DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service
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Memorandum

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Subject: Review of the published literature pertaining to the safety of melatonin for use in conventional foods

I. Introduction

Melatonin is a neurohormone with known physiologic effects (Pandi-Perumal *et al.*, 2006). The use of melatonin in conventional foods has prompted the agency to examine the published literature to determine whether there is available evidence to support the safe use of melatonin in foods.

This memorandum summarizes generally available information from the published literature and other informational sources on melatonin. The memorandum discusses melatonin's role in circadian rhythms and other physiological functions, its chemistry, biosynthesis, absorption, distribution, metabolism and excretion (ADME), and its mode of action; it highlights concerns regarding the potential toxicity and adverse health effects associated with food uses of melatonin.

II. Literature Searches

The following primary literature databases were searched to retrieve scientific data published on melatonin: PubMed, ScienceDirect, Embase, ChemIDplus Advanced, Natural Standard, Natural Medicines Comprehensive Database, Medline, MedlinePlus, and Medical Matrix. The search terms used were melatonin, melatonin in humans, dietary intake of melatonin, daytime ingestion of melatonin, melatonin and sleep disorders, melatonin toxicity, adverse effects of melatonin, pharmacokinetics and metabolism of melatonin, immunomodulation by melatonin, and melatonin and aging. The entirety of the databases from all the years available up to April 2011 was searched revealing more than 16,000 publications. In order to focus on literature most relevant to the use of melatonin as an ingredient in foods, the search was refined to identify studies related to the oral consumption of melatonin with greater emphasis on daytime consumption[1] and potential adverse effects in humans. The literature selected for this review describes some of the effects of melatonin on the endocrine, reproductive, cardiovascular, and central nervous systems, as well as effects on developmental parameters, ocular tissues, and glucose metabolism.

III. Melatonin's Role in Circadian Rhythm and Other Physiological Functions

Melatonin (variously referred to as the hormone of darkness, the photoperiodic molecule, or the pineal hormone) is a neurohormone produced primarily by the pineal gland in the brain. It was isolated in 1958 by Aaron B. Lerner and his colleagues, who thought that melatonin would have dermatological applications as extracts of bovine pineal glands were known to lighten amphibian skin (Lerner *et al.*, 1958).

The most prominent feature of melatonin production is that it exhibits a tightly regulated diurnal pattern and is secreted only during the night (hours of darkness) in all species. It is thus considered to synchronize the body's chronological pacemaker or master biological clock (Pandi-Perumal *et al.*, 2006). In mammals, including humans, circadian rhythms control the timing of many biological processes. The endogenous master circadian "clock" orchestrates characteristic rhythms in neuroendocrine, physiological, and behavioral parameters with a periodicity of approximately 24 hours, and adjusted by the light-dark cycle of the day (Reppert and Weaver, 2002). These circadian rhythms can be altered by changes in environmental temperature, behavioral activity (sleep/wake cycle, fasting/feeding activity), light, or posture (standing or supine position), or by the daytime administration of exogenous melatonin.

Light influences the suprachiasmatic nuclei (SCN) output and thereby suppresses melatonin production (Lewy *et al.*, 1980). Light is captured by the eyes using specialized intrinsic photosensitive retinal ganglion cells (RGCs), and transmitted via a dedicated retino-hypothalamic tract to the SCN of the anterior hypothalamus of the brain (Sadun *et al.*, 1984). In the absence of light, when "dark signals" are received, the pineal gland secretes melatonin. Because the pineal gland is located outside of the blood-brain barrier, melatonin is secreted into the blood vascular

1 While melatonin is typically used to aid in alleviating sleep problems and is consumed right before bed time, foods containing melatonin may be consumed at times when sleep is an inappropriate effect.

system and cerebrospinal fluid and distributed to all organs including the central nervous system. As such, melatonin from the pineal gland acts as an endocrine hormone.

The nocturnal surge of melatonin provides a "time of day" (i.e., onset of night) signal to all the cells, tissues, and organs of the body. The duration of melatonin secretion depends on the photoperiod. A longer night results in a longer duration of melatonin secretion and vice versa. Seasonal variations in the length of the day provide the seasonal clock (Guardiola-Lemaitre, 1997; Claustrat, *et al.*, 2005). Additionally, changes in the production of pineal melatonin with age might also provide the "age clock" (Touitou, 2001).

In animals and humans, melatonin is involved in diverse physiological functions including signaling time of the day or year (i.e., timekeeping function). In its timekeeping function, melatonin is centrally involved in sleep regulation, as well as in other cyclical bodily activities (e.g., autonomic cardiovascular regulation and reproductive functions). Melatonin also acts as an antioxidant and scavenges free radicals. Once it is oxidized, melatonin forms stable products that cannot be reduced back to its former state (Tan *et al.*, 2000). Melatonin also has an anti-apoptotic signaling function (Pandi-Perumal *et al.*, 2006), and immune modulating properties (Carrillo-Vico *et al.*, 2005).

Melatonin plays a key role in reproductive physiology. Available evidence indicates that melatonin regulates the reproductive function in seasonal mammals by its inhibitory action at various levels of the hypothalamic-pituitary-gonadal axis. The pulsatile secretion of gonadotropin-releasing hormone (GnRH), from neurons in the hypothalamus, controls luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion that, in turn, regulates the functional activity of the gonads (Kennaway and Rowe, 1995; Reiter, 1991). In animals and humans, melatonin has been shown to influence the secretion of several hormones, namely LH, FSH, prolactin, corticosteroids, and thyroid hormones (Tamura *et al.*, 2008).

