Medicines & Healthcare products Regulatory Agency

MHRA

Public Assessment Report

National Procedure

Melatonin 1mg/ml oral solution

(melatonin)

PRODUCT LICENCE NUMBER; PL 41344/0050

Colonis Pharma Limited

LAY SUMMARY

Melatonin 1mg/ml oral solution (melatonin)

This is a summary of the Public Assessment Report (PAR) for Melatonin 1mg/ml oral solution. It explains how this product was assessed and its authorisation recommended, as well as its conditions of use. It is not intended to provide practical advice on how to use this product.

For practical information about using Melatonin 1mg/ml oral solution, patients should read the package leaflet or contact their doctor or pharmacist.

What is Melatonin 1mg/ml oral solution and what is it used for?
This application is for a medicine that has a well-established use. This means that the use of the active substance in this medicine has been well-established in the European Union for at least 10 years, with recognised efficacy and an acceptable level of safety.

Melatonin 1mg/ml oral solution is used in the treatment of jet lag in adults.

How does Melatonin 1mg/ml oral solution work?
Melatonin oral solution contains the active substance melatonin. Melatonin is a hormone produced by the body that synchronizes the body's biological day-and-night rhythm. The biological rhythm can be disturbed by travelling across time zones. This is known as jet lag. The symptoms and their severity vary between individuals but are generally worse and last longer the more time zones are crossed. Melatonin oral solution can help restore the normal day-and-night rhythm and reduce the symptoms.

How is Melatonin 1mg/ml oral solution used?
The pharmaceutical form of this medicine is an oral solution and the route of administration is oral.

The recommended dose for adults and the elderly is 3 mg (3 ml) daily for a maximum of 5 days.

When the effect of Melatonin oral solution is inadequate, up to 6 mg (6 ml) daily can be taken.

The first dose should be taken on arrival at the patient's destination at their usual bedtime. On the following day, the medicine should be taken by the patient at their usual bedtime. The oral solution should not be taken before 20:00 hr or after 04:00 hr.

Food should not be consumed 2 hours before or 2 hours after intake of Melatonin oral solution.

Melatonin oral solution may be taken for a maximum of 16 treatment periods per year.

For further information on how Melatonin oral solution is used, refer to the package leaflet and Summary of Product Characteristics (SmPC) available on the Medicines and Healthcare products Regulatory Agency (MHRA) website.

This medicine can only be obtained with a prescription.

The patient should always take the medicine exactly as their doctor/pharmacist has told them. The patient should check with their doctor or pharmacist if they are not sure.

What benefits of Melatonin 1mg/ml oral solution have been shown in studies?
As the active substance melatonin has been in clinical use for over 10 years, data were provided in the form of literature references to show that Melatonin oral solution is a safe and efficacious treatment for jet lag in adults.

What are the possible side effects of Melatonin 1mg/ml oral solution?
The most common side effects with Melatonin 1mg/ml oral solution (which may affect more than 1 in 10 people) are headaches and drowsiness.

For the full list of all side effects reported with this medicine, see Section 4 of the package leaflet or the SmPC available on the MHRA website.

Why was Melatonin 1mg/ml oral solution approved?
It was concluded that the data provided from literature references had shown that Melatonin 1mg/ml oral solution is effective in the treatment of jet lag in adults. Furthermore, use of the active substance melatonin in the European Union has shown that it has a recognised efficacy and an acceptable level of safety. Therefore, the MHRA decided that the benefits are greater than the risks and recommended that it can be approved for use.

What measures are being taken to ensure the safe and effective use of Melatonin 1mg/ml oral solution?
A Risk Management Plan (RMP) has been developed to ensure that Melatonin 1mg/ml oral solution is used as safely as possible. Based on this plan, safety information has been included in the SmPC and the package leaflet, including the appropriate precautions to be followed by healthcare professionals and patients.

Known side effects are continuously monitored. Furthermore, new safety signals reported by patients/healthcare professionals will be monitored and reviewed continuously.

Other information about Melatonin 1mg/ml oral solution
A Marketing Authorisation for Melatonin 1mg/ml oral solution was granted in the UK on 10 June 2019.

The full PAR for Melatonin 1mg/ml oral solution follows this summary.

This summary was last updated in September 2019.

TABLE OF CONTENTS

Contents

I INTRODUCTION

Based on the review of the data on quality, safety and efficacy, the Medicines and Healthcare products Regulatory Agency (MHRA) considered that the application for Melatonin 1mg/ml oral solution (PL 41344/0050) could be approved.

The product is approved for the following indication:

- short-term treatment of jet lag in adults.

Melatonin is a naturally occurring hormone. Melatonin secreted by the pineal gland is involved in the synchronisation of circadian rhythms to the diurnal light-dark cycle. Melatonin secretion and plasma melatonin level increase shortly after the onset of darkness, peaking around 02:00 – 04:00 hr and decline to the daytime nadir by dawn. Peak melatonin secretion is almost diametrically opposite the peak daylight intensity, with daylight being the primary stimulus for maintaining the circadian rhythmicity of melatonin secretion.

The pharmacological mechanism of action of melatonin is believed to be based on its interaction with MT1-, MT2- and MT3 receptors, as these receptors (particularly MT1 and MT2) are involved in the regulation of sleep and circadian rhythms in general.

Melatonin has a hypnotic / sedative effect and increases propensity for sleep. Melatonin administered earlier or later than the nocturnal peak in melatonin secretion can, respectively, advance or delay the circadian rhythmicity of melatonin secretion. Administration of melatonin at bedtime (between 22:00 and 24:00 hr) at destination following rapid trans-meridian travel (aircraft flight) hastens resynchronisation of circadian rhythmicity from ‘departure time' to 'destination time', and ameliorates the collection of symptoms known as jet lag that are a consequence of such de-synchronisation.

Typical symptoms of jet lag are sleep disturbances, daytime tiredness and fatigue, though mild cognitive impairment, irritability, and gastrointestinal disturbances may also occur. Jet lag is worse the more time-zones are crossed, and it is typically worse following eastward travel as people generally find it harder to advance their circadian (body clock) than to delay it, as required following westward travel. Clinical trials have found melatonin to reduce patient-assessed overall symptoms of jet lag by ~44%, and to shorten the duration of jet lag. In 2 studies of flights over 12 time zones melatonin effectively reduced the duration of jet lag by ~33%. Due to the potential for incorrectly timed intake of melatonin to have no effect, or an adverse effect, on re-synchronisation of circadian rhythmicity / jet lag, melatonin should not be taken before 20:00 hr or after 04:00 hr at destination.

Adverse effects reported in jet lag studies involving melatonin doses of 0.5 to 8 mg were typically mild, and often difficult to distinguish from symptoms of jet lag. Transient drowsiness / sedation, headache, and dizziness / disorientation were reported; these same adverse effects, plus nausea, are those typically associated with short-term use of melatonin in reviews of the safety of melatonin in humans.

This application was submitted under Article 10a of Directive 2001/83/EC, as amended, as a well-established use application. No new non-clinical data were submitted, as the data submitted for this application is in the form of literature references.

Since this application was submitted on the basis of well-established use, it was necessary for the Applicant to demonstrate a link between their product and the products described in the literature upon which the claims of safety and efficacy rest. The Applicant has addressed this by performing a single-arm pharmacokinetic study, in order to obtain pharmacokinetic data

and investigate the *in vivo* pharmacokinetic characteristics of the test product oral solution formulation, comparing them to available, extensive literature data.

To further bridge the literature reviews to the proposed (test) product, the Applicant has performed an extensive statistical analysis in order to investigate similarity between the test melatonin oral solution 1mg/ml and the reference product utilised in other Applicant-sponsored bioequivalence studies conducted in the past. These studies used the Hungarian product (Bio-Melatonin tablets 3 mg) as comparator versus other oral solid Melatonin immediate-release test formulations.

The MHRA has been assured that acceptable standards of Good Manufacturing Practice (GMP) are in place for this product at all sites responsible for the manufacture, assembly and batch release of this product.

A Risk Management Plan (RMP) and a summary of the pharmacovigilance system have been provided with this application and are satisfactory.

A national marketing authorisation was granted in the UK on 10 June 2019.

II QUALITY ASPECTS

II.1 Introduction

Each 1 ml of solution contains 1 mg of melatonin.

In addition to melatonin, this product also contains the excipients propylene glycol (E1520,), sorbitol liquid (non-crystallising) (E420), sucralose (E955), strawberry flavour (includes ethanol (E1510) and propylene glycol (E1520)), concentrated hydrochloric acid (E507) and purified water.

The finished product is packaged in an amber, type III glass bottle of 150 ml nominal capacity, with an HDPE child-resistant, tamper-evident screw cap with an LDPE liner. An LDPE, CE marked 10 ml graduated oral syringe with intermediate graduations of 0.5 ml and an LDPE, CE marked "press-in" syringe/bottle adaptor are also provided.

Satisfactory specifications and Certificates of Analysis have been provided for all packaging components. All primary packaging complies with the current European regulations concerning materials in contact with food.

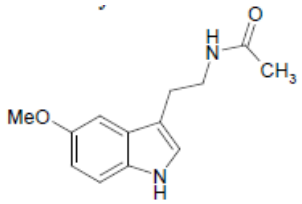
II.2 ACTIVE SUBSTANCE(S)

rINN: Melatonin

Chemical Name: N-[2-(5-Methoxy-*1H*-indol-3-yl)ethyl]acetamide

Molecular Formula: $C_{13}H_{16}N_2O_2$

Chemical Structure:



Molecular Weight: 232.27 g/mol

Appearance: Crystalline powder, ivory to beige

Solubility: Slightly soluble in water; soluble in acetone, ethyl acetate and methanol

Melatonin is the subject of a British Pharmacopoeia monograph.

Synthesis of the active substance from the designated starting materials has been adequately described and appropriate in-process controls and intermediate specifications are applied. Satisfactory specifications are in place for all starting materials and reagents, and these are supported by relevant certificates of analysis.

Appropriate proof-of-structure data have been supplied for the active substance. All potential known impurities have been identified and characterised.

An appropriate specification is provided for the active substance. Analytical methods have been appropriately validated and are satisfactory for ensuring compliance with the relevant specifications. Batch analysis data are provided and comply with the proposed specification. Satisfactory certificates of analysis have been provided for all working standards.

Suitable specifications have been provided for all packaging used. The primary packaging has been shown to comply with current European regulations concerning materials in contact

with food.

Appropriate stability data have been generated supporting a suitable retest period when stored in the proposed packaging.

II.3 DRUG PRODUCT

Pharmaceutical development

A satisfactory account of the pharmaceutical development has been provided.

All excipients comply with either their respective European/national monographs, or a suitable in-house specification. Satisfactory Certificates of Analysis have been provided for all excipients.

No excipients of animal or human origin are used in the finished product.

This product does not contain or consist of genetically modified organisms (GMO).

Manufacture of the product

A description and flow-chart of the manufacturing method has been provided.

A satisfactory batch formula has been provided for the manufacture of the product, along with an appropriate account of the manufacturing process. The manufacturing process has been validated and has shown satisfactory results.

Finished Product Specification

The finished product specification is satisfactory. The test methods have been described and adequately validated. Batch data have been provided that comply with the release specification. Certificates of Analysis have been provided for any working standards used.

Stability

Finished product stability studies have been conducted in accordance with current guidelines, using batches of the finished product stored in the packaging proposed for marketing. Based on the results, a shelf-life of 18 months, with the storage conditions 'Store in the original package in order to protect from light' is acceptable. After first opening, do not store above 25^{o}C and should be used within 2 months.

Suitable post approval stability commitments have been provided to continue stability testing on batches of finished product.

II.4 Discussion on chemical, pharmaceutical and biological aspects

The grant of a marketing authorisation is recommended.

III NON-CLINICAL ASPECTS

III.1 Introduction

This application was submitted under Article 10a of Directive 2001/83/EC, as amended, a well-established use application. With the exception of the data from the bridging studies, no new non- clinical studies were submitted, as the data submitted for this application is in the form of literature references. The literature review provided is satisfactory.

III.2 Pharmacology

Melatonin, also known as N-acetyl-5-methoxytryptamine, is a small lipid and water-soluble hormone of natural origin produced mainly by the pineal gland, which is located behind the

third ventricle in the brain. It plays an important role in the regulation of circadian rhythms, the most important one in humans being the sleep-wake cycle.

The sleep-wake cycle may be pathologically affected in different ways. Furthermore, the sleep may also be disturbed by various processes. The disturbances of the sleep-wake cycle are called circadian rhythms disorders and include the jet lag (time zone change) syndrome, shift work sleep disorder, advanced sleep phase syndrome, non-24h sleep-wake syndrome. In all these, insomnia might appear as a symptom.

Normally, melatonin production begins in the evening and is rapidly released into the blood and the cerebrospinal fluid. Melatonin can advance or delay sleep onset through G-protein coupled melatonin receptors (MT2). Melatonin also has a sleep promoting effect through the more numerous MT1 receptors, as it can induce, maintain, and consolidate fragmented sleep patterns. Functional magnetic resonance imaging shows that both endogenous and exogenous melatonin similarly alter brain activity and induce sleep.

Primary pharmacodynamics

***In vitro* studies**
Melatonin is described in the literature as acting at the central nervous system level, modulating the synchronisation of the biological clock and promoting sleep through stabilisation and phase-shifting effects on the suprachiasmatic nucleus of the hypothalamus. Interaction with melatonin MT1 and MT2 receptor subtypes seem to be involved in the action. MT1 receptors are located mainly in cells of the pituitary pars tuberalis (PT), controlling seasonal prolactin variations in ruminants, whereas there is no evidence to suggest that MT2 receptors are present in the PT. By contrast, both MT1 and MT2 receptors are located in the suprachiasmatic nucleus (SCN). The molecule ^{125}I-melatonin has been used in binding and autoradiographic studies and has enabled detection of melatonin binding sites expressed at low density in most tissues in which effect of melatonin have been reported.

The transduction pathways mediated by these melatonin receptors remain an unsolved and complex issue. The MT1 receptor couples to different G protein, one of which mediates inhibition of adenylcyclase and the other activates phospholipase Cβ. The MT2 receptor couples to phosphoinositide production, the inhibition of adenylcyclase and the inhibition of the soluble guanylyl cyclase pathway. The MT2 receptor mRNA present in human retina and brain is responsible for entrainment of circadian rhythms in the SCN. MT1 and MT2 polymorphisms have been found in humans and may be associated with sleep disorders.

The evidence suggests that melatonin can influence immune cells through nuclear and membrane melatonin receptors. Studies mention these receptors have been identified on macrophages, B cells and T cells. Melatonin can modulate proliferation and cytokine secretion via these receptors on immune cells. In animals, melatonin can inhibit chemically induced tumours, which is increase by pineal suppression (long light phases) or pinealectomy. Pinealectomy stimulates and/or melatonin inhibits the growth and sometimes the metastasis of experimental cancers of the lung, liver, ovary, pituitary, prostate as well as melanoma and leukaemia.

***In vivo* studies**
In mammals, melatonin is mainly synthesised in the pineal gland from serotonin, but it is also formed in the gut and retina. The production is circadian, and it is stimulated by photic stimulus arising after the onset of darkness. Peak melatonin levels are reached in the middle of the night (between 2-4 a.m.) and decrease to low levels in the second half of the night.

A limitation of studies in nocturnal laboratory animals is that melatonin is often administered during the light phase, when it is not endogenously produced but the animals are most likely asleep. Nevertheless, rats display intermittent periods of sleep and wakefulness in both light and dark phases rather than a single consolidated sleep period such as observed in humans. This situation clearly has no analogue in humans; therefore, the conclusions drawn from laboratory studies in rats may be of limited value when extrapolated to other species. In addition, the doses typically employed in rats (i.e. 2–20 mg/kg) produce pharmacological circulating levels, several orders of magnitude greater than what is observed naturally, so like many of the human studies these may not reflect the endogenous physiological role of the hormone.

There are several important similarities between humans and diurnal non-human primates, favouring the use of these animals to model normal and pathological sleep-related processes. These include:
(1) Similar temporal patterns of activation of the major circadian pacemaker, the SCN, relative to the rest-activity cycle in both species, i.e. high activity of the SCN neurons during the day correlates with these species' daytime activity, in contrast to nocturnal animals whose SCN is active during their daytime rest period;
(2) Similar temporal patterns of melatonin production, occurring during habitual night time sleep period;
(3) A consolidated nocturnal sleep episode, with similar sleep architecture, in contrast to the majority of nocturnal or diurnal species which tend to have a polyphasic sleep pattern.

In a study using macaques, the sleep process showed high sensitivity to daytime melatonin administration. Sleep initiation was significantly promoted by a wide range of melatonin doses used and, as in humans, showed a lack of dose dependence of the effect, once the dose (5–20 μg/kg, orally) was sufficient to induce physiologic circulating levels of the hormone (above 50 pg/ml). Lower doses failed to promote sleep in the macaques studied.

