

NTP NOMINATION HISTORY AND REVIEW

MELATONIN

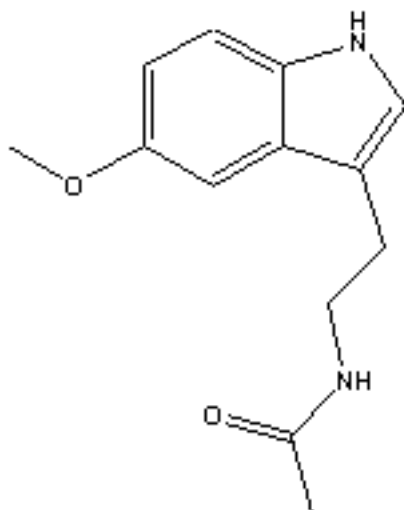
CAS No. 73-31-4

NOMINATION HISTORY

1. Nomination Source: NIEHS
2. Recommendations: Chronic toxicity testing
3. Rationale/Comments: - Potential human exposure (increasing use in chemotherapy and by the general public) - Lack of toxicity studies
4. Priority:
5. Date of Nomination:

SUMMARY OF DATA FOR CHEMICAL SELECTIONCHEMICAL IDENTIFICATION

<u>CAS Registry Number:</u>	73-31-4
<u>Chemical Abstracts Service Name:</u>	Acetamide, N-[2-(5-methoxy-1H-indol-3-yl)ethyl] (9CI)
<u>Synonyms:</u>	Acetamide, N-[2-(5-methoxyindol-3-yl)ethyl]- (6CI, 8CI); N-acetyl-5-methoxy-tryptamine; melatonin; melatonine; Melovine; 5-methoxy-N-acetyltryptamine; Regulin
<u>Structural Class:</u>	Substituted indolealkylamine

Structure, Molecular Formula and Molecular Weight:C₁₃H₁₆N₂O₂

Mol. wt.: 232.28

Chemical and Physical Properties:

<u>Description:</u>	Off-white crystalline solid (Calbiochem, 1995; Sigma, 1996)
<u>Melting Point:</u>	116-118°C (Budavari, 1989)
<u>Solubility:</u>	Soluble in ethanol and benzene (Budavari, 1989; Calbiochem, 1995).
<u>Stability:</u>	Light-sensitive; aqueous solutions stored in sterile vials under vacuum are stable for at least 6 months (Calbiochem, 1995; Cavallo <i>et al.</i> , 1995)

Technical Products and Impurities: Natural melatonin can be isolated from bovine pineal glands, but the risk of impurities is claimed to be greater with the natural material (Anon., 1995a). Synthetic melatonin is available with a purity level ranging from 97 to >99% (Aldrich, 1994; Calbiochem, 1995; Fluka, 1995; Spectrum, 1995). Triple Crown America, Inc., offers bulk powder or bulk tablet products imported from Switzerland at 99.5% (Anon., 1995b). Genzyme Pharmaceuticals advertises its "high quality" melatonin product as Melapure™; bulk quantities from Genzyme have been reported to be 98.5% pure (Genzyme Corp., 1994; Anon., 1996a). Cardiovascular Research, Ltd., offers melatonin at a purity exceeding 99.62% in the following product formulations:

Microtonin capsules, each containing 500 µg of melatonin, in bottles of 60 or 500 capsules;

Multiphasic melatonin S.R. enterically coated tablets, each containing 1.8 mg of melatonin, in bottles of 60 or 500 tablets;

Melatone capsules, each containing 3 mg of melatonin, in bottles of 60 or 1000 capsules;

Oncotonin capsules, each containing 10 mg of melatonin, in bottles of 30 or 500 capsules.

This company recommends that all melatonin products be taken at a frequency of one capsule at bedtime, as needed or as directed by a physician (PDR, 1995). In addition to these four products reported in the *Physicians' Desk Reference* (PDR), melatonin has been prepared for marketing under numerous trade names with a variety of claims regarding benefits to health and wellbeing, such as those presented in the following list (Table 1) (Anon., 1996b; Dialog, 1996b).

Table 1. Trade name products containing melatonin singly or in combination with other agents.

<u>Product name</u>	<u>Source company</u>	<u>Claimed efficacy</u>	<u>Source citation</u>
Melatonin	Natrol, Inc.	dietary aid/sleep and jet lag	US Air <i>In Flight</i> magazine
Melatonin	Nature's Bounty	dietary supplement	Rodman's Drug Store ad.
Melatonin	General Nutrition Ctrs.	dietary supplement	GNC ad.
Circadin	Neurim Pharmaceut.	regulate circadian rhythm	Garfinkel <i>et al.</i> , 1995
Clocktonin	Alfred Lewy	regulate body clock/sleep	<i>The Tan Sheet</i> , 3/1/96
Traveltonin	Alfred Lewy	regulate body clock/sleep	<i>The Tan Sheet</i> , 3/1/96
Somnotonin	Alfred Lewy	regulate body clock/sleep	<i>The Tan Sheet</i> , 3/1/96