In humans, melatonin appears to be involved in the endocrine modulation of sexual maturation. Children with precocious puberty have been found to have lower nocturnal serum concentrations of melatonin than age-matched prepubertal children (Waldhauser *et al.*, 1991), whereas children with delayed puberty present higher nocturnal melatonin concentrations than age-matched normal children (Cohen *et al.*, 1982).

IV. Chemical Structure and Biosynthesis Of Melatonin

The chemical names for melatonin are N-acetyl-5-methoxy tryptamine, 5-methoxy-N-acetyltryptamine and N-[2-(5-methoxy-1H-indol-3-yl) ethyl] acetamide. The chemical formula for melatonin is $C_{13}H_{16}N_2O_2$ and the formula weight is 232. The CAS number is 73-31-4.

Melatonin is an indolamine that is synthesized endogenously in the pineal gland. The synthesis of melatonin starts with tryptophan, taken up from the blood, and proceeds in four enzymatic steps. The major pathway of melatonin synthesis consists of hydroxylation of tryptophan, by tryptophan hydroxylase, to 5-hydroxytryptophan, which then undergoes a decarboxylation by *l*-aromatic amino acid decarboxylase to form serotonin. Serotonin is acetylated by arylalkylamine *N*-acetyltransferase to *N*-acetylserotonin, which in most cases represents the rate-limiting step in melatonin synthesis (low during daytime). *N*-Acetylserotonin is finally converted to melatonin by hydroxyindole *O*-methyltransferase (Pandi-Perumal *et al.*, 2006, Axelrod, 1974).

Melatonin synthesis also takes place in other areas outside of the brain, including the eye, heart, pancreas, skin, bone marrow, lymphocytes and gonads; melatonin produced in these areas influences physiological functions by acting as an autocrine or paracrine hormone or free radical scavenger (Tan *et al.*, 2007). Melatonin is also produced by enteroendocrine cells in the gastrointestinal (GI) mucosa and secreted into the circulation in a post-prandial response, which is influenced by nutrients in the diet such as tryptophan. This results in a short-term increase in the serum levels of melatonin. Melatonin released into the lumen participates in enterohepatic cycling. It is reported that the GI tract is not only a source of melatonin but may also serve as a sink (Huether, 1993; Pandi-Perumal *et al.*, 2006).

V. Biochemical Aspects Of Melatonin (Pharmacokinetics and ADME)

Under normal physiological conditions in humans, circulating levels of melatonin vary from 30–200 picograms/milliliter (pg/ml) during the night and drop to 1–10 pg/ml during day time (Waldhauser and Dietzel, 1985; Guardiola-Lemaitre, 1997).

Orally administered melatonin at doses below 0.3 milligram (mg), given in the daytime (11:45 a.m.) to male volunteers (n=20, ages 18–24), resulted in serum concentrations that are within the normal night-time range (Dollins *et al.*, 1994). In a randomized, crossover study, melatonin administered orally at daytime (between 7 and 9 a.m.) at doses of 2 and 4 mg, with a 1-month washout interval to volunteers (n=12, 6 men and 6 women, aged 37±8 years), resulted in serum concentrations at least 10–30 times higher than the nighttime levels, followed by a decline to baseline in 4–8 hours (hr) (DeMuro *et al.*, 2000). Following oral administration in humans, peak

plasma levels are reached in 22 minutes (min) to 90 min (Aldhous *et al.*, 1985; Vakkuri *et al.*, 1985, Fourtillan *et al.*, 2000, DeMuro *et al.*, 2000). and the mean half-life is around 30–60 min or less (Aldhous *et al.*, 1985; Vakkuri *et al.*, 1985; Di *et al.*, 1997; Fourtillan *et al.*, 2000; DeMuro *et al.*, 2000).

Waldhauser *et al.*, (1984) monitored the pharmacokinetics of melatonin given to five male volunteers at the high oral dose of 80 mg. Sixty (60) to 150 min after administration, peak serum melatonin levels ranged from 350 to 10,000 times those occurring physiologically at nighttime and these levels remained stable for approximately 1.5 hr. Three additional volunteers received three melatonin-containing capsules (80 mg each) at 60-min intervals. This regimen extended the duration of elevated serum melatonin levels to 4–6 hr. Melatonin excretion closely paralleled serum melatonin levels until 9 hr after the hormone's administration, after which urinary levels tended to be higher than those predicted from serum levels. The authors suggested that serum melatonin was elevated over a period of hours as a consequence of saturation of absorption kinetics in the intestine or melatonin distribution into a deeper compartment such as adipose tissue.

In humans, the bioavailability of melatonin after oral ingestion varies widely, ranging from 1% to 76%. The large variations in bioavailability are reported to be dependent on the dose, gender and variations in cytochrome P-450 (CYP450) gene expression in humans (Tan *et al.*, 2007). In one study by Fourtillan *et al.*, (2000), for example, the absolute bioavailability of melatonin in females was reported to be twice that of males (16.8% in females versus 8.6% in males).

Serum melatonin is promptly distributed to the tissues. Melatonin readily passes through tissues and cellular compartments, and penetrates the blood-brain barrier. It is also found in the fluids of the body, including saliva, urine, cerebrospinal fluid, semen, amniotic fluid, and milk. The distribution of melatonin in the body is not homogenous with levels in cells and tissues being orders of magnitude higher than those present in circulating blood (Reiter, 1991). For example, the gut is reported to contain more melatonin than the pineal gland (Bubenik, 2008).

Exogenous melatonin undergoes extensive hepatic first–pass metabolism (Lane and Moss, 1985). Melatonin is metabolized via two pathways, the hydroxylation pathway and the oxidation pathway.