The effect of melatonin on hexobarbital (75 mg/kg, IP)-induced narcosis was investigated in mice using 20 mg/kg IP (low dose) and 100 mg/kg IP (high dose). The onset time for hypnosis and the duration of the sleeping period were measured in all groups. The results are exposed in Table 1 below:

Table 1. Effect of melatonin on hexobarbital induced narcosis in mice

Groups	Hypnotic onset time (min.)	Sleeping time (min).
Control	2.18±0.74	28.8±13.22
20 mg/Kg, ip	5.08±2.09*	43.94±12.52
100 mg/Kg, ip	2.47±1.46	78.51±19.46**

*P<0.05; **P<0.01.

At the dose of 20 mg/kg melatonin delayed the hypnosis induced by hexobarbital and increased the sleeping time of the animals. The animals showed excitation and body rotation before falling asleep. The 100 mg/kg group had an increased duration of sleeping period with the onset time for hypnosis similar (slightly higher) to the one from controls. The results seem to suggest that melatonin potentiated the sleeping effect induced by hexobarbital but increased the onset time for hypnosis (vs controls) for which a plausible explanation was not provided.

The effect of diazepam on the binding profile of ^{125}I-iodomelatonin binding and the effect of melatonin administered in the drinking water on the benzodiazepine and ^{125}I-iodomelatonin binding were evaluated in synaptosomes prepared from the medulla pons and cortex of male CD rats aged 2 months. It was observed that melatonin via drinking water significantly enhanced benzodiazepine (^{3}H-RO 15- 1788) binding in the medulla pons and slightly reduced it in the cortex but did not affect ^{125}I- melatonin binding.
Daily injections of diazepam for 3 weeks reduced markedly ^{125}I-melatonin binding site density in the medulla-ponds but not in the cortex of male rats, whereas benzodiazepine binding was not significantly affected. The combination of melatonin and diazepam reversed the suppression by diazepam of ^{125}I-iodomelatonin in the medulla-pons and the suppression by melatonin of benzodiazepine binding in the cerebral cortex.

Secondary Pharmacodynamics and safety pharmacology
Immune System
There is substantial evidence to suggest that melatonin exerts some of its effects as an immunomodulatory compound, though there is little understanding how melatonin actually regulates the immune system. Some papers suggest that melatonin acts as an immunostimulant, whilst other studies suggest that the molecule exerts anti-inflammatory properties. Some theories suggest that melatonin acts as an "immune buffer", acting as a stimulant under basal or immunosuppressive conditions, or as an anti-inflammatory compound in the presence of exacerbated immune responses such as acute inflammation.

The pineal gland, the primary source of melatonin, is an immune target. Interferon-gamma was shown to increase the production of melatonin from *in-vitro*-cultured rat pineal glands. Studies where administration of recombinant IL-1β inhibited serum melatonin levels in rats through a receptor-mediated mechanism, whereas granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor stimulated the synthesis of melatonin both *in vivo* and *in vitro.*

Lipopolysaccharide (LPS) treatment not only reduced the production of nocturnal melatonin in rats but also enhanced endothelial cell adherence, which was normalised after melatonin administration. LPS was shown to induce tumor necrosis factor-α (TNF-α) production in the rat pineal gland through activating toll-like receptor 4 (TLR-4). Subsequently, the production of TNF-α by pineal gland microglia was found to act on tumour necrosis factor receptor 1 (TNFR1), driving the nuclear translocation of NF-κB, which represses Aa-nat transcription and in turn suppresses melatonin synthesis. Suppression of increased nocturnal melatonin in human mothers with mastitis was highly correlated with increased TNF-α production. Likewise, an increase in TNF-α levels after Caesarean section resulted in the suppression of serum melatonin nocturnal levels.

Melatonin and/or its biosynthetic machinery have been located in a variety of immune tissues, organs and cells, such as rat, mouse and human thymus spleen, bone marrow and circulating leukocytes mast cells, natural killer cells and eosinophils and in several immune cell lines. Rat peritoneal macrophages also produce melatonin *in vitro* after incubation with tryptophan. It has been found that *in vitro*-cultured human lymphocytes not only actively synthesize and release substantial amounts of melatonin, but that this melatonin modulates the IL-2/IL-2 receptor system via receptor-mediated intra-, auto- and/or paracrine actions.

Some studies have shown that melatonin treatment promotes an increase in the weight of immune organs, both under basal and immunosuppressed conditions. Conversely, the anti-proliferative effects of melatonin have been observed *in vitro* in PHA-stimulated human lymphocytes. Melatonin also modulates both the innate and specific immune responses

through regulation of immunocompetent cell proliferation and secretion of immune mediators, such as cytokines.

A study reported that reconstitution of the night-time plasma melatonin peak completely abrogated the humoral and cellular responses in propranolol-immunosuppressed mice. Mice immunosuppressed by lead recovered splenic CD4+ cell numbers and functions after melatonin treatment. Melatonin also averted age-induced immunosuppression in rats by increasing immunoglobulin G1 and M levels. Furthermore, melatonin significantly restored both dexamethasone- and aging-induced immunosuppression in squirrels. Melatonin also increased B cell proliferation and the Th1 response (IL-2 and IFN-γ production) and decreased Th2 cytokines such as IL-10 in old mice. Early *in vitro* studies suggested that melatonin has pro-Th1 effects. Sub-stimulated PBMCs displayed enhanced production of Th1 cytokines, such as IFN-γ and IL-2, after *in vitro* melatonin treatment. The diurnal rhythmicity of human cytokine production indicated that the IFN-γ/IL-10 peak occurs during the early morning; this peak positively correlated with plasma melatonin, suggesting a melatonin/Th1 causality. Splenocyte proliferation in response to the T cell mitogen concanavalin A was also enhanced by the addition of melatonin *in vitro*.

Conversely, melatonin significantly reduced the splenic CD19+ B-cell population in mice with experimental membranous nephropathy and diminished the overexpression of TNF-α, IL-1β and IFN-γ. Further *in vivo* studies have shown the capacity of melatonin to promote a Th2 response in several models. The first report demonstrated that high doses of melatonin enhanced the production of the hallmark Th2 cytokine IL-4 in bone marrow lymphocytes count**.** Early nocturnal sleep induced a shift in the Th1/Th2 cytokine balance towards increased Th1 activity, whereas the Th2 response dominated during late sleep. A robust decrease in TNF-α-producing CD8+ cells was also observed during sleep, suggesting a correlation between melatonin and the Th2 response.

The absence of melatonin due to pinealectomy, polarised rat thymic Th1/Th2 cells towards a Th1 response by increasing the production of IFN-γ and reducing IL-10 levels, implying that melatonin skews the immune response towards Th2 dominance. Chronic administration of melatonin to antigen-primed mice increased the production of IL-10 and decreased the secretion of TNF-α, suggesting a Th2 response. Melatonin inhibited the Th1 response by suppressing IFN-γ and IL-12 in mice with contact hypersensitivity. Furthermore, melatonin protected against experimental reflux esophagitis by suppressing the Th1-mediated immune response. Melatonin also acted as an immunosuppressive agent and reduced Th1 cytokine levels in an experimental model of ovarian transplant in mice, permitting prolonged graft survival.

From the extensive research on the impact of endogenous and exogenous melatonin on the immune response pathways, it has been reported that melatonin possesses an important role in the treatment of a number of different clinical conditions, including as an antiviral, antibiotic and anti-parasitic molecule.

The impact of melatonin has been investigated in auto-immune conditions such as rheumatoid arthritis (RA) where several models have suggested deleterious actions for both endogenous and exogenous melatonin. Fibroblasts from synovial membranes collected from RA patients also show impaired circadian expression of timekeeping genes and pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6. When the *in vitro* data is correlated to human RA patients with active disease who are administered daily melatonin it was reported that low antioxidant profiles were observed along with increased neopterin concentrations and erythrocyte sedimentation rates (inflammation indicators) and no changes

in pro-inflammatory cytokine levels (TNF-α, IL-1β and IL-6), but these effects were not associated with any changes in clinical symptoms.

Other clinical conditions that have been investigated both *in vitro* and *in vitro* that involve auto-immune conditions are multiple sclerosis, Systemic Lupus Erythematosus, Type 1 Diabetes, Irritable Bowel Syndrome/Inflammatory Bowel Disease, Breast Cancer, AIDS. The correlation of the *in vitro* data has not been yet shown to have any significant positive impact on clinical outcomes with humans.

In addition, to the above clinical conditions, melatonin has been extensively studied within the ageing processes and immunosenescence. The immunomodulatory effects of melatonin in aging are evident in the central nervous system, as dietary melatonin was shown to selectively reverse the lack of response to an inflammatory stimulus in the brains of aged mice.

Endocrine and reproductive systems
Melatonin regulates pubertal development in some juvenile mammals. In seasonal breeders, melatonin seems to act as either pro-gonadotrophic or anti-gonadotrophic according to the period of the year (autumn-winter/short days or spring-summer/long days, respectively). Melatonin has also been shown to influence secretion of several hormones in animals and in humans in some situations, namely the luteinizing hormone (LH) and prolactin, corticosteroids, thyroid hormones and insulin.

In adult female rats, it was observed that a single intravenous dose of melatonin (12.8mg/kg) increased serum prolactin levels. In adult males, SC infusion of melatonin decreased serum prolactin levels and (at ~4.8mg/kg) caused a decrease in testes weight and testicular degenerative changes.

In one study, the influence of daily subcutaneous administration of melatonin (5-100 μg/day) on sexual development in prepubertal and pubertal male rats was investigated. This study showed that melatonin administration could inhibit or delay sexual development. A subsequent study confirmed that melatonin (100 μg/day) delays sexual maturation in young male rats when administered daily in the afternoon. It was demonstrated that the inhibitory action of melatonin is most critical between 20 and 30 days of life and is reversible regardless of whether melatonin administration is continued/discontinued after 45 days of life. The suppression of the pubertal peaks of pituitary GnRH receptor number and pituitary and plasma follicle-stimulating hormone (FSH) concentrations in treated rats suggests that melatonin interferes with the pubertal increase in GnRH secretion. The reversibility of the effects was also confirmed in another study.

A study confirmed that chronic melatonin administration (100μg/day) delays sexual maturation of female rats, probably by retarding maturation of hypothalamic GnRH-producing cells. Thus, melatonin could modify basal GnRH secretion of pulsatile release. This study suggested that pituitary and ovarian responsiveness do not seem to be affected since proestrous surges of 17β-estradiol, LH, and FSH occur, albeit at reduced frequency.

A study in adult female hamsters has demonstrated that administration of melatonin (25 μg SC for 8 or 11 weeks) inhibited blood levels of thyroxine, triiodothyronine and thyrotropin. Studies in male rats have demonstrated that administration of melatonin at 30mg/kg SC for 10 days decreases adrenal gland and serum corticosterone levels, and at 8mg/kg SC for 30 days decreases uptake of [3H]testosterone by the prostate. A further study in 10-week old, hypothyroid male hamsters demonstrated that melatonin administration (25μg SC for 10

weeks) led to a decrease in pituitary and serum prolactin, TSH and LH content and decrease in serum thyroxine and triiodothyronine.

Cardiovascular and respiratory systems
A study in rats 30-60 mg/kg melatonin IV caused a dose-related fall of mean arterial pressure, heart rate and of brain serotonin release. Bradycardia was abolished by pre-treatment with bilateral vagotomy thus suggesting that it may be mediated through a parasympathetic action.

Studies in porcine coronary arteries suggest the potential for melatonin to have tensive effects. In baboons, 0.3 to 0.4 mg/kg melatonin IV caused a statistically significant increase of the cardiac output and ventricular ejection associated to a reduction in heart rate.
In rats a dose of 100 mg/kg melatonin caused a slight decrease of heart rate and blood pressure. The QT interval and the respiratory rate were not changed. In humans the evaluation of ECG was performed and reported as not presenting any effects on the QT interval.

Central Nervous Systems
In mice, the Irwin test showed that at doses >8mg/kg melatonin had no behavioural effects. At 16 mg/kg a slight sedation was observed. Sedation was also reported in the repeated dose studies conducted in rats. At doses of 64, 128 and 256 mg/kg decreased fear, reactivity, muscle tone and hypothermia were observed with dose-dependent intensity and duration. At 128 mg/kg it also showed analgesic activity in the four-plate test.

Daily administration of 2.5-10mg/kg melatonin to mice prior to the swimming test significantly reversed the increased immobility period that was observed on chronic exposure to swimming test. This effect was reported to be comparable with that of GABA-benzodiazepine receptor agonists, appearing to involve GABA-benzodiazepine receptors. In other studies, acute administration of melatonin did not reveal antidepressant activity.

From the results of a study conducted in mice it does appear that melatonin has anticonvulsant activity in some of the tests used to screen clinically important anticonvulsants. However, the doses needed to produce an anticonvulsant effect (significant effect *vs* pentylenetetrazole at 200mg/kg; ED50 *vs* 3-MPA, 115 mg/kg; ED50 *vs* ECS, 159 mg/kg) are similar to those which produce signs of motor incoordination in the rotarod test at this pre-dose interval. Thus, the authors suggest that the anticonvulsant action of melatonin may not represent a specific neuropharmacological action but rather an inability of the animal to make the appropriate motor response.

Potential for pharmacodynamic drug interactions
The non-clinical overview includes a discussion of the potential for pharmacodynamic (PD) drug interactions based on the literature. Apart from the potential synergistic effect of melatonin with imipramine and diazepam, which conclude in an anti-depressant effect and anxiolytic actions respectively, melatonin has been shown to enhance tamoxifen's effects. There are no other PD drug interactions data available in the public domain. The limited PD interactions of melatonin indicate a safe profile of the product.

Overall conclusions on pharmacology
The non-clinical overview contains a dedicated section for primary and secondary PD. The pharmacology of melatonin is well known and described in the literature including the potential for PD drug interactions. The information provided in the PD section is adequate.

III.3 Pharmacokinetics

The non-clinical overview discusses published sources of information on the pharmacokinetics (PK) of melatonin, addressing absorption, distribution, metabolism, and excretion, which are briefly summarised below. A non-GLP study investigating permeability of the drug product performed by the Applicant was also provided in the absorption section, however this is viewed as supportive evidence only, in this bibliographic application. The PK section provides an adequate review of the PK of melatonin.

Endogenous melatonin was studied in one dog by taking eleven venous samples over a 24-hour period, with light period between 6am and 6 pm. The pattern of serum melatonin in the dog over a 24-hour cycle displayed a circadian rhythm with low concentrations during the day and highest levels (peak approximately 0.25 nM) during the night at 2 a.m.

Absorption

In vitro absorption

To bridge to published data, the Applicant performed an *in-vitro* study to assess the bi-directional permeability of the test compound to clarify the rate and extent of absorption. Using standardised Caco-2 cells it was determined that the permeability coefficient was 40.3 and 39.9 x 10-6 cms-1 for the A2B and B2A directions respectively. The mean percentage recovery A2B was 99.4% and for the B2A direction it was 97.6%. The calculated efflux ratio was 0.990 thereby indicating that melatonin is not subject to active efflux. It therefore is expected that melatonin is completely absorbed *in vivo*.

In vivo absorption

Two dogs received 10, 20, 40 and 80 mg/kg body weight of melatonin given at 2-hour intervals. The melatonin concentrations in serum increased proportionally with increasing dose, however no exposure parameter (C_{max} or AUC) values were reported. The mean peak concentration after 80 mg/kg was approximately 100 μM. Four dogs were given a single melatonin dose of 40 mg/kg. Melatonin was rapidly absorbed and reached a peak value in serum (circa 5 μM) between 20 to 30 min following its administration. The distribution phase was 3-4 hours and the elimination half time ($t_{1/2}$) was approximately 5 hours. Urinary excretion of melatonin was also investigated in one dog. The total excreted amount of immunoreactive melatonin during the five hours after its administration was 0.25% of the dose.

Oral bioavailability of a 10 mg/kg dose of melatonin in rats was 53.5%, while in dogs and monkeys, it was >l00%. Also, in rats the bioavailability of a 10 mg/kg dose of melatonin administered intraperitoneally was found to be 74%. Since the oral dose used in dogs and monkeys (10 mg/kg) was three-fold higher than the intravenous dose (3 mg/kg), a bioavailability value in excess of 100% may be indicative of nonlinearity and hence dose dependency in the PK of melatonin. To probe the issue of nonlinear PK, oral bioavailability of a 1 mg/kg dose of melatonin was studied in dogs. The results indicate significant dose dependency in the PK, with the plasma AUC and oral bioavailability of the 1 mg/kg dose being disproportionately lower than that of the 10 mg/kg dose (see table below).

Table 2: Summary of pharmacokinetic parameters of exogenous melatonin in rat, dog, and monkey.