			73-31-4 Melatonin
Nighttonin	Alfred Lewy	regulate body clock/sleep	<i>The Tan Sheet,</i> 3/1/96
Timetonin	Alfred Lewy	regulate body clock/sleep	<i>The Tan Sheet,</i> 3/1/96
Sleeptonin	Alfred Lewy	regulate body clock/sleep	<i>The Tan Sheet,</i> 3/1/96
Chronotonin	Alfred Lewy	regulate body clock/sleep	<i>The Tan Sheet,</i> 3/1/96
Mello-tonin	Marlyn Nuetraceut.	enhance rest/relaxation	<i>The Tan Sheet,</i> 2/26/96
Melatonin	Nutraceutical Resour./ Pharmaceut. Resources	nutritional supplement/ and jet lag	sleep <i>The Tan Sheet,</i> 2/19/96
Melatonin/Vitamin B6	Nutraceutical Resour./ Pharmaceut. Resources	nutritional supplement/ and jet lag	sleep <i>The Tan Sheet,</i> 2/19/96
Melatonin/Valerium Power	Nature's Herbs/Twinlab	herbal product	<i>The Tan Sheet,</i> 11/27/95
Melatonin	General Nutrition Cos.	herbal supplement	<i>The Tan Sheet,</i> 11/13/95
Mellodream	Lifewell	dietary supplement	<i>The Tan Sheet,</i> 10/2/95
A Good Day Starts With A Good Night	Lifewell	dietary supplement	<i>The Tan Sheet,</i> 10/2/95
Adjust Your Body Clock To Any Time Zone	Lifewell	dietary supplement	<i>The Tan Sheet,</i> 10/2/95
Turn Back The Clock On Jet Lag	Lifewell	dietary supplement	<i>The Tan Sheet,</i> 9/25/95
Chronosom	Alfred Lewy	regulate body clock/sleep	<i>The Tan Sheet,</i> 6/6/94
Melochron	Alfred Lewy	regulate body clock	<i>The Tan Sheet,</i> 4/11/94
Milkatonin	Alfred Lewy	additive for infant formulas or breast milk to facilitate sleep	<i>The Tan Sheet,</i> 4/11/94
Melosom	Alfred Lewy	to facilitate sleep	<i>The Tan Sheet,</i> 4/11/94

EXPOSURE INFORMATION

Production and Producers: Melatonin can be prepared from 5-methoxyindole as starting material by two different routes (Budavari, 1989). According to Horn (1981) the following synthetic steps illustrate the general preparative method: (See hard copy in Central Files for illustration)

Melatonin is not listed in the EPA TSCA Inventory (Dialog, 1996a). Melatonin is available in the United States from the following companies, according to listings in chemical catalogs and directories; but no annual production statistics were found (Hunter, 1995; Kuney, 1995; Van, 1995; Dialog, 1996d; PDR, 1996; STN, 1996):

3M Pharmaceuticals
Aceto Corp./Pfaltz & Bauer
Aldrich Chemical Co.
Amber Synthesis, Inc.
Bristol-Myers Squibb
Calbiochem-Novabiochem International
Cardiovascular Research, Ltd.
Flavine International, Inc.
Fluka Chemical Corp.
G.C.I. Nutrients
Genzyme Corp.
Hoechst Animal Health/Schering A.-G.
Hoffmann-La Roche & Co.
Interneuron Pharmaceuticals, Inc.
ICN Biomedicals, Inc.
Marcor Development Corp.
Parish Chemical Co.
Regis Technologies, Inc.
Research Organics, Inc.
Sigma Chemical Co.
Spectrum Chemical Mfg. Corp./Janssen Chimica
TCI America
Triple Crown America, Inc. (agent for Biosynth International)
Weinstein Nutritional Products

Melatonin is listed as a chemical in commerce in the U.S. International Trade Commission publications, *Synthetic Organic Chemicals, US Production and Sales, 1990-1993* (USITC, 1991, 1993, 1994a, 1994b). The reporting company for each year was listed as Regis Chemical Co./ Regis Technologies, Inc. of Morton Grove, IL; but no production or sales quantities were included. According to the USITC, separate statistics were not published to avoid disclosure of individual company operations. Although no specific production data were reported, the USITC reporting guidelines specify that each company's report of a chemical represents manufacture of a quantity 4,500 kg (10,000 lbs) or sales \$10,000. Hoffmann-LaRoche of Nutley, NJ, has been reported to be a source of pure melatonin for research; and advertisements for melatonin have been carried in numerous recent issues of the *Chemical Marketing Reporter* for Genzyme Corporation of Cambridge, MA (Anon.,

1996a). However, the only pharmaceutical company participating with the *Physicians' Desk Reference* in making product information available on melatonin is Cardiovascular Research, Ltd. (PDR, 1996). According to another source this company's melatonin products are imported from Japan (Anon. 1994a).

Imports: Synthetic melatonin products available from Triple Crown America, Inc., are reportedly imported from Biosynth International in Switzerland; and products available from Cardiovascular Research, Ltd., are reportedly imported from Japan (Anon. 1994a; Anon. 1995b).