Circulating melatonin is rapidly metabolized by CYP450 enzymes such as CYP1A1, CYP1A2, CYP2C19, and CYP1B1. In the liver, melatonin is predominantly hydroxylated to 6-hydroxymelatonin by the CYP1A2 isoform, with CYP2C19 playing a minor role. 6-Hydroxymelatonin is then conjugated with sulfate to form 6-sulfatoxymelatonin, and, to a lesser extent, with glucuronic acid to form 6-hydroxymelatonin glucuronide; the conjugates are excreted in the urine. CYP1B1 is involved in the degradation of melatonin in extrahepatic tissues such as the GI tract and cerebral cortex (Ma *et al.*, 2005).

In some tissues, especially in the brain, melatonin undergoes oxidative pyrrole-ring cleavage resulting in the formation of N-acetyl-2-formyl-5-methoxy-kynurenamine (AFMK). The leukocytes, lungs, skin and red blood cells are other sites that generate AFMK. AFMK is generated either enzymatically or by a chemical reaction pathway and is subsequently deformylated by either arylamine formamidase or hemoperoxidase to N-acetyl-5-methoxy-kynurenamine, which is excreted in the urine (Tan *et al.*, 2001, 2007).

The concentration of the major urinary metabolite, 6-sulfatoxymelatonin, closely reflects the plasma melatonin profile and is frequently used for evaluation of melatonin rhythm, especially in humans (Schernhammer *et al.*, 2009). Very small amounts of melatonin and free 6-hydroxymelatonin are excreted unchanged in the urine.

In animal studies, the dose normalized bioavailability from a 10 mg/kilogram (kg) oral dose of melatonin was 53.5% in rats and >100% in dogs and monkeys (Yeleswaram *et al.*, 1997). There are numerous pharmacokinetic studies in various animals because of the interest in the effects of melatonin on animal husbandry. For the purpose of this memorandum, these studies were not considered.

In some mouse strains, melatonin has been shown to be metabolized to 6-hydroxymelatonin glucuronide, rather than 6-sulfatoxymelatonin, as determined by metabolite profiling. In CBA, C57/BL6, and 129Sv mouse strains, it was found that 6-hydroxymelatonin glucuronide comprised 75, 65, and 88% of the total melatonin metabolites, respectively. The authors suggested that there was no interspecies difference between humans and mice with regard to CYP1A2-mediated metabolism of melatonin, but a significant difference in phase II conjugation, yielding 6-hydroxymelatonin glucuronide in the mouse and 6-sulfatoxymelatonin in humans (Ma *et al.*, 2008).

VI. Melatonin Receptors

Melatonin exerts its effects mainly through two G-protein coupled, membrane-bound melatonin receptors, MT1 and MT2, expressed in various regions of the central nervous system and peripheral organs. Melatonin receptors exhibit circadian variations in their expression and their responses. They mediate a plethora of intracellular effects depending on the tissue and its cellular milieu. These effects comprise changes in intracellular cyclic nucleotides (cAMP, cGMP) and calcium levels, activation of certain protein kinase C subtypes, intracellular localization of steroid hormone receptors and regulation of G-protein signaling proteins (Pandi-Perumal *et al.*, 2008).

Melatonin also binds to the retinoid-related nuclear hormone receptor family, retinoid Z receptor/retinoid orphan receptor (RZR/ROR) (Steinhilber *et al.*, 1995). The functional significance of these melatonin nuclear receptors is not clear.

Alterations in melatonin receptor expression, as well as changes in endogenous melatonin production, have been associated with circadian rhythm sleep disorders, Alzheimer's and Parkinson's diseases, glaucoma, depressive disorder, breast and prostate cancer, hepatoma and melanoma (Pandi-Perumal *et al.*, 2008) and asthma (Sutherland *et al.*, 2003).

VII. Overview of Studies Regarding the Safety Concerns of the Use of Melatonin in Food

Based on the survey of the scientific literature, a number studies raise questions about the safety for the use of melatonin in foods. A number of studies in the following areas are described below.

1. Reproductive physiology
2. Ocular toxicity
3. Chronobiotic effects
4. Cardiovascular function
5. Glucose homeostasis
6. Tumorigenesis
7. Immunology
8. Neurological complications from pharmacological use
9. Drug, herb and dietary supplement interactions

1. Reproductive physiology

In a study conducted by Okatani *et al.*, (2001), pregnant Sprague-Dawley rats were administered melatonin (2 or 20 microgram(μg)/ml) in drinking water from gestation day 14 until the day of delivery to evaluate the effects on prepubertal secretion of LH, FSH and prolactin in the offspring. The results showed a significant increase in plasma prolactin levels at postnatal day (PND) 15 in male and female offspring, a significant decrease at PND 25 in male offspring, and a significant decrease at PND 25 and 30 in female offspring. The levels of LH and FSH were unchanged. Changes in plasma prolactin levels may be important because other studies have linked low plasma prolactin levels during the pre-pubertal period to delayed maturation of female rats (Advis *et al.*, 1981) and may influence the reported testicular responsiveness to LH in male rats (Zipf *et al.*, 1978).

Singh *et al.*, (2011) conducted a study using two strains of rats administered melatonin (10 mg/kg body weight/day (bw/d) (actual intake)) in drinking water during pregnancy and up to postpartum day 21. In Wistar-Kyoto rats, greater than 50% of the pups died by the age of 3 weeks and 95% died by the age of 6 weeks. In spontaneously hypertensive rats (derived from Wistar-Kyoto rats), no pup mortality was observed. In comparing their results to those of a National Toxicology Program (NTP) study (NTP, 1998; Jahnke *et al.*, 1999) in which pup mortality was not seen in Sprague-Dawley rats administered melatonin by gavage at doses up to 200 mg/kg bw/d, the authors suggested that the observed pup mortality may be strain specific.