Parameter	SD Rat	Beagle Dog		Cyno Monkey
Intravenous Dosing				
Dose (mg/kg)	5.00	2.95		2.98
AUC (mg.hr/L)	2.38	0.81		1.78
Clearance (L/kg/hr)	2.11	3.84		1.68
Half-Life (hr)	0.33	0.31		0.57
Vdss (L/kg)	1.05	1.48		1.2
Oral Dosing				
Dose (mg/kg)	10.00	0.98	10.30	10.00
AUC (mg.hr/L)	2.49	0.05	3.44	8.85
Dose adjusted F	53.5	16.9	>100	>100

Distribution

Melatonin, a hydrophobic molecule, readily penetrates biological membranes, and thus appears in tissues or body fluids in concentration on the same order of magnitude as plasma. Endogenously, melatonin is synthesized from serotonin via N-acetylserotonin, and an enzymatic pathway also exists for reconversion of melatonin to N-acetylserotonin, notably in the retina.

Melatonin has been shown to cross the placenta in rats, sheep, and rhesus monkeys, and can be transferred to rat pups in maternal milk**.** Subcutaneous administration of ^{3}H-acetyl-melatonin to Sprague-Dawley rats on day 18 of gestation (timing of gestation not defined) resulted in detection of radioactivity in whole foetuses and foetal tissues (brain, liver, heart, viscera, skin, muscle, and bone), with the highest concentrations in liver and lowest concentrations in brain. 2-(^{125}I)-iodomelatonin, injected into pregnant Djungarian hamsters, crosses the placenta and is detected by autoradiography at discrete competitive binding sites (i.e. putative melatonin binding sites) within the foetal brain. In near term (151 days of gestation) rhesus monkeys, (3H)- melatonin IV resulted in rapid appearance in foetal plasma, foetal cerebrospinal fluid, and amniotic fluid.

Melatonin seems to distribute fast through tissues in the rat and after injection, rapidly penetrates brain and cerebrospinal fluid. The steady state distribution volumes in the three species as specified in a study, ranged between 1.05 and 1.48 L/Kg at doses of 3-5 mg/kg IV, indicating moderate tissue distribution of melatonin in these animals**.**

The majority of circulating melatonin is bound to albumin.

Metabolism

It is generally accepted that melatonin is primarily metabolised by CYP1A1 and CYP1A2. A study determined from the chromatographic analysis of urinary metabolites obtained in rats administered intraperitoneally with radio-labelled melatonin that there were three distinct peaks. Two of these peaks corresponded to the glucuronic and sulphate conjugates of 6-hydroxymelatonin and the third compound was not completely characterised. It was further determined that the major metabolite accounting for 70%-80% of the radioactivity was the sulphate conjugate of 6-hydroxymelatonin whereas the glucuronic acid conjugate represented 5%. The unidentified metabolite corresponded to 12% of radioactivity.

From *in vitro* metabolism studies using liver microsomes it is suggested that 6-hydroxylation of melatonin is the primary metabolic route. In addition, studies, described how 5-methoxyindoleacetic acid appears to be formed by de-acetylation of melatonin followed by de-amination.

Excretion

Following IV administration of 5 mg/kg, the apparent elimination half-life of melatonin in rats was 19.8 min. The half-life seen in other studies were similar even though the doses employed were significantly lower than the 5 mg/kg within this study (1 -100 μg). A similar half-life estimate was obtained in dogs (18.6 min), while it was longer (33.9 min) in monkeys. A half-life of 30 min has been reported in the rhesus monkey. The calculated clearance values in this study indicate that the beagle dog (CL = 3.84 L/hr/kg) clears melatonin faster than the rat (2.11 L/hr/kg) and the monkey (1.68 L/hr/kg).

The main excretion route of the melatonin metabolites is renal. In rats and rabbits administered labelled melatonin by intraperitoneal injection or stomach tubes, 70 and 20% of the activity was excreted in urine and faeces respectively.

Juvenile
In the neonatal rat, the distribution and metabolism of exogenous melatonin is similar to that in the adult rat. Neonatal rats showed rapid absorption (~90% of total dose within 45 minutes) and metabolism (~60% of total dose within 60 minutes) following intubation of 3H-melatonin. General tissue distribution was similar to that found in adult rats, and the urinary metabolites were primarily the sulphate and glucuronide conjugates of 6-hydroxymelatomnin.

Pharmacokinetic drug interactions
Cytochrome P450 1A2 (CYP1A2) accounts for about 10 to 15% of the total CYP content of human liver and is the major enzyme involved in the metabolism of imipramine, propranolol, clozapine, theophylline, and caffeine. It is also involved in the conversion of heterocyclic amines to their proximal carcinogenic and mutagenic forms, as well as in the metabolism of endogenous substances, including 17 beta-estradiol and uroporphyrinogen III.

One author studied the biotransformation of melatonin and the effects of fluvoxamine on the metabolism of melatonin *in vitro* using human liver microsomes and recombinant human CYP isoenzymes. Melatonin was found to be almost exclusively metabolised by CYP1A2 to 6-hydroxymelatonin and N-acetylserotonin with a minimal contribution of CYP2C19. Both reactions were potently inhibited by fluvoxamine, with a Ki of 0.02 microM for the formation of 6-hydroxymelatonin and 0.05 microM for the formation of N-acetylserotonin. Other than fluvoxamine, fluoxetine, paroxetine, citalopram, imipramine, and desipramine were also tested at 2 and 20 microM. Among the other antidepressants, only paroxetine was able to affect the metabolism of melatonin at supratherapeutic concentrations of 20 microM, which did not reach by far the magnitude of the inhibitory potency of fluvoxamine.

Human hepatic post-mitochondrial preparations were incubated with either melatonin or 6-hydroxymelatonin in the presence and absence of a range of concentrations of interacting drug, and the production of 6-sulphatoxymelatonin monitored using a radioimmunoassay procedure. Of the drugs screened, only the potent CYP1A2 inhibitor 5-methoxypsoralen impaired the 6-melatonin hydroxylation at pharmacologically relevant concentrations and is likely to lead to clinical interactions; diazepam, tamoxifen and acetaminophen (paracetamol) did not impair the metabolic conversion of melatonin to 6-sulphatoxymelatonin at concentrations attained following therapeutic administration. 17-ethinhyloestradiol appeared not to suppress the 6-hydroxylation of melatonin but inhibited the sulphation of 6-hydroxymelatonin, but this is unlikely to result in an interaction following therapeutic intake of the steroid. Species differences in the inhibition of melatonin metabolism in human and rat hepatic post-mitochondrial preparations were evident implying that the rat may not be an appropriate surrogate of human in such studies.

As melatonin's metabolism is mainly mediated by the CYP1A enzymes, there are theoretical interactions that could be possible between melatonin and other active substances as a consequence of their effect on CYP1A enzymes. As melatonin does not induce the CYP1A enzymes *in vitro* at supra-therapeutic concentrations it is unlikely that these interactions would be seen to be significant. Caution should be advised with the concomitant administration with cimetidine, a known CYP2D inhibitor, fluvoxamine, oestrogens, quinolones all potentially increasing melatonin levels. CYP1A2 inducers such as carbamazepine and rifampicin theoretically could reduce the plasma concentrations of melatonin.

Overall conclusions on pharmacokinetics

The non-clinical overview discusses published sources of information on the PK of melatonin, addressing absorption, distribution, metabolism, and excretion. A non-GLP study investigating permeability of the drug product performed by the Applicant was also provided in the absorption section however, this is viewed as supportive evidence only, in this bibliographic application. The PK section provides a brief but adequate review of the PK of melatonin.

III.4 Toxicology

The Applicant has adequately addressed the single dose toxicology. The studies are briefly described below:

Single dose toxicity

The acute toxicity of melatonin was studied in mice and rats, by IV, IP and SC administration. The LD50 by the oral route was shown to be approximately 1250 mg/kg in mice and > 3200 mg/kg in rats (Table 3), which is more than the maximum proposed daily dose 6mg in adults. The main effects observed within these two species at high doses were sedation, lethargy and vasodilatation. The higher doses led to impairment of righting, placing and flexor reflexes, marked reduction in body temperature and respiratory distress preceding death.

Table 3: Acute toxicity (LD_{50}) of melatonin in animals (mg/kg/body weight)

Animal	Oral	Intravenous	Intraperitoneal	Subcutaneous
Mice (Male MFI)	1250 mg/kg	472 mg/kg	1168 mg/kg	>1600 mg/kg
Rats (Male S/D)	>3200 mg/kg	356 mg/kg	1131mg/kg	>1600 mg/kg

Repeated-dose toxicity

Rats

At doses of 15 mg/kg/day IV for 6 days there was no change in blood pressure, heart rate or body temperature. At IV doses of 5 mg/kg complete blood counts were not affected, but there was a significant increase in total protein and AST ($P<0.05$). At 15 mg/kg there was a significant increase in polymorphonuclear cells, a significant decrease in lymphocytes, mononuclear cells and platelets with a significant increase in creatinine, AST and LDH. It was also noticed that there was a significant decrease in body weight over both doses of approximately 5.5%. There was no evidence of organ toxicity (brain, kidney, liver and spleen).

In a 28-day toxicity study, Sprague-Dawley rats received 60 µl/day of vehicle (PEG 400) containing 0.03%, 0.3% or 3% melatonin SC, continuously for 28 days. The dose of melatonin delivered based on weekly group mean body weights (n = 10) was approximately 0.050, 0.50 and 4.8 mg/kg bw/day for the males and 0.074, 0.75 and 7.3 mg/kg bw/day for the females. No deaths or changes in clinical observations were observed. No substance-related effect was noted in body weights, haematology, clinical chemistry, urinalyses or gross pathology. In males, there was a trend toward decreasing serum prolactin concentrations with time at all doses. No difference in serum follicle-stimulating hormone concentrations occurred between treated groups. Most of the samples were at the limit of detection for the serum LH assay (0.157 ng/ml). A dose- related increase occurred in urine 6-sulphatoxymelatonin (the primary metabolite) concentrations in both sexes. No treatment-related organ weight or histopathology changes were noted in rats infused with 0.03% or 0.3% melatonin. Two of 10 males administered 3.0% melatonin had decreased testes weights and testicular degenerative changes composed of reduced or absent spermatogenesis, spermatidic giant cells and oedema.

Melatonin was administered by gavage at doses of 0, 0.005, 0.05; 5.0, 50 or 200 mg/kg bw/day to Long-Evans and Fischer 344 rats in a 90-day toxicity study. Dark-coloured faeces were observed in the two highest dosage groups (50 and 200 mg/kg bw/day). No treatment-related individual organ weight changes were observed during the study. However, mean weight gains over the entire study in all the female Long-Evans Melatonin treated groups were 7 to 10% less than control. A reduction in body weight gain was observed in Fischer rats, though only in dosages starting from 5 mg/kg bw/day. Increases in T3 and T4 were observed at dosages starting from 0.05 mg/kg/day, but these were not clinically significant, since no concurrent effects on thyroid histopathology were observed. Cystic uterine endometrial hyperplasia was observed in a number of treated Long-Evans female rats, but also in their respective control group. Finally, one treatment-related finding in a 50 mg/kg bw/day treated Long-Evans female was a dilated uterus at necropsy.

In a 90-day study in rats at doses of 0.3, 1.2 or 6 mg/kg/day, the only melatonin-related effect reported was a decrease in body weight gain of the animals at mid (males) and high doses (males and females). Decreased testis and increased kidney relative weights were also observed at high dose.

A combined 13-week study with a 4-week recovery period coupled to a 26-week toxicity and a 104-week carcinogenicity phase was conducted in the rat with dose levels of melatonin at 0, 15, 75 or 150 mg/kg/day. In the 13-weeks and the 26-weeks studies increased haemoglobin concentration and platelet counts were observed at 75 and 150 mg/kg/day. Increased liver weights with minor centrilobular hepatocytic hypertrophy were observed. Increased testes, prostate and epididymides weights were seen in mid and high dose males. At 26 weeks, macroscopically dark thyroid was also recorded in several high dose animals. Microscopically, minor liver hypertrophy was seen in some high dose animals but reported as less obvious than in the 13-week group.

<u>Dogs</u>
In a 6-month study in dogs, at doses of 0.4, 1.5 and 8 mg/kg, increased serum glucose levels were observed at some time points of the study. Microscopic examination revealed pituitary gland and parathyroid cysts, adenomyosis of the uterus, capsular fibrosiderosis of the spleen and cytoplasmatic rarefaction of hepatocytes consistent with the presence of glycogen.

Genotoxicity
***In vitro* Ames Test**

The mutagenicity of melatonin and its major metabolite 6-hydroxymelatonin were evaluated using a reduced Ames test, a bacterial reverse mutation test, using three strains of *Salmonella typhimurium*--TA 97, TA 98, and TA 100. Neither compound exhibited mutagenicity whether in the presence or absence of an activation system derived from rats induced with Aroclor 1254. Positive controls were employed throughout and gave the expected response. It was concluded that melatonin, 6-hydroxymelatonin, and their microsomal metabolites are not mutagenic in the Ames test.

The effect of melatonin on the mutagenicity of twelve well-known mutagens and carcinogens was investigated using an *in vitro* Ames test. The 12 mutagens used were 7, 12-dimethylbenz(a)anthracene(DMBA),benzo(a)pyrene,2-aminofluorene,1,2-dimethylhydrazine, bleomycin, cyclophosphamide, 4-nitroquinoline-N-oxide, 2,4, 7-trinitro-9-fluorenone, 9-aminoacridine, N-nitrosomethylurea(NMU), mitomycin C and sodium azide tested in the absence or in the presence of S9 mix. Melatonin alone turned out neither toxic nor mutagenic in the Ames test. In four *Salmonella typhimurium* tester strains TA 97, TA 98, TA 100 and TA 102, melatonin significantly reduced the mutagenicity of chemicals which require S9 activation.

***In vitro* COMET assay**

A Single Cell Gel Electrophoresis assay (COMET assay) was performed on CHOK1 cells. Melatonin itself revealed no genotoxic effect from this test. The SCGE assay showed a slight, but statistically significant ($P < 0.001$), dose-related anticlastogenic effect of melatonin (10-10-10-7 M) was observed. This therefore indicates that melatonin may act as an anti-initiating hormone in NMU-induced carcinogenesis and possess anticlastogenic activity towards NMU in CHOK1 cells.

***In vitro* Chromosome Aberration Test**

Cells in human peripheral blood were treated *in vitro* with increasing concentrations of melatonin (0.5 or 1.0 or 2.0 mM) for 20 min at 37 +/- 1 degrees C and then exposed to 150 cGy gamma-radiation from a 137Cs source. The lymphocytes which were pre-treated with melatonin exhibited a significant and concentration-dependent decrease in the frequency of radiation-induced chromosome damage as compared with the irradiated cells which did not receive the pre-treatment. The extent of the reduction in radiation-induced chromosome damage observed with 2.0 mM melatonin was similar to that found in lymphocytes pre-treated with 1.0 M dimethyl sulfoxide, a known free radical scavenger. Melatonin at 2.0 mM (a 500 x lower concentration) was as effective in decreasing the radiation-induced chromosome damage as dimethyl sulfoxide at 1.0 M.

Carcinogenicity

Four-week-old hemizygous TG.NK female mice with MMTV/c-neu oncogene fed NTP-2000 diet were gavaged with 0.05-0.2 ml of flaxseed oil as the source of omega-3 rich PUFA, or melatonin at 50-200 mg/kg or a combination of 0.10 ml flaxseed oil and 50 mg/kg melatonin for 30 weeks. Melatonin delayed the appearance of palpable tumours and the growth of the tumours with a dose-related statistically significant negative trend for the incidence of tumours. The combination of flaxseed oil and melatonin caused a significant decrease in the number of tumours and tumour weight per mouse compared to the control and to flaxseed oil but not to melatonin alone.

Female HER-2/neu mice starting from the age of 2 months were kept under standard light/dark regimen and were given melatonin with tap water (20 mg/l) during the night-time, 5 times monthly (interrupted treatments) or constantly to natural death. Treatment with melatonin slowed down age-related disturbances in estrous function mostly in the group

exposed to interrupted treatment with the hormone. Constant treatment with melatonin decreased incidence and size of mammary adenocarcinomas, and incidence of lung metastases, compared to controls. The number of mice bearing four and more tumours was reduced in the group with constant melatonin treatment. Interrupted treatment with melatonin promotes mammary carcinogenesis in HER-2/neu transgenic mice. The data demonstrate the regimen-dependent inhibitory effect of melatonin on the development of spontaneous mammary tumours in HER-2/neu mice but not on overall survival with implication about the likely cause of the effect.