Use Pattern: Melatonin, a hormone of the pineal gland also produced by extra-pineal tissues, acts as a biological response modifier; it is postulated to act as a mediator of photic-induced anti-gonadotropic activity in photoperiodic mammals (Budavari, 1989; Calbiochem, 1995). Secretion is reported to increase during the night; and in humans it is implicated in the regulation of sleep, mood, puberty, and ovarian cycles (Anon., 1994b). The synthetic counterpart, which is available as a prescription and over-the-counter (OTC) drug/nutritional supplement as well as a fine organic chemical, has been promoted as an anticancer, radioprotective, contraceptive, antiobesity, antiaging and antifatigue agent, and antidote to jet lag and degenerative diseases (Garcia-Patterson *et al.*, 1996). According to Olin (1996) melatonin is an FDA approved "orphan drug" prescribed for the treatment of circadian rhythm sleep disorders in blind patients. Garcia-Patterson and coworkers (1996) noted that, while experimental data continue to accumulate in support of anticarcinogenic properties for melatonin, its potential clinical use as an oncostatic drug still awaits confirmation. They also described its potential use as an oral contraceptive in combination with progestogen as based on reports of its ability to slightly stimulate prolactin while partially inhibiting gonadotrophins. Voordouw and coworkers (1992) reported on their development of an estrogen-free melatonin-containing oral contraceptive which combines exogenous melatonin with synthetic progestin norethisterone. Interneuron holds a patent for low dose administration of melatonin as a sedative and is said to be developing an OTC version of the drug (1994a). It has also been proposed for co-drug therapy with the glucocorticoid antiinflammatory, dexamethasone, to reduce side effects. Melatonin has been used for a number of years as a veterinary drug (*e.g.* Melovine, Regulin, PRIME-X); it can be administered in the form of implants for enhanced breeding, based on its participation in transmitting photoperiodic information in seasonally breeding animals, and improved pelting, *e.g.* in minks in the fur industry (CVM, 1996).

Human Exposure: Human bodily content of very low levels of natural melatonin as a hormone is virtually universal. Ubeda and coworkers (1995) reported physiological levels of melatonin to be in a concentration range of 10^{-11} to 10^{-10} M. Endogenous levels are highest during young childhood, beginning to decrease around puberty and continuing to fall into senescence when concentrations are minimal, according to Garcia-Patterson and coworkers (1996). They reported that in early childhood there is a strong nyctohemeral rhythm of melatonin secretion over a 24-hour period, with highest levels circulating nocturnally; this nighttime peak decreases gradually with ageing and virtually disappears in old age. Melatonin is rapidly released into the vascular system and due to its high lipophilicity is distributed throughout bodily fluids and cells (Reiter, 1993). Melatonin is reported to be a short-lived hormone in humans, rapidly degraded and eliminated from the body (Pierpaoli & Maestroni, 1987).

There is potential for widespread consumer exposure to synthetic melatonin available as a drug. It is inexpensive and readily available over the counter (OTC) in drug stores, health food stores, and some supermarkets as a health and nutrition supplement. Widely distributed general press articles and marketing sales pitches promote this chemical as a dietary aid for maintaining normal sleep patterns, improving restful sleep, and combating the effects of jet lag. Several subpopulations have been identified in the literature as likely to be exposed to melatonin as a prescribed or OTC medication to adjust the circadian system and avoid circadian disturbances. They include blind persons, night shift workers, and people who fly long distances (to counteract jet lag) (Dawson *et al.*, 1995; Olin, 1995). Ubeda and coworkers (1995) reported that pharmacological levels of melatonin occur in a concentration range of 10^{-9} to 10^{-8} M. However, there is the possibility that a certain percentage of the consuming public will engage in excessive self-administration. Furthermore, in a cancer treatment protocol, Robinson and coworkers (1995) reported administering melatonin at a dose of 200 mg orally per day. Other typical doses administered in various clinical trial protocols for the treatment of advanced cancers have been in the range of 20–50 mg orally per day (Lissoni *et al.*, 1987, 1993, 1994a,b).

No data associating occupational exposures with the manufacture of synthetic melatonin were found in the available literature. Melatonin is not listed in the database of the National Occupational Exposure Survey (NOES), conducted by the National Institute of Occupational Safety and Health (NIOSH) between 1981 and 1983.

Environmental Occurrence: Melatonin is a naturally occurring substance classified as a simple indole alkaloid and derivative of tryptamine (Cordell, 1978). It is a metabolite of the amino acid, tryptophan, and is derived enzymatically from its precursor, serotonin, *via* the intermediate, N-acetylserotonin (Dubocovich, 1988; Hashizume, 1991; Tedesco *et al.*, 1994). It is a hormone produced by the pineal gland as well as extra-pineal tissues in humans and other mammals and is present in many plants. It was first isolated by Lerner in 1958 from bovine pineal glands and reported to have potency as an amphibian skin-lightening factor (Garcia-Patterson, 1996). No information was found in the available literature identifying melatonin in environmental media.