In randomized, double-blind placebo-controlled studies, Cagnacci *et al.*, (1995a, b) gave oral doses of melatonin (up to 3 mg) to normal cycling women (n= 16, ages 25–35 years). The results show an increase in the amplitude of LH pulses, mean LH levels, and enhanced the LH and FSH responses to GnRH stimuli in the follicular phase of the menstrual cycle but not in the luteal phase. The authors cite the work of Apter *et al.* (1993) in concluding that melatonin may play a role in inducing and timing the circadian rhythmicity of gonadotrophin secretion during the follicular menstrual phase and in pubertal, amenorrheic, and postpartum states.

Results from an *in vitro* study using human granulosa-luteal cells, by Woo *et al.*, (2001), indicate that the levels of GnRH and its receptor mRNA decreased with melatonin treatment. The authors stated that their results support the notion that melatonin plays a direct role in regulating ovarian function.

In pregnant women, the daily oral administration of 3 mg of melatonin led to increases in melatonin levels in the serum and the umbilical and maternal veins. Based on these results, the authors concluded that melatonin is transferred from the maternal to the fetal circulation easily (Okatani *et al.*, 1998).

Cieśla (1998) analyzed the adrenocorticotropic hormone (ACTH) and melatonin concentrations in the amniotic fluid (AF) of 20 pregnant women. The results showed a correlation between incidences of developmental abnormalities (e.g. anencephaly, miscarriages) with high AF melatonin levels and low ACTH concentrations. Cieśla hypothesized that disturbances in AF ACTH and melatonin (e.g., high secretion of maternal melatonin) could be associated with an increased risk of bearing a developmentally handicapped child.

Luboshitzky *et al.*, (2002) reported that in healthy men (n=8, ages 23–24 years), the daily oral administration of 3 mg melatonin at 1700 hr for 6 months resulted in a decrease in semen quality. The decrease in semen quality coincided with a decline in seminal fluid, plasma and serum 17-β estradiol (E2) levels and with an increase in the ratio of testosterone:E2 levels. The authors speculated that the observed effect may be mediated through the inhibition of aromatase at the testicular level. In an *in vitro* study, melatonin at concentrations of 150–450 pg/ml had an inhibitory effect on sperm motility (Irez *et al.*, 1992).

FDA's Conclusions:

Based on the animal studies above, exogenous melatonin decreases the plasma prolactin levels in neonatal rats and may delay sexual maturation. Additionally, melatonin also caused high pup mortality in Wistar-Kyoto rats, an effect that may be strain specific.

In women, exogenous melatonin alters the secretion of gonadotropins and may play a direct role in regulating ovarian function. Additionally, in men, the consumption of melatonin decreased semen quality. These observations suggest that exogenous melatonin may have adverse effects on

human reproduction.

2. Ocular toxicity

Retinal melatonin is synthesized in the photoreceptors in a cyclic rhythm with peak levels occurring during the dark period. The daily cyclic changes of retinal melatonin synthesis correlate with circadian events such as photoreceptor outer segment disc shedding and phagocytosis, photomechanical movements, visual sensitivity, and neurotransmitter release. The distinct and differential expression of melatonin receptors in retinal photoreceptors, dopaminergic amacrine neurons, and horizontal cells of the retina, corneal epithelium, stroma endothelium, and the sclera suggest distinct downstream cellular functions of melatonin in these tissues. Disruption of these rhythms (e.g., via exogenous melatonin and/or high intensity (2500 lux)) illumination may lead to the disruption of normal retinal cell function in animals and humans (Wiechmann and Summers, 2008).

Wiechmann and O'Steen (1992) conducted a study in albino Sprague-Dawley rats exposed to high intensity illumination (HII) (1600 lux) and given intraperitoneal injections of melatonin (100 μg) in the late afternoon (at 4 p.m). The results show approximately 30% greater photoreceptor damage in test animals compared to sham-injected controls as measured by the reduced thickness of the outer nuclear layer of the retina. The authors concluded that melatonin treatment increases the susceptibility of retinal photoreceptors to light-induced cell death.

Weichmann *et al.* (2008) undertook a study to determine the potential toxicity of dietary melatonin in retinal receptors under different lighting conditions. Fischer 344 (non-pigmented) and Long-Evans (pigmented) rats were given single daily doses of melatonin in the range of 5-200,000 ug/kg bw/d for 14 days early in the light or dark periods. An additional group of rats exposed to melatonin in the light period were also exposed to high intensity illumination (HII) for two hours. Following treatment, morphometric measurements of the photoreceptor cell layer in the retina were made. In Fischer rats, night time melatonin caused photoreceptor cell death as did day time administration after HII. In contrast, in Long-Evans rats, photoreceptor cell death was only observed in the males when night time melatonin was administered; photoreceptor cell death was not observed in females. The authors concluded that melatonin increases photoreceptor cell death in male and female non-pigmented rats under certain lighting conditions, and in pigmented male rats when treated at night.

Melatonin receptors in the mouse retina are reported to be involved in the regulation of photoreceptor physiology and retinal functions (Baba *et al.*, 2009).

In humans, it has been reported that there is a strong correlation between cone electroretinogram (ERG) recordings and the levels of salivary melatonin indicating that ERG amplitude is lowest when melatonin concentration is highest (Rufiange *et al.*, 2002). In a placebo-controlled, double-blind, crossover study by Gagné *et al.* (2009), 12 (6 males and 6 females) healthy participants were orally administered melatonin (15 mg) during the daytime. ERG measurements taken 30

min after melatonin ingestion show a decrease of about 8% of the cone maximal response. The authors concluded that melatonin appears to reduce input to the retinal cones.