Female Swiss-derived SHR mice were given melatonin with their drinking water (2 or 20 mg/l) for 5 consecutive days every month, from the age of 3 months until their natural death. The results of this study show that the treatment of melatonin did not influence the frequency of chromosome aberrations in bone marrow cells; it did not influence mean life span; and it increased life span of the last 10% of the survivors in comparison to controls. It was also found that treatment with low dose melatonin (2 mg/L) significantly decreased spontaneous tumour incidence (by 1.9-fold), mainly mammary carcinomas, in mice whereas higher doses (20 mg/L) failed to influence tumour incidence as compared to controls. For this reason, it was concluded that the effect of melatonin as a geroprotector is dose dependent.

Spontaneous mammary tumour incidence following melatonin administration was studied in an animal model for human breast cancer, C3H/Jax mice. A group of 39 mice received melatonin (dissolved in ethanol) in drinking water around the clock (25 μg/mouse/day from day 21 to day 44; 50 μg/mouse/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 tumour incidence; 62.5% of control animals developed tumours vs. 23.1% in the melatonin treated group ($P<0.02$).

A study investigated the effect of melatonin administration on the incidence of 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 tumours 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 tumours.

A study investigated the effect of melatonin on oestrogen-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 subcutaneous 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 tumour 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 tumour number was significantly lower than controls. However, when melatonin was administered during the entire promotion phase, both the incidence and numbers of tumours were significantly lower than controls. It was concluded that melatonin inhibits of NMU-induced rat mammary tumorigenesis by acting on the promotion rather than the initiation phase and that melatonin appears to have antiestrogenic properties.

Reproductive and developmental toxicity

Segment I/II – Fertility and Early Embryonic Development
In a study in female CD-1 mice (16/group) melatonin was administered, 100 µg [~3-4 mg/kg] IP) for 19 days prior to cohabitation. Melatonin–treated mice showed disruption of the normal oestrous cycle (longer cycles), primarily due to the greater number of days spent in diestrous. During cohabitation, the daily injection of females continued until mating was confirmed or until 2 weeks had elapsed, whichever occurred first. The proportion of mated females delivering was decreased for melatonin-treated mice (7/16 vs 13/16 for controls) but litter size from fertile matings was not affected.

A study in male Wistar rats administered melatonin 0.8, 2.4, 4.8, or 8.0 mg/kg SC for 30 days (at 1700 h) has suggested that melatonin may have an inhibitory action on the male rat prostate but only at the high dose of 8 mg/kg. Melatonin (8 mg/kg) caused a decreased prostate weight but not testes or other reproductive organs. Lower doses (0.8, 2.4 and 4.8 mg/kg) had no effect. Successive treatment with melatonin (8 mg/kg) produced no effect on testosterone levels in testes and serum nor on the conversion rate of [3H]testosterone to [^{3}H]dihydrotestosterone in prostate but caused a significant decrease in activity of acid phosphatase and uptake of [^{3}H]testosterone by the prostate.

A further study in male Wistar rats has suggested that melatonin inhibits the reproductive behaviour of male rats following melatonin treatment (3.0 or 8.0 mg/kg SC for 30 days at 1700 h) in comparison to vehicle-treated and untreated pinealectomized rats. 5/12 rats dosed at 8mg/kg melatonin did not copulate (compared to 2/12, 1/12 and 0/12 in the 3mg/kg, vehicle control and untreated pinealectomized groups, respectively).

Segment II – Embryofoetal Development
In a study in Balb-c mice, melatonin (200 µg/d [5.7 mg/kg/d in 35g mouse] IP) was administered daily throughout gestation, 3 hours before the end of the light period. The day of confirmed mating was designated as gestation day (GD) 1 and dams were terminated (10 vehicle and 10 treated per timepoint) on GD 6, 12 or 18. No significant differences in dam bodyweight during pregnancy or in the total number of foetuses, number of live foetuses, or number of abortions were reported. However, although mammary gland development, measured according to DNA content and concentration, did not differ on GD 6 or 12, it was significantly behind on GD 18.

Melatonin was administered by gavage to 25 timed-mated Sprague Dawley (CD) female rats on gestation day 6 to 19, at doses of 50, 100 and 200 mg/kg/day. No maternal deaths were observed, and the clinical signs reported were classified as minimal. Transient reduction of the body weight gain and relative decreased food intake were observed at the high dose group. Increased relative maternal liver weight was also observed in the animals from mid and high dose. Absolute liver and gravid uterine weights were not affected. The endpoints related to embryo/foetal growth, viability or morphological development were not modified by melatonin treatment. Based on the lack of embryo/foetal toxicity, the developmental toxicity NOAEL of melatonin was considered as 200 mg/kg/day. Based on the slight maternal toxicity reported at 200 mg/kg/day treated animals, the maternal toxicity NOAEL was considered as 100 mg/kg/day (NTP).

A study of the embryo-foetal development in the NZW rabbit with oral administration of melatonin at 0 (control), 15, 50 and 150 mg/kg/day from days 7 to 19 of gestation. There were no dose-related maternal effects at any dose. No effects were observed on pre or post-implantation loss and mean number of foetuses/female. Foetal, litter and placental weighs were not affected by treatment. Visceral and skeletal malformations and/or variations were observed in all groups including controls. Some of such malformations/variations showed a

trend or a significant increase in the treated groups, such as absence of lung or iliac alignment/caudal shift of vertebrae at high dose corresponding to an approximate AUC of 24000 to 45000 ng.h/ml. When compared to the AUC values to be achieved in man (<4 ng.h/ml), very high exposure ratios were reached in this study.

Segment III – Prenatal and Postnatal Development

A study in female Wistar rats (19-20/group) administered melatonin (2.5 mg/kg/d SC) throughout gestation has shown altered reproductive maturation of female offspring. Injections of melatonin were given 2 hours prior to the end of the light phase under a constant photoperiod (12:12, lights off at 1200). At birth, litters were standardised to 12 offspring per litter. Vaginal opening was significantly delayed in female offspring of melatonin-treated *vs* saline control rats (mean of 40.63 *vs* 37.25 days, respectively). On the day of vaginal opening, lower LH levels were observed in the melatonin group, but no effects were noted for bodyweight, melatonin levels, organ weights (absolute or relative for ovary, pineal, and pituitary), or % off-spring in each phase of the estrous cycle.

A subsequent study has investigated the reproductive development in both male and female offspring following gestational exposure to melatonin in rats entrained for 3 weeks to a 12:12 light:dark cycle with lights on at 2400 hours. Female Wistar rats (34-38/group) were injected (route not specified) with 2.5 mg/kg/d melatonin throughout gestation at the end of the light phase and allowed to deliver naturally. Melatonin exposure was associated with a significantly shorter gestational period (mean 20.9 *vs* 21.5 days for controls), but did not affect maternal weight gain, litter size, or male/female ratios per litter. Offspring were evaluated at 5 (neonatal), 15 (infantile), 25 and 30 (juvenile), or 55 (pubertal) days of postnatal age to evaluate developmental patterns for reproductive hormones. Plasma levels of LH and prolactin but not FSH were affected in female offspring. In male offspring, developmental patterns for all 3 hormones were affected.

In the study 24 pre-mated females were treated with 0, 15, 55 and 200 mg/kg/day of melatonin from Day 6 of gestation to Day 21 post-partum, inclusive. The treatment had no effect on parturition and the outcome of pregnancy, but the subsequent growth and viability of the high dose offspring was slightly reduced during lactation. At weaning, a slight reduction of offspring maturity was observed in all dose groups, but the subsequent F1 development was not modified. Therefore, melatonin intake during lactation should be avoided.

Other toxicity studies

In rats melatonin (5 mg/kg bw) was injected prior to a single dose of 10 mg/kg bw lipopolysaccharide (LPS) and thereafter at 6-h intervals up to 72 h. The number of micronucleated polychromatic erythrocytes increased significantly after LPS administration both in cells from peripheral blood and bone marrow. Melatonin administration to LPS-treated rats highly significantly reduced micronuclei formation in both peripheral blood and bone marrow cells beginning at 24 h after LPS administration and continuing to the end of the study (72 h). In blood, the increase in micronuclei formation was time-dependent in LPS-treated rats with peak values being reached at 36 – 48 h. According to the authors, the ability of melatonin to reduce LPS-related genotoxicity is likely related to its antioxidant activity.

In an *in vivo* micronucleus test in mice, the protection afforded by melatonin against paraquat-induced genotoxicity in both bone marrow and peripheral blood cells was tested using micronuclei as an index of induced chromosomal damage. Melatonin (2 mg/kg bw) or an equal volume of saline were injected intraperitoneally (i.p.) into mice 30 min prior to the i.p. administration of paraquat (2 injections of 15 mg/kg bw; given with a 24-h interval) and thereafter at 6-h intervals to the end of the study (72 h). Paraquat treatment increased the

number of micronucleated polychromatic erythrocytes (MN-PCE) at 24, 48 and 72 h, both in peripheral blood and bone marrow cells, while no differences were observed in the polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) ratio. Melatonin inhibited the paraquat-induced increase in MN-PCE by more than 50% at 48 and 72 h. The proposed mechanism of action of melatonin is its free radical scavenging ability.

Melatonin (10 mg/kg) or an equal volume of saline were administered IP to mice 30 min prior to an IP injection of paraquat (20 mg/kg x2), and thereafter at 6-h intervals until the conclusion of the study (72 h). The number of the MN-PCE increased after paraquat administration both in peripheral blood and bone marrow cells. Melatonin administration to paraquat-treated mice significantly reduced micronuclei formation in both peripheral blood and bone marrow cells; these differences were apparent at 24, 48 and 72 h after paraquat administration. The induction of micronuclei was time-dependent with peak values occurring at 24 and 48 h. The reduction in paraquat-related genotoxicity by melatonin is likely due in part to the antioxidant activity of the indole. No effects of melatonin over paraquat in paraquat+melatonin groups incubated at 0, 60 and 120 min were observed.

Overall conclusions on toxicology
The Applicant has adequately addressed the single dose toxicology. Discussion on repeat dose toxicity in rats for 6, 28 and 90 days has been provided. Additional data from a published source was provided on a combined 13- and 26-week toxicity study in rats and a 6-month study in dogs, as supportive information only. Given the intended short duration of treatment with melatonin, the data provided on repeat dose toxicity in rats can be accepted, discussion from the published literature has been provided on repeat dose toxicity in a non-rodent species.

The Applicant has provided adequate discussion on the potential for genotoxicity of melatonin concluding that melatonin is not mutagenic or clastogenic. The section on carcinogenicity describes transgenic mouse studies which do not indicate carcinogenic effect but rather a protective effect against mammary tumours. No discussion of long-term studies in rats is provided, however given the posology of melatonin this is acceptable.

The studies presented by the Applicant indicated that melatonin may have some effects on reproductive behaviour and sexual maturation. The SmPC, section 4.6 of the SmPC includes the appropriate wording and states that the use of melatonin by pregnant women and women intending to become pregnant is not recommended.

III.5 Ecotoxicity/Environmental Risk Assessment
A suitable justification for the absence of a full environmental risk assessment has been provided, based on the expectation that introduction of this product onto the market is unlikely to result in an increase in the combined sales of melatonin containing products, which in turn is unlikely to increase exposure of melatonin to the environment.

III.6 Discussion on the non-clinical aspects
The grant of a marketing authorisation is recommended.

IV CLINICAL ASPECTS

IV.1 Introduction
With the exception of the data from the bridging bioavailability studies, no new clinical studies were submitted, as the data submitted for this application is in the form of literature references.

Data from three pharmacokinetic bridging studies have been submitted. The studies were conducted in-line with current Good Clinical Practice (GCP).

The Applicant has performed a single-arm pharmacokinetic study, in order to obtain pharmacokinetic data and investigate the *in vivo* pharmacokinetic characteristics of the test product oral solution formulation, comparing them to available, extensive literature data.

To further bridge the literature reviews to the test product, the Applicant has performed an extensive statistical analysis in order to investigate similarity between the test product melatonin oral solution 1mg/ml and the reference product utilised in other bioequivalence studies conducted by the Applicant in the past. These two studies used the Hungarian product (Bio-Melatonin 3mg tablets) as comparator versus other oral solid melatonin immediate-release test formulations.

Background on condition to be treated

The Applicant has provided the following background on the condition to be treated:
The sleep-wake cycle may be pathologically affected in different ways. Furthermore, the sleep may also be disturbed by various processes. The disturbances of the sleep-wake cycle are called circadian rhythms disorders and include the jet lag (time zone change) syndrome.
Disorders of the sleep-wake schedule or Circadian Rhythm Sleep-Wake Disorders (CRSWDs) are classified under G47.2 of ICD-10 version 2016. The International Classification of Sleep Disorders (ICSD) 3rd Revision, 2014, includes Jet lag disorder (ICD-10-CM code: G47.25) in which insomnia might appear as a symptom.

Exogenous melatonin reportedly induces drowsiness and sleep and may ameliorate sleep disturbances, including the nocturnal awakenings associated with old age. Daytime administration of exogenous melatonin (when it is not present endogenously) promotes sleep in humans and results in sleep-like brain activity patterns at specific areas such as the precuneus and hippocampus.

Melatonin's two well-established physiological effects - promotion of sleep and entrainment of circadian rhythms - are both mediated by two specific receptor proteins in the brain, and not by the gamma-aminobutyric acid (GABA) receptors through which most hypnotic agents act. This difference probably explains why, unlike the GABA-agonist drugs, which are true "sleeping pills," exogenous melatonin does not suppress rapid eye movement (REM) sleep nor, in general, affect the distribution of sleep stages.

Jet lag disorder, also known as time zone disorder, is a common complaint of travellers who fly across a number of time zones. The symptoms of jet lag are primarily daytime fatigue and sleep disturbance, but also include loss of mental efficiency, weakness and irritability. Jet lag is caused by desynchronization between the body's circadian system and the new day-night cycle at the traveller's destination. The sleep loss caused by the travel itself often contributes to jet lag. After a flight through six or more time zones most travellers will take 4-6 days to re-establish a normal sleeping pattern and not to feel tired during the day. The severity of jet lag symptoms largely depends on the number of time zones crossed and the direction of travel. They are worse the greater the number of zones crossed. Westbound travel generally causes less disruption, as it is easier to lengthen than to shorten the natural circadian cycle. These symptoms consist of daytime fatigue, impaired alertness, insomnia, loss of appetite, poor psychomotor coordination, reduced cognitive skills, and depressed mood. Eastbound travel tends to cause difficulties in falling asleep, whereas westbound travel interferes with sleep maintenance.

The disruptive effects of jet lag have been documented at the molecular level of clock genes present in the SCN and peripheral tissues. Eastbound travel causes phase advances in the body's circadian rhythms, while westbound flight induces phase delays in circadian rhythms. As a consequence, jet travellers are forced to synchronize their bodily rhythms; synchronization occurs at a speed of approximately 1.5 h a day after westward flights and approximately 1 h a day after eastward flight irrespective of whether their travel occurs during daytime or night. Regardless of the direction of air travel, there is also travel fatigue due to factors such as the cramped seats, altered feeding schedule, poor air quality, and inability to sleep. These factors aggravate the symptoms of jet lag.

A number of pharmacological interventions have been tried to minimise the effects of jet lag. Treatment for jet lag disorder can begin before travel. Beginning to adjust the circadian clock to the new time zone before travel may be desirable for some travellers, especially if they want to be functioning at their best immediately upon arrival in the new time zone. Studies in the laboratory have shown that starting circadian interventions about 3 days before the day of travel, combining advancing the sleep schedule with appropriately timed bright light and melatonin administration can phase advance the circadian clock by about 2.5 hours and is also beneficial for sleep and well-being. The patient would start by altering their sleep – wake schedule and go to bed an hour earlier each day. They would also aim to get approximately an hour of bright light (four 30-minute pulses of 5000 lux) in the morning, and to take low-dose melatonin (1 – 3 mg) 5 hours before their usual sleep time.

The efficacy of melatonin in preventing or reducing jet lag has been reviewed in four systematic reviews that included numerous randomised controlled trials,

Melatonin, taken close to the target bedtime at the destination (10 pm to midnight), decreased jet lag from flights crossing five or more time zones. Daily doses of melatonin between 0.5 and 5 mg are similarly effective, except that people fall asleep faster and sleep better after 5 mg than 0.5 mg. Doses above 5 mg appear to be no more effective. The relative ineffectiveness of 2 mg slow-release melatonin suggests that a short-lived higher peak concentration of melatonin works better. The benefit is likely to be greater the more time zones are crossed, and less for westward flights. In summary, Melatonin is effective in preventing or reducing jet lag, and its occasional, short-term use appears to be safe. It can be used by adult travellers flying across five or more time zones, particularly in an easterly direction, and especially if they have experienced jet lag on previous journeys. Travelers crossing 2-4 time zones can also use it if needed.

IV. 2 Pharmacokinetics

Published data were provided to support the application. An acceptable overview of the PK of melatonin was presented.