Regulatory Status: No standards or guidelines have been set by NIOSH or OSHA for occupational exposure to or workplace maximum allowable levels of melatonin. The American Conference of Governmental Industrial Hygienists (ACGIH) has not recommended a Threshold Limit Value (TLV) or Biological Exposure Index (BEI) for this compound.

Melatonin is marketed as a widely available, inexpensive nutrition and health aid; and as such falls under the Food and Drug Administration's (FDA's) Dietary Supplement Health and Education Act (DSHEA) category of "other dietary substance". Since it was available on the U.S. market prior to enactment with an assumption of generally recognized as safe (GRAS), FDA's position with regard to its status is uncertain because of the lack of certain animal toxicology data in pilot trials (Anon., 1995c). Therefore, in spite of any claims of medicinal effects, such as antioxidant properties, put out by the health food industry, FDA has the burden of proof in terms of possible toxicity or health hazard before any action can be taken to regulate the substance or restrict its availability in the marketplace.

As a veterinary supplement for use in a variety of fur-bearing and farm animals, melatonin falls under the jurisdiction of FDA's Center for Veterinary Medicine. It is a substance identified in New Animal Drug Application (NADA) # 140-846 with restrictions placed on its use under the Federal Food, Drug and Cosmetic Act (FFDCA), 21 CFR, New Animal Drug (NAD), part 500, subparts C/D: regulatory limits for added poisonous or deleterious substances reserved/naturally occurring poisonous or deleterious substances reserved. Under this regulation, the maximum dosage of melatonin allowed in silicon plastic implants for kits and mink is 2.7 mg of melatonin, with one implant allowed per animal; and use in food-producing animals is proscribed (CVM, 1996).

In addition, melatonin is classified by FDA as an "orphan" drug which is prescribed for the treatment of circadian rhythm-related sleep disorders in blind patients (Olin, 1996). Based on the exclusivity of the market, it can be made available on a pre-approval basis as a drug intended for a small, specific population.

Actions taken by other governments to restrict the use of melatonin include the following:

- Melatonin is not authorized for sale in France because of lack of clinical trials and toxicity data (Anon., 1995d).
- Belgium has also restricted sales of melatonin (Anon., 1996c).
- The United Kingdom through the authority of its Medicines Control Agency has ended the unrestricted sale of melatonin (Anon., 1995e).

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: Although melatonin was first isolated and identified as the principal hormone of the pineal gland in 1958, the full dimensions of the physiologic role of the pineal gland in humans and the physiologic and pharmacological effects of melatonin continue to be investigated (Singer *et al.*, 1995). Dubocovich (1988) reported that melatonin binding sites have been found in most areas of the human brain, but they appear to be most abundant in the hypothalamus. There are many reports in the literature relating sleep disturbances to lower levels of nightly melatonin excretion, a common occurrence in old age which has also reportedly been a finding in patients with some types of cancer. While there are many claims of beneficial effects associated with melatonin, some unsubstantiated or clearly exaggerated, little information can be found in the literature about undesirable or toxic effects of this chemical or potential adverse interactions with other substances. However, the following precautions are included with one or two brands of melatonin: keep out of reach of children and consult a physician before using if pregnant or nursing or if a preexisting condition exists, including diabetes, depression, leukemia, epilepsy or autoimmune disease (Anon. 1995f; PDR, 1996).

Nordlund & Lerner (1977) administered melatonin orally at a high dose of 1 g per day (250 mg, 4 x day) to 5 humans with hyperpigmented skin for a prolonged period (25-30 days) to study effects on skin color and release of pituitary hormones. They reported that, while melatonin depressed the level of luteinizing hormone (LH) in serum and may have inhibited to some extent the release of pituitary growth hormone in response to stress or L-dopa stimulation, aside from increased drowsiness, there was no evidence of any toxic effect on eyes, liver, kidneys or bone marrow. Based on clinical trials in which high doses of melatonin (200 mg: 50 mg 4 x day) were administered orally to patients with metastatic cutaneous malignant melanoma, Robinson and coworkers (1995) reported that, while melatonin lacked significant therapeutic benefits, there were also only minimal side effects, including no significant effects on sleep patterns, mood, or biorhythms in their studies. The authors observed that melatonin showed little toxicity even at very high daily oral doses.

No epidemiological studies or case reports investigating the association of exposure to melatonin and cancer risk in humans were identified in the available literature. However, researchers have noted altered levels of melatonin in patients with some cancers. For example, Tamarkin and co-workers (1982) reported that the nocturnal plasma melatonin peak was depressed in patients with estrogen-receptor positive breast cancer. Numerous

reports were identified in the available literature which suggest an association between melatonin administration and a suppression of tumorigenesis (anticarcinogenesis).