FDA's Conclusions:

The studies above demonstrate that exogenous melatonin in combination with exposure to natural or artificial light may result in retinal photoreceptor damage in animals and humans. Based on this concern, FDA notes that specialized studies may be needed to further assess exogenous melatonin's potential to cause ocular toxicity in humans.

3. Chronobiotic effects

A practical definition of chronobiotic would be 'a substance that adjusts the timing of internal biological rhythms' or more specifically 'a substance that adjusts the timing of the central biological clock.' Melatonin would be classified as a chronobiotic since it has effects on mammalian circadian rhythms and one of its major physiological roles is the initiation and regulation of sleep (Arendt and Skene, 2005).

Many studies have confirmed that exogenous melatonin administration shifts the phase of the circadian clock, either delaying (morning administration) or advancing (evening administration) the phase. Melatonin alters the timing of bodily rhythms, including sleep, core body temperature, endogenous melatonin and cortisol rhythms (Reid *et al.*, 1996; Arendt and Skene, 2005; Cardinali *et al.*, 2006; Lewy *et al.*, 1992).

Certain conditions (e.g., shift work and jet lag, sleep disorders, blindness, aging) can cause alterations in the circadian rhythm. It has been widely reported in the literature that melatonin is taken to correct these alterations (Srinivasan *et al.*, 2008; Arendt and Skene, 2005; Dahlitz *et al.*, 1991; Nagtegaal *et al.*, 2000; Sack *et al.*, 2000; Lewy *et al.*, 1998; Zisapel, 2007).

A double-blind placebo-controlled study in twenty healthy male subjects (19–39 years) given doses of melatonin (10, 20, 40, 80 mg) at noon on five separate occasions increased feelings of sleepiness, fatigue, and confusion at the lowest dose compared to placebo (Dollins *et al.*, 1993).

FDA's Conclusions:

FDA notes that based on the above reports, the consumption of foods containing added melatonin could lead to drowsiness, fatigue, disorientation, confusion, and reduced alertness. This may lead to deleterious consequences for individuals, particularly those who are not aware of melatonin's effects, and individuals whose activities or jobs require elevated levels of alertness (e.g., machine operators, truck drivers).

4. Cardiovascular function

Cardiovascular function has a distinct daily rhythm with heart rate (HR), blood pressure (BP), and vascular tone decreasing at night. There have been epidemiological reports noting a peak in the occurrence of cardiovascular incidents, including ischemic strokes, myocardial infarction (MI), sudden cardiac death, and ventricular arrhythmias in the morning hours (Muller, 1999; Gupta and Shetty, 2005). Changes in behavior or sleep/wake cycle might worsen any pre-existing conditions if they occur at a phase when cardiac function is more susceptible to cardiac incidents. Disruption of the sleep/wake cycle is the hallmark of shift work and repeated jet-lag. Epidemiological studies indicate that higher incidence of coronary heart disease (CHD), diabetes and obesity occur among shift workers, especially night shift workers, due to the disruption of their circadian rhythm resulting from the variations in their sleep/wake cycle and fasting/feeding times (Rüger and Scheer, 2009).

An *in vivo* mouse study by Tailleux *et al.* (2002) examined the effect of high doses of melatonin on atherosclerosis. They reported that feeding hypercholesterolemic mice (transgenic mice with human apolipoprotein B) diets supplemented with 0.02% melatonin (200 mg/kg feed or approximately 30 mg/kg bw/d) for 16 weeks significantly increased the development of atherosclerotic lesions in the proximal aorta and enhanced the susceptibility of atherogenic proteins to oxidative stress during the fasting period. The authors expressed caution in the use of high dose melatonin supplementation in humans.

The effects of oral administration of melatonin (2 mg, in the morning) on heart rate variability (HRV), blood pressure and plasma catecholamine levels were examined in healthy men (melatonin group, n=13; placebo group, n=13; ages 24–35 years) who were kept awake during the study. The measurements were recorded both in supine and standing positions before and after the administration of melatonin. HRV is a measure of the interbeat intervals and of the activity of the autonomic nervous system. The results indicated that subjects in the melatonin group had increased interbeat intervals, decreased BP, and lowered plasma norepinephrine and dopamine levels compared to the placebo group, when assessed in the supine position 60 min after melatonin administration. However, in the standing position such differences were not found. The authors concluded that the daytime administration of melatonin increased the cardiac vagal tone in men who were awake in the supine position (Nishiyama *et al.*, 2001).

Fourteen (14) healthy men (23–29 years old) received either melatonin or placebo on study day one and the alternate treatment on the next day. The results indicated that the single administration of 1 mg melatonin during daytime (2:30-5:30 p.m.) significantly decreased systolic BP (115 versus 106 millimeters of mercury (mm Hg)), diastolic BP (72 versus 68 mm Hg), pulsatility index in the internal carotid artery (1.40 versus 1.16) and plasma norepinephrine levels within 90 min compared to the placebo. The authors concluded, based on their data, that melatonin may blunt the activity of the cardiovascular system (Arangino *et al.*, 1999). With the same treatment, similar results were also observed in 17 healthy women (ages 23–29 years) in the early follicular phase of the menstrual cycle (days 4–6) (Cagnacci *et al.*, 1998).

