

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

# Appendix B Overview of experimental animal studies: Carcinogenicity

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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.

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#### Table 1. Alternating light model studies

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

				Altered LD
	LL vs. LD or DD	Dim light vs. LL and/or LD	Intermittent light in dark phase	(short/long days, natural light <sup>a</sup> )
DMH – colon cancer	<i>Tumors</i> : growth, aberrant crypt foci incidence <i>Biomarker</i> : apoptosis, serum melatonin, Cox-2 expression <i>Co-exp<sup>c</sup></i> : melatonin			
DEN liver nodules	<i>Tumor</i> : liver nodule incidence; GST-pi <sup>+</sup> lesion incidence			
Urethane – lung tumors				Tumors: incidence, growth
NMU – mammary tumor	Tumors: incidence, onset		<i>Tumors</i> : incidence, multiplicity, size <i>Biomarker</i> : nocturnal serum melatonin <i>Co-exp<sup>c</sup></i> : melatonin	
Xenografts/tumor growth				
Human MCF breast tumors	<i>Tumors</i> : growth <i>Biomarkers</i> : nocturnal melatonin, FA uptake, LA uptake, 13-HODE	<i>Tumors</i> <sup>d</sup> : growth, onset <i>Biomarkers</i> : 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 <i>Co exp</i> <sup>c</sup> : melatonin		
Mouse mammary cell lines or tumors			<i>Tumors</i> : growth <i>Biomarkers</i> : 6- sulfatoxymelatonin, DNA	<i>Tumors</i> : growth <i>Biomarkers</i> : 6- sulfatoxymelatonin, DNA

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

<sup>b</sup>Coexposure of different light exposures with melatonin.

<sup>c</sup>Natural light from Northern Russia.

<sup>d</sup>One study exposed animals to increase light intensity in the dark phase, ranging form dim to bright light

	Altered LD		
	(light cycle inverted)	Jet lag (advancing time on light cycle)	Jet lag and meal timing
Spontaneous tumors			
Mammary gland	<i>Tumors<sup>a</sup></i> : onset		
	<i>Biomarkers</i> : serum corticosterone, vitamin D, sleep, clock gene expression		
Initiation promotion			
DEN – primarily liver nodules,		<i>Tumors</i> : incidence, multiplicity, and size	
		<i>Biomarkers and other endpoints</i> : 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	
		<i>Biomarkers</i> : body temperature, serum corticosterone, immune function clock and cytokine gene and expression	
Mouse osteosarcoma		Tumors: growth	Tumors: growth
		<i>Biomarkers and other endpoints</i> : plasma corticosterone, body temperature, locomotor activity, clock gene and expression of genes related to carcinogenicity, survival	Biomarkers and other endpoints: clock gene and expression of genes related to carcinogenicity

#### 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
Ehrlich carcinoma or sarcoma	<i>Tumor</i> : growth		
	<i>Biomarkers and other effects</i> : immune rhythms and effects, survival		

<sup>a</sup>Transgenic animal model.