Anticarcinogenicity

Altered circulating melatonin levels have been noted in patients with cancer, but existence of a causal relationship or etiological significance continues to be investigated. For example, Tamarkin *et al.* (1982) reported investigating plasma melatonin concentrations in 20 women with stage I or II breast cancer. In 10 patients with estrogen receptor (ER) positive tumors, nocturnal plasma melatonin increase was much lower than in 8 controls. Lowest peak plasma melatonin concentrations corresponded to occurrence of tumors with highest ER concentrations. The authors concluded that low nocturnal melatonin concentrations may indicate the presence of hormonally dependent breast cancer and postulated that absence of a nocturnal rise/peak in melatonin plasma concentration could serve as a biochemical marker for increased risk of developing ER positive breast cancer. Furthermore, melatonin has been attributed with the ability to inhibit tumor growth *in vivo* and *in vitro*, and the pineal gland which produces it has been referred to as the oncostatic gland. Recent studies have shown that melatonin can profoundly influence tumor progression induced by chemical carcinogens and modulate enzymes involved in xenobiotic metabolism and detoxification (Tan *et al.*, 1993).

Melatonin, both alone and in combination with other agents, has been studied for anti-carcinogenic or carcinostatic activity against a variety of human cancers. A number of clinical trials have been undertaken to assess therapeutic melatonin administration to patients with advanced or metastatic cancers where standard therapies had been tried and failed. Some results medical researchers have reported from clinical studies are briefly summarized as follows.

- A group of 19 patients with advanced solid tumors received melatonin at a dose of 20 mg/day im for 2 months followed by a lower maintenance dose in patients experiencing remission, stabilization or performance status (PS) improvement. Evident improvement was reported in 6 out of 10 patients with higher PS, whereas 7 of 9 patients with very poor PS died during the first 2 months of therapy (Lissoni *et al.*, 1987).
- A group of 35 patients with advanced digestive tract tumors and a high rate of metastases and 41 of a randomized trial group of 80 patients with locally advanced or metastatic solid tumors other than renal cell cancer or melanoma received oral melatonin (50 mg/day for 2 chemotherapy cycles; 40 mg/day) concomitantly with low sc doses of interleukin-2 (IL-2). The authors reported that in the first trial melatonin was well tolerated and of low toxicity and produced an overall beneficial response in 8/35 (23%) and in the second trial combinatorial treatment with melatonin produced a demonstrated increase in the efficacy of IL-2 therapy (Lissoni *et al.*, 1993; Lissoni *et al.*, 1994a,b).

Very high dose and lower dose (700 mg and 200 mg/day orally) melatonin was administered to groups of patients with metastatic malignant melanoma, but the authors reported that little therapeutic benefit was derived from the use of melatonin (Robinson *et al*, 1995).

Another area of current investigations relates to findings that *in vivo* oncostatic action of melatonin could be exerted, in part, through modulation of the levels of gap junctional intercellular communication linked to the regulation of cell proliferation and differentiation (Ubeda *et al.*, 1995a). Furthermore, there is recent evidence that extremely low frequency (ELF) magnetic fields (MF) may act as cancer promoters and co-promoters. *In vivo* exposures to ELF-MF in several different organisms have been shown to alter melatonin secretion by the pineal gland; and it has been reported that 60 Hz MF blocked the melatonin-induced inhibition of cell proliferation *in vitro* in human breast cancer cells (Ubeda *et al.*, 1995b).

Animal Data:

Acute

The acute toxicity of melatonin in rats and mice was determined by Sugden (1983). He reported that administration of large doses by several routes (oral, sc, ip, iv) caused similar behavioral effects in both species. A high dose (400 mg/kg) typically produced vasodilation, piloerection, ptosis, muscle relaxation, reduced motor activity and ataxia; while higher doses led to impairment of righting, placing and flexor reflexes, marked reduction in body temperature, and respiratory distress preceding death. Calculated LD₅₀ values for male Sprague-Dawley rats and MFI mice from Sugden (1983) are presented in Table 2. Numerous TD_{LO} values for melatonin in various animals by various routes of administration were identified in RTECS, mainly in conjunction with efficacy studies (NLM, 1996b). Some values also cited in Sax and Lewis (1989) are included in Table 2.

Table 2. Acute toxicity data for melatonin

Route	Species	Toxicity Value	Reference
oral	rat (S-D)	LD ₅₀ = >3200 mg/kg	Sugden (1983)
	rat (ID pre)	TD _{LO} = 27 mg/kg	Sax & Lewis, 1989/NLM, 1996
	mouse (MFI)	LD ₅₀ = 1250 mg/kg	Sugden (1983)
subcutaneous (sc)	rat (S-D)	LD ₅₀ = >>1600 mg/kg	Sugden (1983)
	mouse (MFI)	LD ₅₀ = >>1600 mg/kg	Sugden (1983)
	mouse (20W-1)	TD _{LO} = 4200 mg/kg	Sax & Lewis, 1989/NLM, 1996
	hamster (60D male)	TD _{LO} = 12 mg/kg	Sax & Lewis, 1989/NLM, 1996

intraperitoneal (ip)	rat (S-D) mouse (MFI)	LD ₅₀ = 1131 mg/kg LD ₅₀ = 1168 mg/kg	Sugden (1983) Sugden (1983)
intravenous (iv)	rat (S-D) mouse (MFI) mouse	LD ₅₀ = 356 mg/kg LD ₅₀ = 472 mg/kg LD ₅₀ = 180 mg/kg	Sugden (1983) Sugden (1983) Sax & Lewis, 1989/NLM, 1996

Subchronic

Two animal studies, in which adults received long-term administration of melatonin, are summarized briefly as follows. Enhanced longevity was a reported result in both; there were no observations of toxic or carcinogenic effects related to melatonin administration.