In a study with 5 healthy male subjects (age not reported), the influence of oral administration of melatonin (3 mg, between 10:20 and 11:35 a.m.) on autonomic function was investigated by measuring BP, heart rate (HR), and burst rate of muscle sympathetic nerve activity (MSNA). The results show that melatonin decreased both systolic (124.3 versus 110 mm Hg) and diastolic (65 versus 54 mm Hg) BP. Melatonin did not significantly change heart rate or MSNA. The authors concluded that melatonin showed a significant hypotensive effect and that the normal nocturnal rise in melatonin might, at least in part, account for the nocturnal decrease in blood pressure (Kitajima *et al.*, 2001).

Cook *et al.* (2011) studied the effect of melatonin (3 mg, daytime) on blood flow to various vascular beds in humans (5 males and 5 females, age 29 years). Melatonin decreased renal blood flow velocity and renal vascular conductance significantly compared to placebo. In contrast, melatonin increased forearm blood flow and forearm vascular conductance compared to placebo. However, melatonin ingestion did not alter cerebral blood flow, mean arterial BP, or HR. The authors stated that the different vascular effects observed with melatonin are attributed to the relative distribution of MT1 and MT2 receptors. The author commented on the conflicting effects of exogenous melatonin on BP that have been reported in other studies and the authors stated that this appears to be dose-related, as studies with 1 mg dose decreased blood pressure (Arangino *et al.*, 1999; Cagnacci *et al.*, 1998) while another study with 3 mg dose did not (Ray, 2003).

The effect of melatonin on lipoprotein metabolism was investigated in normolipidemic postmenopausal women (n=15, ages 48–55 years). The women received an oral daily dose of 6 mg melatonin between 21:00 and 22:00 hr for 2 weeks. Measurements were made assessing the total lipid profile (low density lipoprotein (LDL), high density lipoprotein (HDL), very low-density lipoprotein (VLDL), total cholesterol and triglycerides), and the activities of three enzymes involved in lipid metabolism (plasma lipoprotein lipase, hepatic triglyceride lipase and plasma lecithin cholesterol acyltransferase). The results revealed that melatonin increased the plasma level of triglycerides by 27%, VLDL-cholesterol by 37%, VLDL-triglycerides by 62%, VLDL-protein by 30%. The concentrations of cholesterol, triglycerides and protein in the LDL and HDL fractions were not significantly affected. Melatonin inhibited the activity of plasma lipoprotein lipase by 14%, but had no significant effect on the activities of hepatic triglyceride lipase and plasma lecithin cholesterol acyltransferase. The authors concluded, based on their findings, that melatonin consumption may affect lipid metabolism unfavorably in normolipidemic women (Wakatsuki *et al.*, 2001).

FDA's Conclusions:

The results of the above studies show that in both men and women, melatonin treatment may blunt the cardiovascular response. Although the results are inconsistent, some studies indicate that the daytime consumption of melatonin may decrease BP. The effects of melatonin on HR are also variable, with some studies showing a decrease and other studies showing no change. FDA notes that based on the above results, consumption of foods containing melatonin may pose a

health risk to individuals, particularly those with hypotension or other cardiovascular concerns such as hyperlipidemia. More research is needed to further elucidate the effects of exogenous melatonin on parameters that affect cardiovascular function, and on melatonin's potential interactions with medications used for treating cardiovascular or other conditions (see also section 9).

5. Glucose homeostasis

The studies discussed in this section show the interrelationship between exogenous melatonin and insulin and its functional implications.

In a study by Peschke *et al.* (2010), melatonin at a dose of 2.5 mg/kg bw/d, delivered in drinking water, (with the concentration adjusted weekly based on the nightly water consumption) was provided during the dark period (for 12 hr) for 9 weeks to normoglycemic Wistar rats and type 2 diabetic Goto-Kakizaki (GK) rats. Melatonin administration decreased the expression of pancreatic melatonin receptor-mRNA and increased the pineal insulin receptor expression in both strains of rats. The results also show that melatonin decreased plasma insulin levels significantly in the Wistar rats and nonsignificantly in the GK rats. The pancreatic expression of glucagon, GLUT2 (glucose transporter 2), and glucokinase was decreased in GK rats. The authors concluded that melatonin administration decreased plasma insulin levels and suggested that an insulin-melatonin antagonism exists.

In order to understand the observed age-related decline in melatonin and its possible modulatory effects on fat metabolism, Rasmussen *et al.* (1999) examined the effects of daily administration of melatonin in Sprague-Dawley rats over 48 weeks of treatment. The rats were given either a high dosage (4 μg/ml or 300-400 μg/kg bw) or low dosage (0.4 μg/ml or 30-40 μg/kg bw) of melatonin in their drinking water (92% consumed at night) from 10 months of age (middle-aged) until 22 months of age. The results indicated that melatonin treatment at both dose levels decreased visceral (retroperitoneal plus epididymal) fat mass, and plasma levels of insulin when compared to the control group. The plasma levels of leptin decreased at both doses compared to the control group.

Daytime oral administration of melatonin (1 mg) to postmenopausal women, including some who were on hormonal replacement therapy, reduced glucose tolerance and insulin sensitivity but did not affect the plasma levels of leptin (Cagnacci *et al.*, 2001; 2002).

FDA's Conclusions:

The studies discussed above show how alterations in the melatonin levels by consumption of exogenous melatonin could contribute to changes in glucose homeostasis and increase the risk of acquiring type 2 diabetes.

6. Tumorigenesis

Anisimov *et al.* (2001) studied the effect of melatonin on spontaneous tumor incidence and life span. The results showed that the long-term nightly administration of melatonin to female CBA mice, at a dose of 20 mg/liter (3–3.5 mg/kg bw/d) in drinking water, 5 days a week, starting from 6 months of age until their natural death, significantly increased both the survival time of the mice and the malignant tumor incidence (lung adenocarcinomas and lymphomas). Melatonin failed to influence physical strength or fatigue and slightly decreased locomotor activity and body temperature in these mice. This mouse strain was selected for the study because of unequivocal evidence of pineal melatonin production.