As there is currently an approved melatonin product, Circadin, informal reference is made to the SmPC for this product with regard to the PK data in order to avoid inconsistencies between the two products in the reported PK characteristics. Circadin was approved more than 10 years ago and the data exclusivity period has expired, however it is not probable to expect that the clinical efficacy and/or safety data might be extrapolated from Circadin (prolonged release tablets) to the current oral solution product. In clinical studies, melatonin was typically administered orally, sublingually, or intravenously. Until now, the PK of melatonin has primarily been investigated in healthy volunteers following oral and intravenous administration of melatonin, but findings have been inconsistent. Melatonin is synthesised in the pineal gland during the dark phase of the light/dark cycle and is rapidly delivered to the body via the systemic circulation. Tryptophan is converted to serotonin (5-hydroxytryptamine), then acetylated (N-acetylserotonin) and finally converted to melatonin,

which is an indole (N-acetyl-5 methoxytryptamine). Several studies have been performed and have demonstrated that the dynamic pattern of melatonin secretion is fundamental for its time-giving function. The peak of melatonin levels is reached in the middle of the night (between 2 - 4 a.m.) and decrease to low levels in the second half of the night. In young adults, the average daytime levels of melatonin are 10 pg/ml and the peak night-time level is 60 pg/ml. Endogenous production of melatonin is reduced in the elderly. The rhythmic pattern of melatonin secretion is important because it brings to organisms information about time that allows them to adapt some of their physiological functions to the daily and seasonal variations of their environment.

Absorption

The absorption and bioavailability of orally administered (exogenous) melatonin in humans has been extensively reported in the literature. Melatonin is rapidly absorbed following oral administration of instant release forms, with T_{max} ranging between 0.25 - 1.0 hours. A recent study has shown oral melatonin to have a $T_{1/2}$ as low as 6 minutes.

In some high oral dose studies of melatonin, the average absorption half-life for an 80 mg oral dose, when administered to five adult volunteers, was seen to be 24 minutes (range 19 - 29) with peak serum levels 350 - 10,000 times higher than the endogenous night-time peak within 60 - 150 minutes of dosing was observed. Following the administration to a single adult female of a high oral dose of melatonin (75 mg), peak serum levels of 110 ng/ml approximately 300 minutes post dosing were observed. However, significant intra-subject variability has been reported in a number of studies, in exposure parameters has been reported within the literature. In the case where 2 x 3 mg immediate release tablets were evaluated in pre and post-menopausal healthy female volunteers, the rate of absorption, C_{max}, ranged from 2.827 to 29.289 ng/mL in premenopausal women and from 1.892 to 40.488 ng/mL in postmenopausal women, whereas for AUC all values ranged from 2.640 to 39.735 ng·h/mL and 3.072 to 53.132 ng·h/mL for pre- and postmenopausal women, respectively. In a study, high plasma melatonin concentrations were determined also in the case of melatonin formulated as an oral solution.

In studies to ascertain the absolute bioavailability of two strengths of oral melatonin dosing (2 mg and 4 mg), it was found that the absolute bioavailability of melatonin was only approximately 15 %. However, this study showed that there was little between-subject variability.

Subject and gender variability following an oral melatonin solution of 250 µg was reported in another study. The absolute oral bioavailability ranged from 1 to 37 % (mean ± SD values: 8.6 ± 3.6 % for males and 16.8 ± 12.7 % for females, respectively). D7 melatonin was used and the authors were able to separate exogenous melatonin from endogenous melatonin. From a retrospective analysis on multiple studies that used intravenous melatonin or oral preparations (but not both in the same subjects) and estimated oral bioavailability to range from 3 to 76 %.

Basal serum melatonin levels were studied in conjunction with the administration of a low oral dose of melatonin (0.3 mg) in a group of healthy young adults (mean age 29.2 ± 6.5 years) and in a group of older adults (mean age 60 ± 8.8 years). Serum melatonin levels were measured at 30 minutes intervals over a 10-hour period. Time to peak melatonin levels was 48 ± 4.9 minutes in the younger group and 45 ± 6.7 minutes in the older group. Systemic exposure parameters, C_{max} and AUC (mean ± SD), did not differ significantly between the younger and older groups: 170.2 ± 22.0 pg/ml versus 254.9 ± 45.7 pg/ml and 441.9 ± 21.07 versus 595.8 ± 12.09 pg/ml.h, respectively. Peak melatonin levels following administration of 0.3 mg melatonin were significantly greater than that observed during endogenous secretion:

170.2 versus 101.1 and 254.9 versus 49.4 pg/ml, young and old groups respectively.
A comparison of the endogenous and exogenous melatonin levels was conducted in 23 healthy subjects, 12 young and 11 older adults, of both genders. In the same blood sample, they were able to distinguish endogenous melatonin from exogenously administered D7 melatonin, a molecule in which seven deuterium atoms replace seven hydrogen atoms. All subjects participated in two experiments: one with 250 μg of oral D7 melatonin at midday and, after a washout period of 1 week, one with 250 μg of oral D7 melatonin at midnight. In addition, the young subjects participated in a third study, involving a 23- mg D7 melatonin infusion. Significant gender differences and between subject variability in exposure parameters were reported. Following oral dosing with 250 μg of D7 melatonin, mean ± SD C_{max} was 243.7 ± 24.6 pg/ml and 623.6 ± 575.1 pg/ml, whereas AUC was 236±07 pg.h/ml and 701 ± 45 pg.h/ml, in males and females respectively. However, there were no significant differences in total body clearance normalised to body weight: 1.27 ± 0.20 L/h/kg and 1.18 ± 0.22 L/h/kg for males and females respectively.

In a cohort crossover study, the pharmacokinetic parameters of oral and intravenous (i.v.) melatonin in healthy volunteers were investigated. The volunteers received either 10 mg oral melatonin or 10 mg intravenous melatonin on two separate study days. Blood samples were collected at different time points following oral administration and short i.v. infusion, respectively. Twelve male volunteers completed the study. Baseline melatonin plasma levels did not differ significantly between the study days (P = 0.067). It was concluded that the bioavailability of oral melatonin was only 3 %.

A randomised controlled-placebo trial, demonstrated that exogenous administration of melatonin with a loading dose of 3 mg (as solution through subjects' feeding tube), followed by an hourly dose of 0.5 mg, results in supraphysiological and sustained concentrations of serum melatonin during 12 hours overnight in subjects (in critically ill patients). These findings support the notion that despite a first-pass effect or pharmacological interactions on the enteral absorption of melatonin in critically ill patients, the enteral administration of melatonin is a feasible option with excellent oral bioavailability.

Oral solution of melatonin (10 mg) was also administered in patients, who had undergone a tracheostomy in a randomised double-blind placebo-controlled trial. Melatonin appeared to be rapidly absorbed from the oral solution and peak concentrations were higher than those reported for comparable doses in healthy individuals. After oral dosing, the C_{max} is affected by the solubility of melatonin in the formulation, alterations in bioavailability, and clearance. Orally administered melatonin is subject to an extensive 'first-pass effect', with bioavailability reported to be approximately 15 %, although there is high variability due to factors such as cytochrome P450 1A2 (CYP1A2) activity and co administration of interacting drugs.

Bioavailability
Oral melatonin is almost completely absorbed from the gastrointestinal tract, though bioavailability is low (~ 15%) due to extensive first-pass metabolism. Variability in the extent of first-pass metabolism appears to be the primary process underlying inter-individual variability in bioavailability.

Based on literature data, the 3 – 6 mg oral dose of immediate-release melatonin can be expected to have a T_{max} of ~ 50 minutes. T_{max} is essentially constant with typical therapeutic doses of melatonin. The 3 and 6 mg oral dose of immediate-release melatonin can be expected to result in AUC values of ~ 380 and ~ 680 ng/mL·min respectively, though values are subject to considerable inter-individual variation. The 3 mg oral dose of immediate-

release melatonin can be expected to result in a C_{max} of ~ 3400 pg/mL. This value is ~ 60-times the peak nocturnal (endogenous) plasma melatonin C_{max}, though both values are subject to considerable inter-individual variation.

Melatonin is a highly permeable drug that is almost completely absorbed from the gastrointestinal tract, and as the high solubility of the drug substance has also been shown, it is considered a BCS-class I drug. Measurement of absolute bioavailability is not useful in this situation because of high (and variable) first pass metabolism. Data on renal excretion support the high permeability of melatonin (extent of absorption ≥ 85 %). A paper reports that 'over 90 % of the administered radioactivity ([β-14C melatonin) was recovered in the first 24 h urine sample and the remainder in the next 24 h.' This is considered sufficient to support absorption as >85%, as required to use a BCS biowaiver. However, a BCS biowaiver is not applicable in the case of this Melatonin 1mg/ml oral solution application as it is an oral solution, hence the Applicant has provided a bridge to the literature studies and their product by performing a single-arm pharmacokinetic (PK) study and 2 pivotal studies as supportive data.

The Applicant investigated the *in vivo* PK characteristics of the test product melatonin oral solution 1 mg/mL (3 ml solution), in order to obtain PK data and investigate the *in vivo* PK characteristics of the test product oral solution formulation, comparing them to available, extensive literature data. A bioavailability single-arm PK study was conducted in healthy volunteers after single oral administration of the test product under fasting conditions. The Applicant has provided a comparison between the PK results of the bioavailability study and the data available for a number of liquid and solid oral formulations containing melatonin, evaluated in the 2 pivotal studies.

STUDY- single arm PK study
This study was an open label, single-treatment, single-period, single-dose, bioavailability study of the test product Melatonin 1mg/ml oral solution in healthy adult human subjects under fasting conditions.

PIVOTAL STUDY 1
An open label, balanced, randomised, two-treatment, three-period, three-sequence, partial replicate, crossover, single oral dose, comparative bioavailability study of Melatonin 3 mg coated-tablets with the reference product Bio-Melatonin® 3 mg film-coated tablets in healthy, adult, human subjects under fasting conditions.

PIVOTAL STUDY 2
An open label, balanced, randomised, two-treatment, four-period, two-sequence, full replicate, crossover, single oral dose, comparative bioavailability study of a Melatonin 3 mg solid dosage form versus the reference product Bio-Melatonin® 3 mg film-coated tablets in healthy, adult, human subjects under fasting conditions.

Table 4: Summary of pharmacokinetic data for Melatonin 1mg/ml Oral Solution
The following table summarises the pharmacokinetic parameters of the test product. For baseline corrected data, primary pharmacokinetic parameters are C_{max}, AUC_{0-t} and AUC_{0-inf} secondary parameters are AUC_{0-t}/AUC_{0-inf}, Residual area, T_{max}, Kel and $t_{1/2}$.

Pharmacokinetic parameter	Arithmetic Means (±SD)-Test product
AUC(0-t) (pg*hr/mL)	11839.216 ± 9780.866
AUC(0-inf) (pg*hr/mL)	11990.474 ± 9962.512
Cmax (pg /mL)	8774.235 ± 4922.787
tmax (hrs)	0.333 (0.167, 1.000)
Kel	0.915 ± 0.335
t1/2 (hrs)	0.817 ± 0.195
Residual Area (%)	0.959 ± 0.743
AUC0-t/ AUC0-inf Ratio (%)	99.041 ± 0.743

Table 5: Summary of the variability in pharmacokinetic results of pivotal study 1 and pivotal study 2 versus the reference product

Study	Total Variability		Intrasubject Variability	
	AUC_{0-t}	C_{max}	AUC_{0-t}	C_{max}
Pivotal study 1	90.6%	92.0%	36.7%	43.3%
Pivotal study 2	132.4%	126.4%	39.3%	51.6%

The analysis of the plasma melatonin concentrations was performed using a validated method. The concentration of samples was found to be above the upper limit of quantification (ULOQ), these samples were repeated by applying dilution under code A, the clinical overview describes this method sufficiently. Testing of the samples was repeated using dilution factors. Concentrations of all these samples after dilution were found within the validated curve and concentrations were found below ULOQ. The Applicant has provided a comparison between the PK results of the bioavailability study and the data available for a number of liquid and solid oral formulations containing melatonin, including the 2 pivotal studies (refer to tables 4 and 5 above).

Based on literature data, it appears that linear PK may be observed for the strengths from 1 mg to 10 mg. The mean values of the PK parameters AUC, C_{max}, t_{max} and $t_{1/2}$ obtained in the study single-arm pharmacokinetic bioavailability study were compared to the mean values calculated using the literature data available for melatonin at the strengths 0.25 mg - 10 mg, although non-linearity was observed at strengths <1 mg.High variability was observed in the single-arm PK study as the CV% was 82.6 and 56.1 for AUC and C_{max}, respectively. In addition, in some literature data a CV% >100 appears to be estimated for both C_{max} and AUC.

The Applicant has provided a discussion of the high variability observed for the PK of melatonin. The intrasubject variability appears lower than the total variability observed in the studies conducted by the Applicant. Regarding the comparison with the published data, the mean values seems to be within the range of variability reported in literature.

Faster absorption (lower median t_{max} value) is observed for the test oral solution compared to the literature for both liquid and solid oral formulations.

Moreover, the Applicant has provided a comparison between the mean plasma concentrations-time profiles observed in the study and those available in the literature at strengths from 0.1 mg to 10 mg and extrapolated to the 3 mg dose. PK profiles available in

both fasting and fed condition were included in the comparison although the food seems to affect the systemic exposure of melatonin.

Although high variability is acknowledged for melatonin (further discussion on this is provided below), overall the shape of the mean PK curve seems to be consistent with those observed for a number of other liquid oral formulations. However, the C_{max} appears higher than most of the products included in the comparison.

In addition, higher bioavailability and systemic exposure (AUC and C_{max}) seems to be expected compared to the majority of the oral solution and solid dose formulations. It appears that the C_{max} is expected to be reached earlier (lower t_{max}) after administration of the test oral solution with respect to the literature data. However, the PK profiles extrapolated at strengths <1 mg (i.e. outside the linearity range) may not be a reliable representation of the expected plasma melatonin concentration-time profile at the dose 3 mg.

The studies included for the pharmacokinetic comparisons displayed high variability with respect to both AUC and C_{max}. This is in accordance with literature data. The high total variability seems to be almost equally distributed among inter- and intrasubject variability, with ~ 30-50 % of the total variability being attributed to within subject variability. The remaining 50-70 % is therefore attributed to differences between individuals.

The intrasubject variability, which contributes to about 50-70 % of the total variability, can be attributed to a number of factors. Melatonin exhibits unstable absorption from the gastrointestinal tract and extensive first-pass hepatic metabolism. Consequently, its oral bioavailability varies widely among subjects. The variations observed in clinical studies may be caused by individual differences in absorption, distribution, metabolism (CYP1A2 activity) and elimination of melatonin. No significant relation (direct ‘’dose-response relationship’’) between specific melatonin plasma concentration levels and actual clinical effects (or adverse effects) has been fully established; several different formulations and posology have been administered in clinical studies and have been proven effective and safe. The differences in study designs, dosages, drug formulations and melatonin assay analysis (different methods of analysis) may also potentially affect the variability of the pharmacokinetic parameters and result in some cases, in values >100 (CV % (coefficient of variation)). Regarding the use of different analysis assays, this might be a significant factor contributing to the observed variability of the results. In summary, the reported variability of the single-arm pharmacokinetic study is on the lower end of reported variability values for melatonin, either in other studies or literature data, possibly due to the nature of the formulation (oral solution).

The variability of melatonin was taken into consideration in the statistical comparison between the single-arm study and other studies.

Regarding the comparison with literature data, although the variability is high, the mean values lie within the range reported in literature. For example, the F% value of the single-arm study, which has been calculated using the DeMuro intravenous data, is 29 %. In literature the mean value is 20 % ± 14%. Taking into consideration the limitations of this methodology, this value does not have a statistically significant difference from literature data. For all PK parameters, with the exception of T_{max}, the reported values for the single-arm PK study remain within the ±SD range of literature values. Furthermore, the high variability of melatonin PK parameters in literature is offset by the high number of studies, which provides some assurance that the pharmacokinetic profiles of the product under assessment is within the range of values reported on the public domain.

Although the high variability of melatonin makes comparisons within and between studies more difficult, the wide therapeutic range of the molecule ensures that there are no safety concerns for the patient. Some degree of individualisation of treatment may be required, which is already described in the SmPC.

The conducted *in vivo* clinical program focused on investigation of comparative bioavailability and PK is sufficient to bridge the new formulation in terms of efficacy and safety, to the melatonin products administered in the clinical trials of this bibliographic dossier. Appropriate SmPC wording have described the PK characteristics of the oral solution product.

The Applicant has further discussed the link between the melatonin pharmacokinetic data obtained for their oral solution and the data provided in the literature studies for oral solution and solid dose formulations. Overall it appears that up to an approximately 2-fold increase in the systemic exposure of melatonin may be expected after administration of melatonin 3 mg oral solution compared to the PK data available in literature for other oral formulations. This is briefly discussed below.

Table 6: Normalised pharmacokinetic data for melatonin, excluding fed studies.