- Pierpaoli and Regelson (1994) reported the effects in BALB/c female mice of melatonin administration, 10 µg (dissolved to 0.01% in ethanol)/ml of tap water during a fixed cycle of darkness starting at 15 months. Their findings included average survival, median survival, and absolute upper limit of survival in melatonin-treated animals (843 days, 28.1 months, 29.4 months) compared with controls (715 days, 23.8 months, 27.2 months). They reported an equal variance *t* test for unpaired normal samples treated vs. controls of $P < 0.001$ and no significant weight differences between control and melatonin-treated mice throughout the study. They also cited prior studies in which New Zealand Black (NZB) female mice were given melatonin in drinking water day and night starting at 5 months and C57BL/6 male mice were given melatonin in drinking water during a dark cycle starting at 19 months of age (*i.e.* at the expected onset of age-related deaths). Both studies showed life prolongation of dark-cycle melatonin-treated animals when compared with controls.
- Oaknin-Bendahan and coworkers (1995) studied the effects of long-term (16 month) oral administration of melatonin to adult CD rats for evidence of increased survival and modulation of age-related reduction of testosterone levels and brain melatonin binding sites. CD rats reportedly have a maximum life span of 30-35 months, with 20% mortality at 20-25 months. A treatment group of 15 rats (11-13 months old) receiving 4 mg (dissolved in 100 µg dimethylsulfoxide) /L *ad lib* in drinking water and a vehicle control group of 16 rats were observed for up to age 29 months, when surviving animals were sacrificed. The authors reported that melatonin supplementation markedly increased the number of rats which survived to the age of 27-29 months (87%) while survival of the control group was in agreement with the life expectancy of the species (43%).

Chronic/Carcinogenicity

No 2-year carcinogenicity studies of melatonin in animals were identified in the available literature. However, Malakhova and Raushenbakh (1986) reported a slight increase (26% compared with 18% in controls) in the frequency of transplacental neoplasms in the offspring of C57BL/6 mice injected subcutaneously with melatonin.

Anticarcinogenicity

Melatonin treatment has been reported to decrease the growth of mammary glands in pubertal and pregnant mice. Based on *in vitro* organ culture studies, Recio and co-workers (1994) reported that melatonin inhibited murine mammary gland growth at μM concentrations and increased it at pM concentrations (which may correspond to an association with physiologic pubertal development). They summarized the complexities of melatonin's modulatory role in mammary gland growth, based on the published findings of a number of researchers. A possible anticarcinogenic role for melatonin either alone or in combination with another substance has been investigated in the following studies.

- Subramanian and Kothari (1991) studied spontaneous mammary tumor incidence in C3H/Jax mice, an animal model for human breast cancer, following prolonged oral melatonin administration. A group of 39 mice received melatonin (dissolved in ethanol) in drinking water around the clock (25 $\mu\text{g}/\text{mouse}/\text{day}$ from day 21 to day 44; 50 $\mu\text{g}/\text{mouse}/\text{day}$ from day 45 to sacrifice at 1 yr). They reported that melatonin modulated the degree of development of mammary epithelium and significantly reduced spontaneous mammary tumor incidence; 62.5% of control animals developed tumors vs. 23.1% in the melatonin treated group ($P < 0.02$).
- Tamarkin and coworkers (1981) investigated the effect of melatonin administration on the incidence of 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced mammary adenocarcinoma in Sprague-Dawley rats. They reported that, when a control group and a treatment group of 30 50-day old rats given a 15 mg dose of DMBA by intragastric intubation were put on a regimen of daily ip injections of 500 μg melatonin for the next consecutive 90 days, delayed onset and reduced incidence of tumors occurred. The animals were observed for 50 days after discontinuation of melatonin (140 days after dosing with DMBA), at which point 79% of the control animals, but only 20% of the melatonin treated animals had developed breast tumors.
- Blask and coworkers (1991) investigated the effect of melatonin on estrogen-responsive rat mammary carcinogenesis caused by the direct acting DNA-alkylating agent, N-nitroso-N-methylurea, a mammary tumorigen in which the successive stages of initiation and promotion are well delineated. When female Sprague-Dawley rats received daily sc injections of melatonin (500 μg) only during the initiation phase of NMU mammary tumorigenesis (melatonin from age 37 days to 60 days and 2 doses of NMU administered on day 50 and day 60), the hormone was ineffective in altering tumor incidence or number over a 20-week observation period. When melatonin administration was delayed for 4 weeks after NMU injection and then continued throughout the remainder of the promotion phase, only tumor number was significantly lower than controls. However, when melatonin was administered during the entire promotion phase, both the incidence and numbers of tumors were significantly lower than controls. The authors concluded that melatonin inhibits of NMU-induced rat mammary tumorigenesis by acting the promotion rather than the initiation phase and that melatonin appears to have antiestrogenic properties.