Sharman *et al.* (2011) analyzed the results of a series of experiments they conducted over a nine-year period in male B6C3F1 mice for the effect of dietary melatonin (40 parts per million (ppm), equivalent to 6 mg/kg bw/d) on cancer. Their analysis showed that melatonin-fed mice aged 26 months or more at sacrifice had significantly fewer tumors with lower severity compared to controls. In younger mice, 25 months of age or less, melatonin treatment did not significantly affect cancer incidence.

FDA's Conclusions:

Results of the available studies on the effect of melatonin on tumorigenesis in mice are conflicting. Melatonin increases tumor incidence in CBA mice while it decreases tumor incidence in B6C3F1 mice. FDA notes that in the study by Anisimov *et al.* (2001), it is not clear if the increased tumor incidence in CBA mice is due to the increased survival rate or to the administration of melatonin. Further studies are required to elucidate the potential effect of melatonin on tumorigenesis.

7. Immunology

Evidence in the literature suggests that melatonin administration acts on the immune system and regulates cytokine production. Several studies have shown that melatonin enhances pro-inflammatory cytokines such as interleukin (IL)-2, IL-6 and gamma-interferon (IFN-γ) in monocytes/macrophages, TNF-α and IL-12 and drives a Th1 (T helper cells) response (Carrillo-Vico *et al.*, 2005).

Immunoenhancing effects of melatonin have been demonstrated in experimental models of ulcerative colitis and collagen-induced arthritis in Wistar rats (Marquez *et al.*, 2006; Jimenez-Caliani *et al.*, 2005).

Melatonin administration has been reported to have adverse effects on Crohn's disease,and ulcerative colitis (Calvo *et al.*, 2002; Maldonado and Calvo, 2008). Melatonin administration may also adversely impact patients with other diseases that have an immunological basis (Carrillo-Vico *et al.*, 2005).

In individuals with nocturnal asthma, Sutherland *et al.* (2003) reported a significant elevation and phase delay of peak serum melatonin levels that was correlated with an increase in the severity of airway inflammation. The study authors state that exogenous melatonin is pro-inflammatory and might be deleterious to patients with asthma.

FDA's Conclusions:

FDA notes that published literature indicates that melatonin affects the immune system, however these effects are not well understood. More studies are needed in order to better understand melatonin's role in the effects noted above, and to relate such effects to the general population.

8. *Neurological complications from pharmacological use*

Melatonin is used therapeutically to manage sleep in certain neurological disease conditions. However, in certain instances, administration of melatonin may further complicate the disease condition.

Sheldon (1998) reported the effects of an oral dose of 5 mg melatonin on six children (ages 9 months-18 years) with severe neurological disabling conditions, seizures and chronic severe sleep disturbances. Although melatonin had a positive effect on the patients' sleep disorders, it increased seizure activity in four of the six children. The seizure activity returned to baseline after discontinuation of melatonin. When three of these children were re-challenged with a lower dose of melatonin (1 mg), seizure activity was again observed. It should be noted that because of the increase in seizure activity, the study was terminated early. The authors cautioned against recommending melatonin in view of the possible pro-convulsant potential of melatonin and the limited human studies in patients with epilepsy.

Whittom *et al.* (2010) studied the effects of exogenous melatonin and the suppression of endogenous melatonin by bright light exposure on the severity of the sensory and motor manifestations of restless legs syndrome (RLS) in eight subjects. Each subject was studied in three conditions: at baseline, after oral administration of 3 mg melatonin at 19:00 hr and during bright light exposure (3000 lux) from 19:00 hr to midnight. The two latter conditions were separated by one week. The authors reported that after oral administration of melatonin, the severity of the motor symptoms increased as evidenced by measurement of periodic leg movement during wakefulness while bright light exposure produced small, but significant, improvement of leg discomfort. The authors concluded that exogenous melatonin may have a detrimental effect on the motor manifestations of RLS.

FDA's Conclusions:

In spite of melatonin's efficacy in the management of sleep disturbances associated with

neurological diseases in children, it is apparent that melatonin treatment can cause serious unanticipated adverse effects in this patient population. Similarly, unanticipated results occurred in adult patients with RLS. These results indicate that melatonin should not be used indiscriminately in individuals with medical conditions and that, if used at all, medical supervision is indicated.

9. *Drug, herb, and dietary supplement interactions*

In a double-blind, randomized, placebo-controlled, cross-over study, nighttime administration of 5 mg of melatonin (at 22:30 hr) for 4 weeks in hypertensive patients (n=47, ages 38–65 years) on treatment with nifedipine (a calcium channel blocker), showed increased systolic and diastolic BP and HR. As melatonin impaired the antihypertensive efficacy of nifedipine, the authors cautioned against the uncontrolled use of melatonin in hypertensive patients on calcium channel blocker medication (Lusardi *et al.*, 2000).