Dose (mg)	Number of administrations pooled	C_{max} (pg/ml) [normalised to 3 mg]	T_{max} (min)	$T_{1/2}$ (min)	AUC (pg*min/ml) [normalised to 3 mg]	F %
1.0-2.0	6	4594	43	47	409,245	17
3.0-4.0	9	5928	33	57	601,812	24
5.0-6.0	8	5763	40	66	554,471	23
10.0	2	4492	30	88	459,004	19

It is generally accepted that melatonin has linear PK over the 1-10 mg range, and possibly even lower. According to the Public Assessment Reports and SmPCs of EU approved products, the following linearity ranges have been accepted by the EU authorities:

Table 7: Linearity range reported in the SmPCs of the EU approved melatonin products

Melatonin products	Linearity Range (mg)
Melatonin Orion 3 mg kalvopäällysteiset tabletit (Finland 2016, Orion Oyj)	2-8
Melatonin Vitabalans 3 mg & 5 mg tabletit (Finland 2016, Vitabalans Oy)	0.1-8
Melatonin Pharma Nord 3 mg film-coated tablets (Netherland 2018, Pharma Nord Aps)	3-6 (according to SPC) & 0.1-5 (according to PAR)
Melatonine Tiofarma 1 mg, 3 mg, 5 mg tabletten (Netherland 2018, Tiofarma BV)	0.1-10
Circadin XL 2 mg Tablets (Neurim Pharma, EU Procedure No. EMEA/H/C/695, 2007).	2-8

The Applicant has performed a literature search to retrieve relevant information for 0.1 mg dose. A study mentioned in a literature review depicts 0.1 mg, 0.5 mg, 1 mg and 5 mg being administered to four groups of eight healthy adults. The authors concluded that the mean peak plasma level increased in a dose-dependent manner, however, no mention to AUC has been made. Both studies demonstrated that the plasma levels of melatonin increased in a dose-proportional manner.

It is reasonable to assume, that the administration of sub-therapeutic doses of melatonin (< 1 mg) would result in unreliable AUC calculations, due to the more pronounced effect of endogenous melatonin levels. This is a possible explanation for the lack of linearity at this range.

Literature data on the effect of food at or around the time of intake of melatonin on its PK are limited. A literature review depicts a study that has determined plasma concentrations of melatonin in fasting and fed conditions. The study demonstrated that melatonin concentrations were higher in the fed than in the fasted state with both preparations examined (oral solution and immediate release (IR) capsules), however the study documented similar T_{max} values between these groups and $T_{1/2}$ ranged from 32 (fasting state) to 40 min (fed state). For the oral solution the T_{max} values under both conditions were the same (30 min).

The increased AUC in the fed state is unlikely to be of clinical significance particularly in view of the large inter-individual variations.

Additionally, the SmPCs of the EU approved Melatonin products state the following, regarding the administrative conditions of melatonin:

Melatonin products	Method of administration
Bio-Melatonin, (Pharma-Nord, Hungary, 2003)	The presence of food delays the absorption of melatonin
Melatonina Medicplast 5 mg Tabletki (MedicPlast zoo, Poland, 2003)	The food increases the absorption of melatonin
Melatonin-LEK-AM 1 mg, 3 mg & 5 mg Tabletki (Lek, Poland, 2005)	The food increases the absorption of melatonin
Tonasen, 5 mg, tabletki (Poland 2003)	The food increases the absorption of melatonin
Melatonin Orion 3 mg kalvopäällysteiset tabletit (Finland 2016, Orion Oyj)	The food decreases the absorption of melatonin and the T_{max}
Melatonin Vitabalans 3 mg & 5 mg tabletit (Finland 2016, Vitabalans Oy)	Melatonin should be taken 2 hours before or 2 hours after food consumption
Melatonin Pharma Nord 3 mg film-coated tablets (Netherland 2018, Pharma Nord Aps)	Tablets should be swallowed whole with fluid. It is recommended that food is not consumed approximately 2 h before and 2 h after intake of melatonin
Melatonine Tiofarma 1 mg, 3 mg, 5 mg tabletten (Netherland 2018, Tiofarma BV)	Melatonin tablets can be taken with or without food. However, it is recommended that no food is consumed 2 h before to 2 h after taking melatonin.
Circadin XL 2 mg Tablets (Neurim Pharma, EU Procedure No. EMEA/H/C/695, 2007).	The presence of food delays the absorption of melatonin resulting in later and lower plasma concentration in the fed state.

It should be noted that the most recently approved EU products as depicted in the table above (Melatonin Pharma Nord 3 mg Tablets and Melatonin Tiofamra 1, 3, 5 mg tabletten) recommend avoiding food consumption 2 hours before to 2 hours after taking melatonin. The same recommendation has also been made for this product.

It is acknowledged that food delays and increases the absorption of melatonin, the Applicant has included a warning on the concomitant food intake during melatonin administration. The proposed warning, which is in accordance with other melatonin products is considered adequate to avoid administration under fed conditions.

Bioequivalence

Different oral dosage forms of melatonin have been used in the cited literature articles and clinical trials. Melatonin oral solution has been studied in a number of articles; however, the majority of the trials have been conducted with immediate release tablets. PK data to bridge the Applicant's Melatonin 1mg/ml oral solution to products used in the studies from the literature is provided in the bioavailability section. The Applicant has submitted acceptable justification for waiving the requirement for bioequivalence studies to support this bibliographic application.

In order to bridge the literature data with the proposed pharmaceutical product, the following facts should be taken into consideration:

Melatonin satisfies the criteria to be classified as a highly soluble. It has linear PK over the 1 mg to 10 mg dosage range. Melatonin has a high permeability. The relatively low bioavailability is attributed to the extensive first pass effect.

The Applicant undertook a validated in-vitro study to assess the extent of permeability of melatonin across Caco-2 monolayers and efflux from Caco-2 cells. The data showed that Melatonin is highly permeable in the apical to basolateral direction within the Caco-2 cells and when compared with the reference propranolol, it suggests that human intestinal

absorption of melatonin would be greater than 90 % (Papp 40.3 x 10-6 cms-1 for A2B, Papp 39.9 x 10-6 cms-1 for B2A).

Melatonin is therefore a BCS I active substance, with linear PK. The pharmacokinetic profiles of immediate release oral dosage form, such as oral solution and tablets, should be comparable.

Distribution
A study has reported that melatonin reaches all tissues of the body within a very short period. Melatonin half-life is bi-exponential, with a first distribution half-life of 1.4 min and a second of 28.4 minutes. Melatonin released to the cerebrospinal fluid via the pineal recess attains, in the third ventricle, concentrations up to 20 – 30 times higher than in the blood. These concentrations, however, rapidly diminish with increasing distance from the pineal, thus suggesting that melatonin is taken up by brain tissue. The author depicted how in one healthy volunteer, bolus i.v. administration of 14C melatonin was shown to rapidly cross the Blood Brain Barrier, interact with brain structures and quickly disappear from the brain, suggesting rapid diffusion and turnover.

It has been estimated that the mean steady state volume of distribution (Vdss) in healthy adult volunteers, following an intravenous infusion of D7 melatonin, to be 0.98 L/kg distribution. No gender difference in the Vdss normalized to body weight was observed: 0.99 ± 0.063 L/h/kg and 0.97 ± 0.13 L/h/kg in males and females, respectively.

The *in vitro* plasma protein binding of melatonin is 61.2 %. Melatonin is mainly bound to albumin, alpha1- acid glycoprotein and high-density lipoprotein. The level of melatonin binding appears to be constant over range of different serum concentrations. Data from the literature indicates that melatonin is distributed in all body fluids and is accessible at all tissues. The mean binding of melatonin to erythrocytes is 49.0 %. Melatonin is not strongly or extensively bound to plasma proteins, therefore protein binding effects on PK should not be expected to be significant.

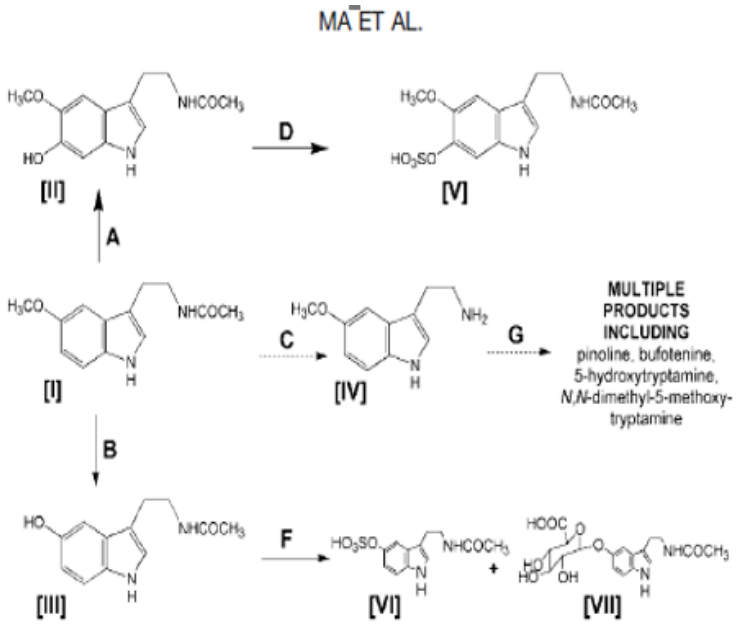
The plasma protein binding of exogenous melatonin is expected to be in the range 50 – 60%, with the upper value more representative of binding associated with the peak plasma melatonin concentrations achieved during the first 1 – 2 h post-ingestion, and the lower value more representative of melatonin concentrations approaching physiological levels. Melatonin binds primarily to albumin, a high-capacity, low affinity plasma binding protein, with limited binding to other plasma proteins, though high-affinity binding to a minor fraction of circulating α1- acid glycoprotein has been reported. Melatonin is not strongly or extensively bound to plasma proteins, therefore significant effects of protein binding on melatonin PK are not expected.

Metabolism
The literature provides information regarding the metabolic fate of melatonin. Circulating melatonin is metabolised primarily in the liver where it is first hydroxylated in the C6 position by cytochrome P450 mono-oxygenases (isoenzymes CYP1A2, CYP1A1 and, to a lesser extent, CYP1B1) and thereafter conjugated with sulphate to be excreted as 6-sulfatoxymelatonin (aMT6S); glucuronide conjugation is extremely limited. CYP2C19 and, at lower rates, CYP1A2 also demethylate melatonin to N- acetylserotonin, being otherwise its precursor. It has also been suggested that melatonin may be developed as an alternative to caffeine as a probe drug for CYP1A2 phenotyping.

The metabolism in extrahepatic tissues exhibits substantial differences. Tissues of neural

origin, including the pineal gland and retina, contain melatonin-deacetylating enzymes, which are either specific melatonin deacetylases or less specific aryl acylamidases; as eserine-sensitive acetylcholinesterase has an aryl acylamidase side activity, melatonin can be deacetylated to 5-methoxytryptamine in any tissue carrying this enzyme. Melatonin can be metabolized non-enzymatically in all cells, and extracellularly, by free radicals and a few other oxidants. It is converted into cyclic 3-hydroxymelatonin when it directly scavenges two hydroxyl radicals. From one of the studies reviewed, it appears that repeated dose administration does not alter the metabolic profile of melatonin.

Figure 1 the common metabolic pathways of melatonin:



A substantial fraction of melatonin is metabolised to kynuramine derivatives in the brain. This is of interest as the antioxidant and anti-inflammatory properties of melatonin are shared by these metabolites, N1-acetyl-N2- formyl-5-methoxykynuramine (AFMK) and, with considerably higher efficacy, N1-acetyl-5-methoxykynuramine (AMK). AFMK is produced by numerous non-enzymatic and enzymatic mechanisms; its formation by myeloperoxidase appears to be important in quantitative terms.

Experimental data suggest that isoenzymes CYP1A1, CYP1A2 and possibly CYP2C19 of the cytochrome P450 system are involved in melatonin metabolism, with the liver being the primary site of metabolism. The primary melatonin metabolite is 6-hydroxymelatonin, which constitutes ~ 80 – 90% of melatonin metabolites recovered in the urine. 6-hydroxymelatonin is almost exclusively excreted in conjugated forms: sulphate (~ 70%) and glucuronide (~ 30%). Metabolism of melatonin to 6-hydroxymelatonin is rapid, with serum / plasma 6-hydroxymelatonin level rising within minutes of exogenous melatonin entering the systemic circulation. N-acetylserotonin, conjugated with glucuronide or sulphate, appears to be the primary minor metabolite. 5-methoxytryptamine, 2-hydroxymelatonin, and kynuramine derivatives are other more minor metabolites; little is known about their PK or possible PD actions.

Excretion and elimination

The primary metabolite of melatonin, 6-sulphatoxymelatonin, accounts for 80 % of the dose excreted in the urine. The other main metabolite results from melatonin O-demethylation, yielding N-acetylserotonin.

Following intravenous infusion of 23 mcg, total body clearance in healthy males and females was 1.27 ± 0.20 L/h/kg and 1.18 ± 0.222 L/h/kg respectively. The half-life of melatonin

following single intravenous and oral doses in healthy volunteers has been reported to be approximately 1 hour. Elsewhere, the elimination half-life has been reported as 43.6 minutes following intravenous administration in human subjects. It has also been determined that the half-life following an intravenous infusion to be 36.0 and 41.4 minutes in males and females respectively, and after oral dosing, 36.0 and 45.0 mins, respectively.

One author used a population pharmacokinetic turnover and surge-function model for describing the circadian disposition of melatonin in healthy male subjects. A median acrophase at 04:00 was observed, although their model estimated typical value was at 02:00. The elimination half-life was estimated to be 2.7 h, longer than 0.5 to 1.0 h reported after exogenous intravenous and oral melatonin administration to healthy adults. This difference may reflect the continuous formation and release of melatonin while hormone synthesised earlier was undergoing elimination from the bloodstream, thereby leading to an underestimation of the terminal phase slope.

Special populations

The PK of oral IR melatonin in the range 0.3 – 6 mg is generally comparable in younger and older adults, though the range of values for a given parameter tends to be greater in the elderly. There do not appear to be significant differences in the PK of oral IR melatonin in men and women, though C_{max}, AUC and F may be higher in women; no such tendency is evident for $T_{½}$. Data for the influence of race and genetic factors are limited but are not considered to suggest any major concerns for efficacy or safety. Limited data indicate that hepatic impairment can reduce the clearance of exogenous melatonin, though the data are inadequate to allow correlation between degree of hepatic impairment and impact on clearance. Data regarding the effect of renal impairment on the clearance of exogenous melatonin are not available, though only a very small fraction (less than ~ 1%) of exogenous melatonin is excreted untransformed in urine. However, as melatonin metabolites are predominantly excreted in the urine, renal impairment can be expected to reduce their elimination.

As melatonin is metabolised mainly by CYP1A isoenzymes there is potential for PK interactions with other drugs for which CYP1A is an important route of metabolism. Drugs that have been found to reduce metabolism of exogenous melatonin – resulting in a clinically significant increase plasma melatonin level – include fluvoxamine, methoxypsoralens, and estrogens, with caffeine having a lesser effect. Other drugs that, due to their route(s) of metabolism, can also be expected to reduce metabolism of melatonin, but for which no data for exogenous melatonin were identified, include quinolones (such as ciprofloxacin) and cimetidine. Drugs that, due to their capacity to induce cytochrome P450 isoenzymes, can be expected to increase metabolism of melatonin – and thus increase plasma melatonin level – but for which no data for exogenous melatonin were identified, include carbamazepine and rifampicin. No PK interactions clearly involving drug transporters were identified.

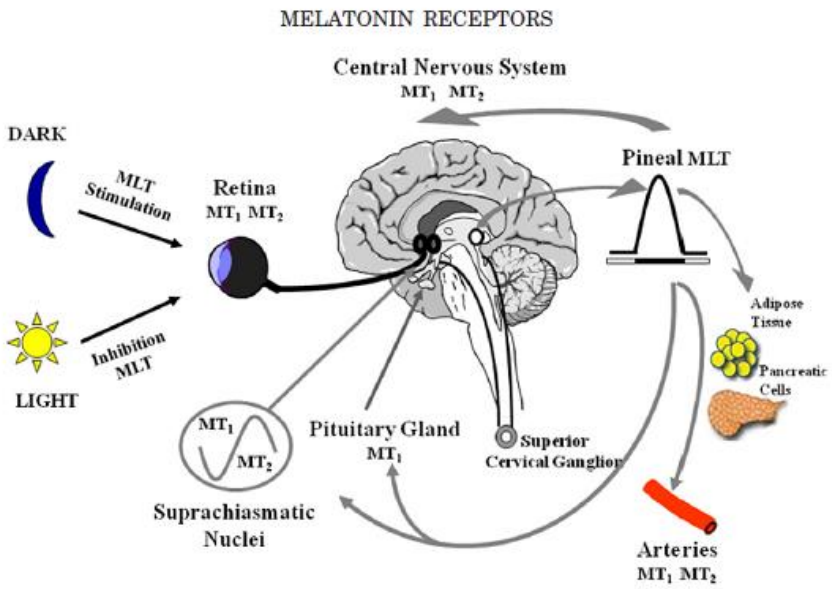
IV.3 Pharmacodynamics

The clinical PD properties of melatonin is well established. No new PD data have been submitted for this application and none were required. The PD section of the clinical overview is considered acceptable. A bridge to the supporting literature has been established and the literature review that has been submitted can be accepted. The literature evidence presented in the clinical overview is sufficient to demonstrate the safety of the individual active ingredients for the proposed indications.

