, vitamin D, sleep, clock gene expression		
Initiation promotion			
DEN – primarily liver nodules,		*Tumors*: incidence, multiplicity, and size *Biomarkers and other endpoints*: body temperature, locomotor activity, liver toxicity, survival	
Xenografts/tumor growth			
Rat plasmacytoma		*Tumors*: growth *Biomarkers and other endpoints: estrous cycle, survival*	
Mouse lung carcinoma		*Tumors*: growth, metastasis *Biomarkers*: body temperature, serum corticosterone, immune function clock and cytokine gene and expression	
Mouse osteosarcoma		*Tumors*: growth *Biomarkers and other endpoints*: plasma corticosterone, body temperature, locomotor activity, clock gene and expression of genes related to carcinogenicity, survival	*Tumors*: growth *Biomarkers and other endpoints*: clock gene and expression of genes related to carcinogenicity

	Altered LD (light cycle inverted)	Jet lag (advancing time on light cycle)	Jet lag and meal timing
Ehrlich carcinoma or sarcoma	*Tumor*: growth *Biomarkers and other effects*: immune rhythms and effects, survival		

[a]Transgenic animal model.