There are reports of melatonin interacting with other drugs, herbs and dietary supplements. For example, fluvoxamine (an antidepressant), that inhibits CYP1A2 isoenzyme increased the bioavailability of melatonin resulting in increased serum levels of melatonin. All the study subjects reported remarkable drowsiness after oral intake of 5 mg of melatonin and this effect was even more pronounced with fluvoxamine co-administration (Härtter *et al.*, 2000). Co-administration of melatonin (10 mg/kg, intraperitoneal (i.p.)) and imipramine (40 mg/kg, i.p.), another antidepressant, resulted in an additive antidepressant effect in female mice (Ergün *et al.*, 2008). It has been reported that co-administration of melatonin (80 mg/kg, i.p.) with diazepam (0.25 mg/kg, i.p., ineffective on its own) can potentiate the anxiolytic effects of diazepam in rats (Loiseau *et al.*, 2006). A Cochrane review of melatonin, described 6 case reports of possible interaction with warfarin (Herxheimer, 2002). The authors suggested a need for further investigation. Herbs such as valerian and hops and the fixed valerian-hops extract combination, named Ze 91019, have been reported to bind to both the melatonin receptors, MT1 and MT2, as well as to the serotonin receptor subtype (5-HT_6) explaining their sleep inducing property (Abourashed et al., 2004). As such, melatonin may alter the biological effects of drugs and herbs and cause undesirable outcomes.

FDA's Conclusions:

There are reports of interactions of melatonin with drugs, herbs, and dietary supplements that indicate that melatonin can alter the effects of these substances. More information is needed on the possible interaction of melatonin and drugs, particularly those used to treat cardiovascular conditions or epilepsy, and those with sedating properties. Similarly, more information is needed on the interactions of melatonin with herbs and dietary supplements as these substances are commonly used in combination with melatonin (e.g., melatonin and valerian root) and without medical supervision.

Overall Conclusions

As discussed above, melatonin is a neurohormone predominantly secreted during the night by the pineal gland. It is involved in the regulation of various circadian rhythms in many target organs and influences a wide variety of biological functions. Exogenous melatonin alters reproductive/developmental physiology, ocular health, chronobiotic systems such as the sleep/wake cycle, cardiovascular functions, glucose homeostasis, responses of the immune system, and possibly tumorigenesis.

FDA is aware of the therapeutic uses of melatonin. For example, melatonin is used to treat individuals with sleep disorders and insomnia, conditions that are associated with decreased levels of endogenous melatonin (Nagtegaal *et al.*, 2000). It is also used to treat other conditions, such as certain types of seasonal depression, cluster headaches, and cancer to name just a few (Pandi-Perumal *et al.*, 2006, Leone *et al.*, 1996). However, the strength of the evidence regarding the safety and efficacy of such diverse uses is variable and some of melatonin's uses are not supported by available data (Dolberg *et al.*, 1998) or are contraindicated by the data (Sheldon, 1998). In any event, the recognized uses for melatonin are typically short-term and require oversight by a physician.

FDA considers that the indiscriminate use of melatonin as an ingredient in conventional foods for use by the general population is not supported by the available safety data. Melatonin ingestion during the daytime results in mimicking an "artificial darkness" condition and shifts the phase of the circadian clock by either advancing or delaying it, depending on the time of consumption. A predictable effect of melatonin is the induction of sleep, and therefore, melatonin's indiscriminate use will result in reduced alertness, disorientation, fatigue and confusion in a significant number of consumers. This alone is enough to question the safety of food ingredient uses of melatonin. However, FDA is further concerned because there are ample publications in the literature that raise more specific issues regarding the safe use of melatonin as an ingredient in food.

As discussed above in the overview of studies regarding the safety of the use of melatonin in foods, published literature indicates that melatonin may decrease blood pressure under certain conditions and it may increase atherosclerotic plaque buildup in humans. These effects are adverse for the general population and especially so for individuals with cardiovascular disorders. In postmenopausal women, low doses of melatonin were reported to reduce glucose tolerance and insulin sensitivity. In human subjects, alterations of the pituitary-ovarian function have been reported, as well as decreased sperm count, suggesting potential adverse effects on human reproduction. Other adverse effects reported in animal and human studies include ocular damage and neurological complications. In addition, melatonin has been shown to interact with a number of other drugs, herbs, and dietary supplements and co-administration of these substances with melatonin may lead to negative consequences. Data are either lacking or inadequate to resolve these concerns.

With regard to the available studies on melatonin given orally, many have only a small number of subjects so that their interpretive value is limited. Most of the studies were not designed to assess the safety of melatonin for use in food and so potential adverse effects were not rigorously assessed. Adverse effects have not been reported for some doses of melatonin and there are limited dose response data in animals or humans leading to uncertainty about an upper safe limit of exposure. Other studies yield conflicting results, such as studies conducted in mice to determine the tumorigenic potential of melatonin. These results need to be better understood in order to relate them to melatonin's potential to cause human cancer. In spite of the vast literature on melatonin, there are significant data gaps.

FDA is aware that the short-term uses of low doses of melatonin in healthy people are ordinarily well-tolerated (Buscemi *et al.*, 2005). Such information, however, is not adequate to establish the safety of melatonin for use as an ingredient in food. It should be emphasized that, because a food ingredient in a conventional food may be consumed by the entire population over a lifetime, assurance of safety requires an evaluation of potential effects of long-term use. FDA is particularly concerned because there are no long-term studies that have been conducted in animals or humans for use in assessing the long-term safety of melatonin as an ingredient in food.

A safety determination for a substance that will be used as an ingredient in conventional food must be based on scientific studies appropriate to establish the safety of the substance under the conditions of its intended use. For the use of melatonin as an ingredient in food, FDA considers that such data are lacking. On the contrary, various reports in the scientific literature, including those described above, raise safety concerns about the use of the substance. In light of these safety concerns and given the inadequacy of the available data (i.e., through review of published literature) to establish safe use conditions, there is no basis to conclude that the use of melatonin as an ingredient in conventional food is generally recognized as safe (GRAS).

Moreover, there is no food additive regulation in effect that provides for the safe use of melatonin, and FDA is not aware of any information to indicate that melatonin is the subject of a prior sanction. Therefore, FDA considers melatonin an unapproved food additive when used as an ingredient in conventional foods.

Melatonin - 19

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