PD data derived from the literature is presented below.

The mechanism of action of melatonin can be seen in the figure below which describes the regulation of melatonin production and receptor function. Neuron signals form SCN follow a multi-synaptic pathway to the superior cervical ganglia. The precise mechanism of action of melatonin is not known, although it seems that MT1 receptors in the SCN and MT2 receptors in the retina and the hypothalamus are involved. However, other mechanisms of action, including those that do not involve the MT1 and MT2 receptors, cannot be excluded (e.g. serotonin receptors in the SCN).

Figure 2: The mechanism of action of melatonin



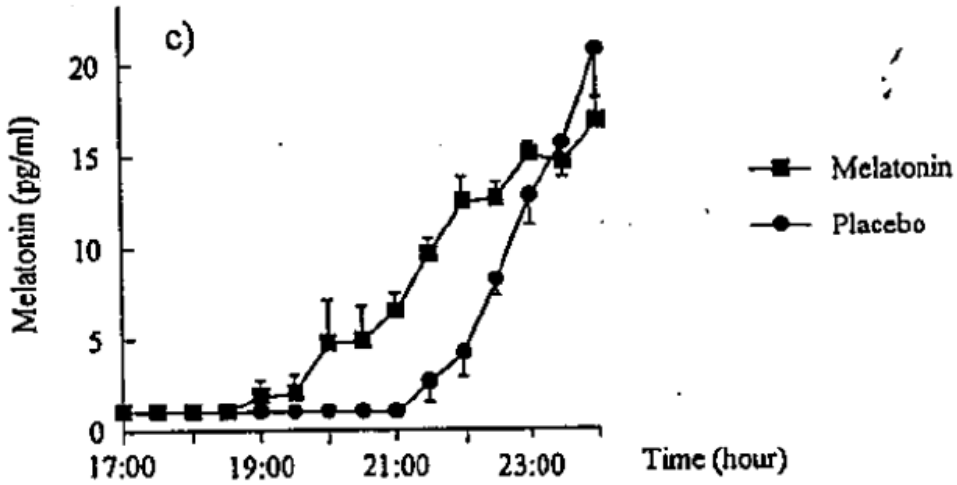
Primary Pharmacology

Exogenous melatonin can be expected to limit and treat symptoms of jet lag by hastening the re-entrainment of circadian rhythmicity, including the rhythmicity of endogenous melatonin secretion, and via a direct sleep-inducing / maintenance action. Oral melatonin at typical pharmacological doses shifts circadian rhythms in humans according to a phase response curve that is nearly opposite the phase-response curves for light exposure. Melatonin delays circadian rhythmicity when administered after dim light melatonin onset (DLMO) and advances it when administered before DLMO.

Effects on endogenous melatonin rhythmicity

A crossover study depicted in a literature review, examining the association between reduction in core body temperature and the acute phase-shifting effects of melatonin found that 5 mg oral IR melatonin administered to 8 young men at 17:00 hr significantly advanced DLMO (mean ± SD) 1.14 ±0.49 h) the following day (see *Figure 3 below*). The degree of phase-shift was correlated with the degree of temperature decrease.

Figure 3: Time course of endogenous melatonin secretion as assessed by saliva melatonin concentration during the evening after administration of 5 mg melatonin at 17:00 hr the previous day.



An author reported in a study of melatonin for jet lag in which 10 women and 7 men ingested 5 mg melatonin (probably IR) or placebo at bedtime for 2 days pre-flight, on the day of departure, and for 4 days post-flight (eastward over 8 time zones), found resynchronisation of melatonin rhythm (determined via measurement of the primary urinary melatonin metabolite at the end of the treatment) to be more rapid in subjects receiving melatonin.

A second study of melatonin for jet lag in which 26 men and 10 women ingested 5 mg melatonin (probably IR) or placebo at bedtime for 7 days following eastward flights crossing 6 – 7, 8 – 9, or 10 – 11 time zones did not find a clear effect of melatonin on the rate of re-synchronisation of endogenous melatonin rhythm (determined via measurement of salivary melatonin and the primary urinary melatonin metabolite at the end of the 5-day treatment phase).

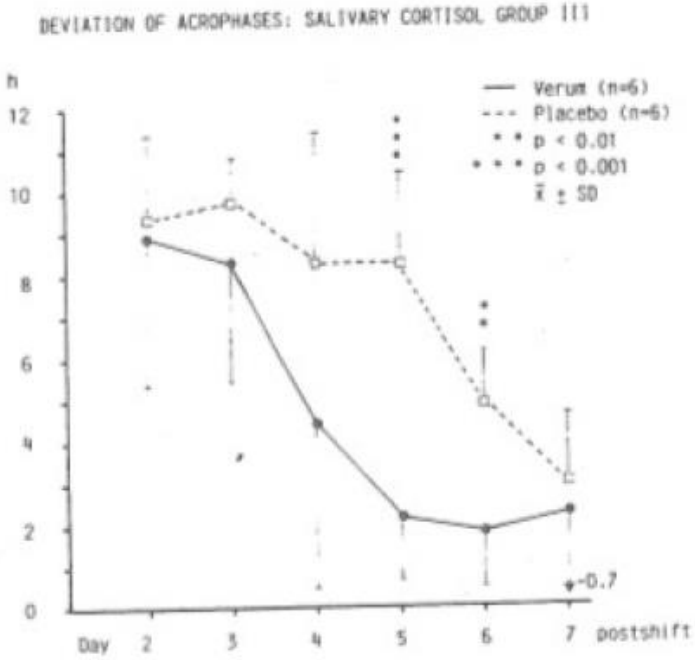
A study in which an open study of melatonin for jet lag found intake of 3 mg melatonin at bedtime (23:00 hr) on Days 2 – 4 after an 8-h eastward flight to result in more rapid resynchronisation of endogenous melatonin rhythm than when the same subjects had not taken melatonin on a previous, comparable flight (daily shift in re-entrainment 76 minutes with melatonin vs. 31 minutes without).

Effects on cortisol rhythmicity

A study has reported that administration of melatonin during the day has been found to advance the phase but not to have a significant effect on the amplitude of diurnal plasma cortisol profile.

Figure 4 below depicts the results found in a study that reported the effect of 5 mg oral melatonin on the mean post-shift acrophase of salivary cortisol relative to the baseline (pre-shift) acrophase following an eastward flight over 10 – 11 time zone.

Figure 4



A third jet lag study found ingestion of 5 mg melatonin daily before, during, and after an eastward flight over 7 time zones to hasten resynchronisation of cortisol rhythm during the 4 days post-flight.

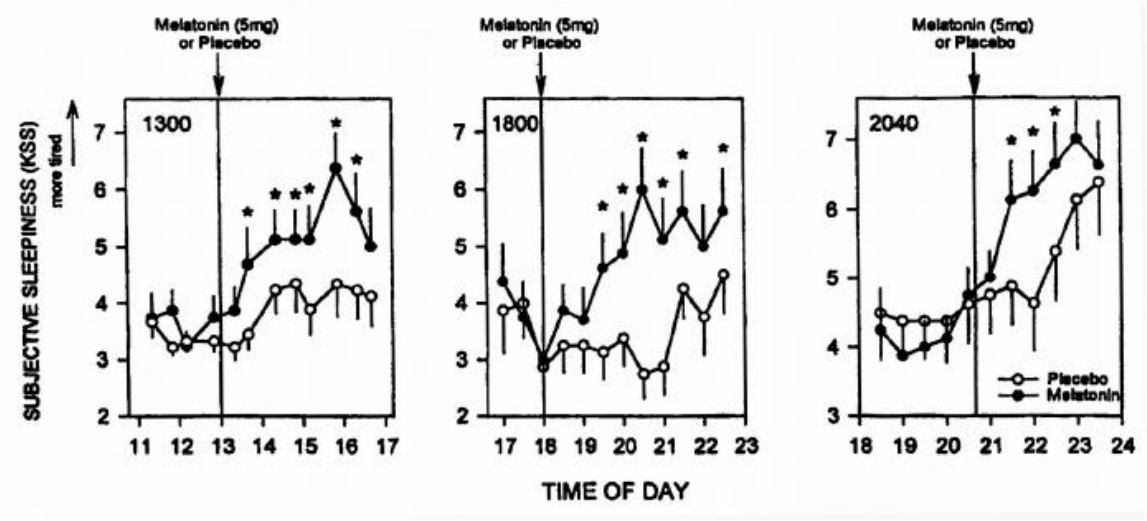
Sleep-inducing / maintenance effects

A study examined the effect of melatonin on daytime sleepiness and theta-alpha activity in the waking electroencephalography (EEG) in 2 crossover RCTs. In one experiment, 8 young men ingested 5 mg IR melatonin or placebo at 13:00 hr, while in a second experiment 8 young men ingested 5 mg IR melatonin or placebo at 18:00 hr. The subjects had to remain in supine position and awake from 10:00 to 17:00 hr or from 16:00 to 23:00 hr in the respective experiments. During these periods, half hourly self-ratings of fatigue (Visual Analog Scale, VAS) were obtained, and subjects completed the Akerstedt (Karolinska) Sleepiness Symptoms Check List and the Akerstedt (Karolinska) Sleepiness Scale. Waking EEG power density was measured in the range 0.25 – 20 Hz; measurement of EEG whilst awake permits repeated, non-invasive assessment of sleepiness. Subjective sleepiness increased following ingestion of melatonin, reaching significance 40 minutes after intake at 13:00 hr and 90 minutes after intake at 18:00 hr, and in lasting for 3 h and 5 h, respectively. Alpha-theta activity in the EEG increased immediately after ingestion of melatonin (appearing before the subjective symptoms of sleepiness were manifest) and remained elevated in parallel with the increase in subjective sleepiness. There was a significant correlation between the increase in salivary melatonin level and the timing of the increase in subjective sleepiness.

In a third experiment, generally comparable in design to the first 2 experiments, 8 young men received 5 mg melatonin or placebo at 20:40 hr and were asked not to close their eyes for 3 h before and 4 h after administration. The author reported the increase in alpha-theta activity in the EEG with melatonin did not differ significantly from that seen for placebo, probably as endogenous melatonin level had started to increase at the time of administration. Self-rated sleepiness was, however, significantly greater with melatonin. Subjective sleepiness when melatonin was administered at the 3 time-points is presented in *Figure 4*. The author also reported that heat loss due to melatonin (5 mg at 13:00 hr) was reduced when posture

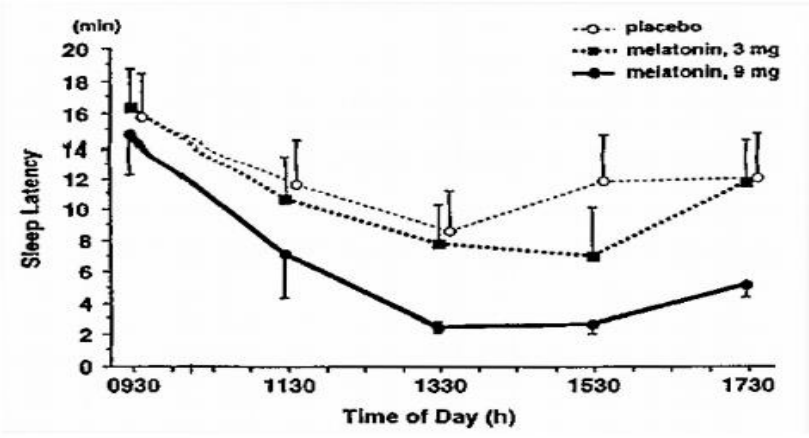
changed from supine to an upright position, and that the postural change counteracted melatonin's soporific effect, reflected both in subjective measures of sleepiness and in the EEG power spectrum.

Figure 5 Effect on subjective sleepiness as rated by the Karolinska Sleepiness Scale of 5 mg melatonin ingested at 13:00, 18:00 and 20:30 hr**.** Values are mean ± SEM, n = 8; * = p < 0.05 compared to placebo



A crossover study investigated the hypnotic / sleep-inducing and hypothermic action of 3 or 9 mg IR melatonin or placebo ingested at 09:30 hr by 6 young men (mean age 22.5 years). A multiple sleep latency test (MSLT) was performed every 2 h after administration of melatonin until 17:30 hr. Core body temperature (CBT) was recorded every 5 min from 10:00 to 17:30 hr. The subjects were seated throughout the study. The 9 mg dose significantly reduced sleep latency while a tendency for a reduction (p = 0.08) was seen with the 3 mg dose (see *Figure 5*).

Figure 6 Effect on sleep latency of ingestion of 3 or 9 mg melatonin at 09:30 hr. Values are means ± SEM; n = 6.

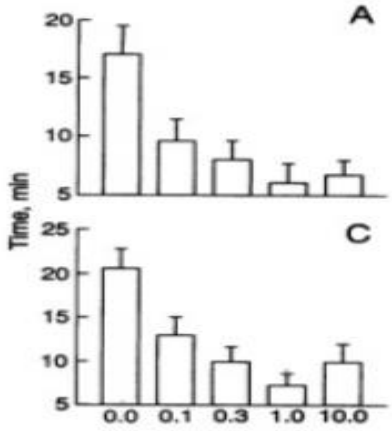


In a crossover study examining the effects of 5 mg oral melatonin (probably IR) and/or exposure to bright light (5000 lux from 21:00 to 24:00 hr) in 10 young men (mean age 27 years), the normal evening rise in sleepiness was accelerated after ingestion of melatonin and protracted by bright light, with these changes reflected in alterations in the theta-alpha range

of EEG. Melatonin shortened sleep latency and rapid eye movement (REM) sleep latency, and improved sleep quality, whereas bright light increased sleep latency. Melatonin could not cancel the phase-delaying effect of the bright light exposure.

In another study designed to investigate the effect of increasing pharmacological doses of melatonin on sleepiness, body temperature, mood and performance, 22 healthy men (23 ± 4 years) ingested 0.1, 0.3, 1.0 or 10 mg IR melatonin or placebo at 11.45 hr during 5 test session (from 09:30 to 17:30 hr) separated by 5 days (balanced Latin square design). A sleep test was performed between 13:30 and 14:00 hr, and a performance task and mood questionnaire were completed. Melatonin reduced SOL, and increased sleep duration and self-reported sleepiness significantly in a dose-dependent manner, though the effect of the 10 mg dose was less than that of the 1 mg dose.

Figure 7 Effect on measured sleep onset latency (A) and self-reported sleep onset latency after ingestion of placebo or melatonin doses in the range 0.1 – 10 mg at 11:45 hr. Values are means± SEM; n = 20.



Another crossover study found administration of 5 mg IR melatonin at 17:00 hr to 8 men (23 – 28 years) to improvement subjective sleep quality.

In summary, oral melatonin has been found to reduce sleep onset latency, with some evidence that it improves sleep quality and sleep architecture. The sleep-inducing effect is evident regardless of the time of administration, though not unexpectedly there is a degree of attenuation with administration around DLMO. The effects of oral melatonin on sleep may be mediated by a reduction in core body temperature, and possibly also by potentiation of the effects of gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter of the CNS44, via direct interaction with GABA receptors.

The capacity of oral melatonin to improve sleep parameters in jet lag is addressed in the efficacy section. In brief, 5 of 7 studies found a significant improvement (1 a strong tendency) in sleep quality, and 3 of 5 studies a significant improvement in sleep onset latency and daytime tiredness. A jet lag study examining 0.5 and 5 mg doses of oral IR melatonin reported a dose-dependent reduction in sleep onset latency and the 'ease' with which subjects fell asleep.

A series of randomised, placebo-controlled studies in young adult healthy subjects indicates that saliva/serum peak levels of melatonin occur within 60-90 minutes and elevated levels may last as long as 8 hours after ingestion of 3-9 mg of exogenous melatonin. In these studies, 5-9 mg of melatonin seems to result in increased sleepiness and reduced (REM)

sleep latency. In a study depicted in the literature reviews, an effect on sleep duration was also observed.

No significant effects were found for 3 mg melatonin. These studies thus suggest that 5-9 mg melatonin is likely to result in a dose-dependent reduction of sleep latency.