Short-Term Tests

Mutagenicity

There is limited information in the available literature on the genotoxicity of melatonin.

The following test results indicate that melatonin exhibits a lack of mutagenicity or very weak mutagenicity under specific conditions.

- Melatonin, at doses of 5, 50, 500 and 5000 $\mu\text{g}/\text{plate}$, was reported negative in an Ames assay in *Salmonella typhimurium* strains TA97, TA98 and TA100 both with and without metabolic activation with rat liver S9 (Neville *et al.*, 1989).

- Melatonin was reported negative at doses up to 500 µg per plate in an Ames assay in *Salmonella typhimurium* strain TA100 without metabolic activation. The test was repeated after treatment with nitrite under acidic conditions to evaluate whether melatonin showed potential to be nitrosated to a mutagen; after nitrite treatment melatonin was reported to show low mutagenic activity at doses of 100, 200 and 500 µg per plate in strain TA100 without metabolic activation (Hashizume *et al.*, 1991).

Antimutagenicity

Melatonin has been described as having antimutagenic and antioxidant properties. Tan and coworkers (1993) reported testing for melatonin's ability to protect against DNA-adduct formation by the rat liver carcinogen, safrole. They coadministered safrole with a lower and higher dose of melatonin to 4-month old Sprague-Dawley rats as follows: 4 groups of 6 animals each received vehicle only (vegetable oil ip), safrole (300 mg/kg ip) or safrole with melatonin (0.2 mg/kg or 0.4 mg/kg sc in three equal doses over a 20 hour period). The animals were sacrificed at 24 hours and liver tissue examined for DNA-adducts. No adducts were found in livers of the untreated (control) group. While DNA-adducts were abundant in liver tissue of the safrole treated rats, melatonin at both dose levels significantly reduced safrole-induced DNA-adduct formation, with a greater effect observed in the higher dose group. The authors noted a dose-response relationship between melatonin levels and the ability of safrole to induce DNA-adducts, reporting an association of lowest DNA-adduct formation corresponding to highest circulating levels of melatonin.

According to Reiter (1993) melatonin is an antioxidant which affords protection to macromolecules, especially DNA within the nuclei of cells, against oxidative damage. He reported that melatonin acts as a more powerful free radical scavenger than vitamin E or glutathione, especially effective as a scavenger of hydroxyl radicals, and stimulates glutathione peroxidase activity, which is involved in the metabolism of hydroxyl radical precursor, hydrogen peroxide, to water. Additional antioxidant properties attributed to melatonin summarized as follows: highly potent antioxidant against lipid peroxidation and DNA damage due to the radiolysis of water or chemical carcinogen administration (Reiter *et al.*, 1993, 1995).

Antiproliferation

Hill and coworkers (1992) reported that physiological concentrations of melatonin exerted an antiproliferative effect when tested with the MCF-7 breast cancer cell line and that striking morphological changes accompanied the growth inhibition. They also reported

the results of an investigation of the effects of melatonin on the proliferation of 10 human breast cancer cell lines, including MCF-7 and two other estrogen-responsive cell lines. Melatonin inhibited to varying degrees the proliferation of all three estrogen-responsive cell lines but failed to inhibit any of seven estrogen-unresponsive breast tumor cell lines. The melatonin effect on the MCF-7 cell line was mitigated in the presence of tamoxifen, and there was a lack of evidence of any synergistic or additive action of melatonin on tamoxifen's inhibition of MCF-7 cell growth. The authors postulated that the antiproliferative effects of melatonin are mediated through the estrogen-response pathway.

Metabolism: Williams (1959) did not address the metabolism of melatonin itself but reported that parent compound, indole, is metabolized in animals by an oxidation pathway to 3-hydroxy indole (indoxyl) which is excreted as conjugates of glucuronic and sulfuric acids. Indoxyl occurs normally in human urine as the sulfate, with daily output of 5-20 mg of indican (potassium indolyl-3-sulfate). Webb and Puig-Domingo (1995) reported that melatonin is metabolized in the liver to 6-hydroxymelatonin, which is conjugated to either glucuronide (20-30%) or sulfate (60-70%). The main metabolite excreted is urinary 6-hydroxymelatonin sulfate (6-sulfatoxymelatonin). Citing studies by a number of researchers they stated that an excellent correlation has been observed between pineal and circulating melatonin, urinary 6-hydroxymelatonin sulfate, and even salivary melatonin. Several recent studies which identified 6-hydroxymelatonin sulfate as the principal metabolite of melatonin described measurement of urinary levels of this metabolite as a method of studying pineal melatonin synthesis (Garfinkel *et al.*, 1995; Reiter, 1996). Stieglitz and coworkers (1995) reported that, based on studies in the Djungarian hamster, the pineal gland is the major source of urinary 6-hydroxymelatonin sulfate and that its excretion varied according to a marked photoperiodic rhythm. Reiter (1996) reported observing a low daytime/high nighttime excretion of the sulfate metabolite in healthy humans, with 75 % of endogenously secreted melatonin accounted for as urinary 6-hydroxymelatonin sulfate. The plasma half-life of exogenous melatonin in rats is reported to be 27 minutes (Mauviard *et al.*, 1995). Praast and coworkers (1995) reported that *in vitro* biotransformation in Fisher rats of melatonin to 6-hydroxymelatonin and conjugation to its sulfate is strongly inducible by phenobarbital and to a lesser degree by 7,12-dimethylbenz[a]anthracene and other polycyclic aromatic hydrocarbons with dramatic depletion of circulating melatonin. The authors suggest that this accelerated metabolic depression of plasma melatonin indicated by measuring levels in the liver homogenates of treated mature female animals may be associated with a decrease in melatonin's protection against cancer. Circulating nocturnal melatonin has been found to be lower in women with

breast cancer exhibiting positive estrogen receptors (Garcia-Patterson, 1996). The following pathway, adapted from Shellard and coworkers (1989), depicts the biotransformation of melatonin to its principal metabolite, 6-hydroxymelatonin: (See hard copy in Central Files for illustration)

Other Biological Effects: There is an extensive body of literature addressing melatonin's possible involvement with endocrine systems and impact on secretions of numerous other hormones as well as its relationship to reproductive and immune system functions. Moreover, melatonin has also been reported to act as a radioprotector against the DNA-damaging effects of ionizing radiation (Reiter *et al.*, 1995). The possibility that melatonin may influence human reproductive function is tentatively explored along with other aspects of melatonin's neurohormonal interactions in Pierpaoli and Maestroni (1987), Reiter (1991), and Garcia-Patterson *et al.*, (1996), several reviews with extensive lists of references.

Structure/Activity Relationships: Two compounds structurally similar to melatonin, 6-hydroxymelatonin and 5-methoxyindoleacetic acid (5-MIAA), were screened for relevant information associating these related chemicals with a mutagenic or carcinogenic effect. A summary of mutagenicity and carcinogenicity information found on melatonin and these two structurally related chemicals is presented in Table 3.

Malakhova and Raushenbakh (1986) reported a more than twofold increase in the frequency (52%) of development of transplacental neoplasms in the offspring of C57BL/6 mice treated with 5-MIAA during pregnancy compared with controls (18%). After administration of 5-MIAA (2.5 mg in 0.25 ml water 3 x week over a lengthy period for a total dose of up to 100 mg per mouse) injected subcutaneously into adult female mice during the prenatal period, 26 of 32 offspring developed neoplasms (mainly hemoblastoses, especially lymphosarcomas). The tumors were larger and appeared sooner (at 17-21 months vs 21-22 months for comparable spontaneous neoplasms in controls). 5-MIAA showed much stronger transplacental carcinogenic potential than melatonin.

Pierrefiche and coworkers (1993) have attributed the antioxidant activity of melatonin, both *in vivo* and *in vitro*, to the indole structure of the molecule; moreover, they reported that the principle hepatic metabolite, 6-hydroxymelatonin, shows greater antioxidant activity than melatonin. Other pineal indoles structurally related to melatonin have been reported to have antiproliferative and antitumorigenic effects on cultured tumor cell lines.

Sze and coworkers (1993) examined the proliferative effect of each of seven related compounds on a variety of tumor cell lines, including melanoma (B16), sarcoma (S180), macrophage-like cell line (PU5), fibroblasts (3T3), and choriocarcinoma (JAR). They reported the inhibitory potency of these indoles in descending order as follows: methoxytryptamine > melatonin, methoxytryptophol, hydroxytryptophol, and methoxyindoleacetic acid > serotonin and hydroxyindoleacetic acid.

Table 3. Summary of Information on Melatonin and Two Structurally Related Compounds

Chemical name [CAS RN]	Carcinogenicity data	Mutagenicity data
Melatonin [73-31-4]	no data found (NDF) supporting association with a carcinogenic effect in humans slight increase in frequency of transplacental neoplasms in offspring of C57BL/6 mice injected sc during pregnancy (Malakhova & Raushenbakh, 1986) possible anticarcinogenic role demonstrated in humans and animals based on studies of mitigating effects toward tumorigenicity (see text for details)	negative with or without S9 at 5, 50, 500 and 5000 µg/plate in <i>S. typhimurium</i> strains TA97, 98 and 100 (Neville <i>et al.</i> , 1989) mutagen precursor, weakly positive after nitrite treatment in <i>S. typhimurium</i> strain TA100 without S9 (Hashizume <i>et al.</i> , 1991) antimutagenic based on ability to reduce safrole-induced DNA-adduct formation in the livers of Sprague-Dawley rats with a dose-response relationship (Tan <i>et al.</i> , 1993)
6-hydroxymelatonin [2208-41-5]	NDF	negative with or without S9 at 0.1, 1, 10 and 100 µg/plate in <i>S. typhimurium</i> strains TA97, 98 and 100 (Neville <i>et al.</i> , 1989)
5-methoxyindole-3-acetic acid [3471-31-6]	significant increase in frequency of transplacental neoplasms in offspring of C57BL/6 mice injected sc during pregnancy (Malakhova & Raushenbakh, 1986)	NDF

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