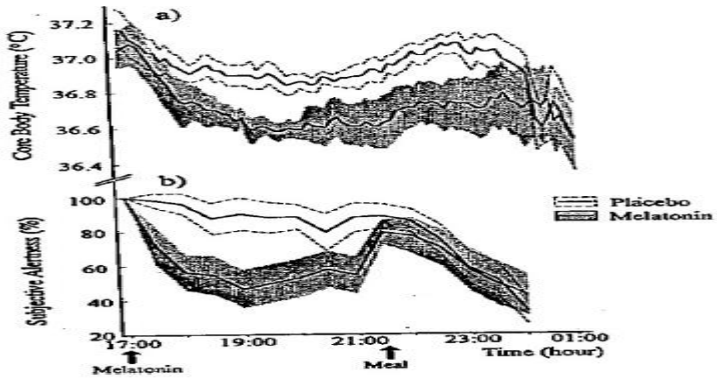
Effect on body temperature

The circadian rhythm of CBT in humans is characterised by maximum values during the day and minimum values during the night and is normally coupled with the sleep-wake cycle. For example, in young, healthy women, serum melatonin remained at a low and constant level between 08:00 and 20:00 hr while body temperature gradually increased to peak at 18:20 hr, after which it declined and serum melatonin level increased, with a strong inverse correlation. The latter changes preceded the onset of sleep by ~ 3 h. Melatonin reinforces the nocturnal decrease of CBT, an event which facilitates sleep propensity. Melatonin receptors have been identified in peripheral vasculature, such that the nocturnal decrease CBT may be at least partly due to peripheral vasodilation (and subsequent heat loss) mediated by melatonin receptor stimulation.

A study summarised in the previous section, which found single oral doses of 0.1 – 10 mg IR melatonin administered at 11:45 hr to 22 young men to shorten sleep onset and enhance sleep duration, also found melatonin to decrease oral temperature in a significant, dose-dependent manner.

In a cross-over study designed to examine the association between the acute phase-shifting effects of melatonin and reduction of CBT, 5 mg IR melatonin or placebo was administered to 8 healthy men (23 – 28 years). Each phase of the study was performed over 5 days. Days 1 and 2 served to establish normal sleep-wake cycle, on Day 3 subjects received melatonin or placebo at 17:00 hr. On Day 3 and Day 4 subjective drowsy-alert scores were rated, while sleep quality was rated for the nights of Days 2 – 4. Rectal temperature was measured continuously from 08:00 hr on Day 2 until 08:00 hr on Day 5. Melatonin induced an acute, transient reduction in CBT between 17:00 and 24:00 hr, with ANOVA identifying a significant treatment effect on temperature between 18:12 and 20:30 hr, with a mean ± SD) fall in temperature of 0.26 ± 0.19 °C (see *Figure 7*). A significant treatment effect on alertness between 17:00 and 2100 hr was also identified, with mean ± SD) reduction of 31.1 ±1.1%.

Figure 8 Time course of suppression of core body temperature and subjective alertness following ingestion of 5 mg melatonin.



An author depicted the results from a cross over study that studied the hypothermic effect of 0.1, 0.5, 1.0 or 5.0 mg IR melatonin on CBT in 21 men and 11 women. Following 8 h bed

rest (08:00 – 16:00 hr) melatonin was ingested at 16:00 hr. CBT was measured during the 8 h prior to intake of melatonin and for 4 h afterwards. T_{max} for blood melatonin did not differ significantly between the 4 melatonin doses. Fisher's PSLD analysis indicated that, in general, CBT was reduced in a significant, dose-dependent manner by melatonin: mean maximum decline in CBT was 0.08, 0.15, 0.20 and 0.25 ^{o}C for the 0.1, 0.5, 1.0 and 5.0 mg doses, respectively. As CBT did not decline in some subjects receiving the 2 lowest doses, the authors suggested that to improve sleep onset and maintenance melatonin doses between 1.0 and 5.0 mg should be used.

A crossover study in which 6 young men ingested 3 or 9 mg IR melatonin or placebo at 09:30 hr found both doses to significantly decrease CBT, with the greatest effect between 11:30 and 12:00 hr; the effect was not dose dependent. The study also found that melatonin induced a significant reduction of sleep latency, and it was suggested that exogenous Melatonin exerts its circadian phase-shifting effect via a transient hypothermic action.

In summary, the studies depicted in the literature reviews the effect of exogenous melatonin on the melatonin receptors on the peripheral vasculature may result in peripheral vasodilatation and reduction of the core body temperature, which in turn may cause a circadian phase shift and sleep induction. In a series of randomised, placebo-controlled trials convincing data were presented showing dose dependent (0.1-10 mg melatonin) reductions in CBT with significant body temperature changes only for doses between 1- 10 mg melatonin. One study reported that a lowering of the core body temperature preceded the onset of sleep by 3 hours.

Secondary and safety pharmacology

In addition to its role as a chronobiotic, including its capacity to hasten re-entrainment of circadian rhythmicity following rapid transmeridian travel (i.e. its proposed use in the treatment of jet lag), *exogenous* melatonin has been found (or been suspected) to affect the nature of a number of physiological processes. The potential for exogenous melatonin to adversely affect the trio of systems in the core safety battery (i.e. nervous, respiratory, and cardiovascular systems) is limited, as reflected in the fact that such effects have generally not been raised as concerns in reviews addressing the safety of melatonin (see section on safety). The submission dossier provides a detailed bibliographic review of secondary PD actions of exogenous melatonin and its effects on metabolic function (specifically glycaemic control), cardiovascular function, immune system function, reproductive function, sexual development, and analgesic effect.

Overall conclusion on the Pharmacodynamic effects of melatonin

Melatonin is a hormone, produced primarily by the pineal gland, that has an important role in regulating circadian rhythms. The circadian pacemaker ('body clock') in the SCN of the hypothalamus modulates melatonin synthesis and secretion from the pineal gland. Once melatonin appears in the plasma it enters the brain and binds to melatonin receptors (MT1 and MT2) in the hypothalamus (and other areas of the brain), forming a feedback loop. The high density of melatonin receptors in the SCN suggests that melatonin affects the sleep-wake cycle by acting on these receptors. The presence of melatonin receptors in multiple sites in the body, and the capacity of many tissues to synthesise melatonin (that does not appear to enter the systemic circulation) indicates important roles of melatonin.

The circadian production of melatonin underlies its chronobiotic influence on biochemical and physiological processes including the endocrine and non-endocrine rhythms. The most significant regulatory factor in the production of pineal melatonin is the daily alternation of light and darkness; (day)light registered and signalled by the retina suppresses melatonin

synthesis. In humans, the circadian rhythm of pineal melatonin release is highly synchronised with the habitual hours of sleep, and the daily onset of melatonin secretion correlates with the subsequent onset of nocturnal sleepiness.

Regarding primary PD, exogenous melatonin can be expected to limit and treat symptoms of jet lag by a) hastening the re-entrainment of circadian rhythmicity, including the rhythmicity of endogenous melatonin secretion, and b) via a direct sleep inducing/ maintenance action.

Oral melatonin at typical pharmacological doses shifts circadian rhythms in humans according to a phase-response curve that is nearly opposite the phase-response curve for light exposure. Melatonin delays circadian rhythmicity when administered after DLMO and advances it when administered before DLMO. The former occurs when melatonin is taken following a westward flight, while the latter occurs following an eastward flight. Likewise, administration of melatonin during the day advances the phase of the diurnal plasma cortisol profile. Peak in melatonin production coincides with the nocturnal trough in CBT, and the evening fall in CBT is strongly correlated with the rise in plasma melatonin level following DLMO.

Oral melatonin reduces sleep onset latency, with some evidence that it improves sleep quality and sleep architecture (percentage REM sleep). The sleep-inducing effect is evident regardless of the time of administration, though – not unexpectedly – there is a degree of attenuation with administration around DLMO. The effects of oral melatonin on sleep may be mediated by a reduction in CBT, and possibly also by potentiation of the effects of gamma-aminobutyric acid (GABA) via direct interaction with GABA receptors.

Regarding secondary PD and safety pharmacology, typical therapeutic doses of melatonin do not appear to adversely affect the 'core safety battery' (i.e. nervous, respiratory, and cardiovascular systems). Limited data suggests that ingestion of melatonin in close proximity to a meal (especially if carbohydrate-rich) may transiently impair glycaemic control, though longer-term (minimum 3 weeks) ingestion (typically at bedtime) of melatonin does not appear to adversely affect fasting blood- glucose or lipids in healthy persons, nor fasting blood- glucose, HbA1c or lipids in Type 2 diabetics. Exogenous melatonin does not appear to have an adverse effect on the immune system in general, though limited data suggest that it may potentially exacerbate or induce autoimmune disorders. Typical therapeutic doses of melatonin may have mild analgesic and anxiolytic effects, though neither is considered a concern in relation to use of the Applicant's product. Melatonin is a potent free radical scavenger and has anti-inflammatory effects via downregulation of pro-inflammatory cytokines, inhibition of nitric oxide and methylenedioxyamphetamine (MDA) production, and modulation of prostaglandin profile.

As melatonin is the body's primary phase-setting hormone, changes in the timing of melatonin secretion can be expected to affect the diurnal rhythm of other hormones, including reproductive hormones. There is good evidence that administration of melatonin during the day can alter the *timing* of the diurnal secretory patterns of other hormones, however typical therapeutic doses of melatonin have, at most, only a slight, transient effect on the *level* of other hormones. Ingestion of typical therapeutic doses of melatonin for 4 weeks is not associated with adverse changes to female or male endocrine function, particularly reproductive endocrinology. This also appears to be the case with longer-term use of the melatonin-receptor agonist, ramelteon.

The synthesis and presence of melatonin at multiple sites in the ovary and testes reflect its potential role in intra-, auto-, and paracrine regulation of reproductive physiology, though

effects of melatonin on reproductive processes (such as ovulation and fertility) in humans appears attenuated compared to animals. No clear adverse effects on female or male fertility, or on pregnancy, have been observed with typical therapeutic doses of melatonin, though data are limited. When effects have been seen (at very high doses), they tend to be consistent with the capacity of endogenous melatonin to decrease gonadotrophin (LH, FSH) production. Exogenous melatonin has been found to ameliorate oxidative stress in luteinised granulosa cells, and to have beneficial effects when taken by women undergoing IVF treatment.

Administration of melatonin to pregnant rats and ground squirrels has been found to delay sexual maturation of their male and female offspring, and to reduce reproductive organ weights in the latter species. These data suggest that exogenous melatonin may influence the ontogeny and activation of the hypothalamic-pituitary-gonadal (HPG) axis. These species are seasonal breeders, in which the annual rhythmicity of melatonin secretion modulates gonad hypertrophy and reproductive activity in general. It is not known to what extent exogenous melatonin modulates the HPG axis pre-puberty in humans, which are not seasonal breeders. Limited clinical studies in children have not confirmed any safety concerns, however specifically designed studies have not been performed.

IV.4 Clinical efficacy

No new efficacy data have been submitted for this application and none were required. The clinical efficacy of melatonin is well known and adequately discussed in the clinical overview. The literature reviews have adequately demonstrated the efficacy for the jet lag indication.

The Applicant has provided an appropriate package of information to justify well established use of melatonin in the EU in the claimed indication. The Hungarian product Bio-melatonin has been approved for substantially more than 10 years. The prescribing data clearly shows extensive use of this and other immediate release melatonin products, including in countries such as the UK where there is no licensed product, so that melatonin is supplied according to national rules on the supply of unlicensed medicines. The high degree of scientific interest in the use melatonin in jet lag was already accepted as demonstrated by the extensive literature. All of the requirements for the demonstration of a well-established use of melatonin, required for an application under Article 10a of Directive 2001/83, are considered to be fulfilled. The literature reviews submitted as part of the bibliography dossier include reviews and summaries of the key published studies. The bibliographic dossier is considered to be sufficiently comprehensive and its content adequately justified.

Ten randomised, placebo-controlled trials to evaluate the effect of oral melatonin in preventing or alleviating jet lag associated with transmeridian air travel were included in the review. These studies, plus several more published subsequently, form the core of the bibliography submitted in support of this application for the jet lag indication which are briefly described below in relation to clinical efficacy.

Randomised controlled trials in jet lag

Study 1

The impact of various dosage forms of melatonin and placebo on jet lag symptoms was evaluated by an author in a double-blind, randomised trial. The efficacy of melatonin was evaluated by electronic medication event monitoring system and questionnaires. The study showed that 5 mg melatonin significantly alleviated the jet lag syndrome, improved self-rated sleep quality, shortened sleep latency and reduced fatigue. Additionally, melatonin proved more effective than a slow-release formulation (2 mg controlled release formulation). Lower (0.5 mg) physiological doses were almost as effective as pharmacological doses (5.0 mg).

Only the hypnotic properties, such as sleep latency, were significantly greater with melatonin 5.0 mg.

Study 2
An author studied the effects of slow-release caffeine (SRC) and melatonin on sleep and daytime sleepiness after a seven-time zone eastbound flight. In a double-blind, randomised, placebo-controlled study, each of three groups of nine subjects was given either 300 mg SRC on recovery day 1 (D1) to D5 (0800) or 5 mg melatonin on pre-flight D-1 (1700), flight day D0 (1600), and from D1 to D3 (2300), or placebo (placebo) at the same times. Night-time sleep was evaluated by polysomnography and daytime sleepiness from measurements of sleep latencies and continuous wrist actigraphy. Compared with baseline, they found a significant rebound of slow-wave sleep on night 1 (N1) to N2 under placebo and melatonin and a significant decrease in rapid eye movement sleep on N1 (placebo) and N1–N3 (melatonin). Sleepiness was objectively increased under placebo (D1–D6) and melatonin (D1–D3). SRC reduced sleepiness but also tended to affect sleep quality until the last drug day.

Study 3
The efficacy of oral melatonin in alleviating jet lag in flight crew after a series of international flight has been investigated in a literature review. A double-blind placebo-controlled trial resulted in reduced feelings of jet lag and a more rapid recovery of sleep and energy levels. The timing of melatonin dose seems also crucial. In aircrew returning from a duty that includes a large number of time-zone changes over 1 week or more, melatonin taken a few days prior to returning home results in a worse adjustment. One explanation for this finding is that it may be caused by the natural circadian rhythm being so disrupted at this end of the duty that melatonin started before arrival does not re-entrain unless it is taken in the context of a stable day-night cycle. Another possible explanation comes from recent work that suggests melatonin shifts circadian rhythms according to a phase-response curve.

Study 4
In another study by the same author as study 3, subjects taking melatonin reported less jet lag and took less time to recover from their shift across 12 time zones. Subjects reported also that they were less tired during the day and required less time to establish a normal sleeping pattern and reach their normal level of energy. The lack of adverse side effects in subjects taking melatonin suggests that it is well tolerated at the dose used.

Study 5
A study examined melatonin’s ability to transduce light-dark information, its hypnotic effects in man and its low toxicity in a double-blind study. Subjects took a daily dose of melatonin (5 mg in gelatine lactose) or placebo. Subjects were asked to rate their jet lag on a 10 cm visual analogue scale from 0 (insignificant) to 100 (very bad). Jet lag was deliberately not defined as its nature and severity vary from person to person, but it was considered to be present at scores of 50 or above. Fisher's exact test for small sample sizes indicated that jet lag was significantly less severe among subjects treated with melatonin. In another study of the same group, it has been reported that in sensitive individuals, melatonin can induce rapid drowsiness after late afternoon ingestion and hence detection of treatment. Most subjects reported no significant jet lag. The rate of resynchronisation of aMT6s rhythms was consistent with that previously reported in an earlier study.

Study 6
The effects of oral melatonin in alleviating jet lag and its effects on subjects who had flown from London to Eastern Australia, 10 time-zones to the east, have been also examined in a

study. Melatonin (5 mg/ day-1) or placebo capsules were administered to 14 experimental and 17 control subjects, respectively, in a double-blind study; the time of administration was in accord with the current consensus for maximizing its hypnotic effect. The greatest amount of adjustment occurred in the first 3 days. There was also a significant time-of-day effect, jet lag being higher in the afternoon and evening than in the morning and at noon. The authors hypothesized that melatonin works only in those individuals in whom fatigue is high and motivation is low; in the current study, all subjects were motivated to be active in the new environment, and many were determined to `throw off' any negative effects due to sleep loss, for example.

Study 7
A new rating scale for measuring severity of jet lag was validated by an author in a randomised, double-blind trial of placebo and three alternative regimens of melatonin (5.0 mg at bedtime, 0.5 mg at bedtime, and 0.5 mg taken on a shifting schedule) for jet lag. Despite the finding of no group differences, the validity of the measures (summary jet lag item and total jet lag score) is supported by their ability to demonstrate gradual improvement in the severity of jet lag over time.

Study 8
In this study, the combined use of slow-release caffeine and melatonin improved several jet lag symptoms during an eastbound flight. For travel of 11 - 13 hours, whether eastbound or westbound, available data from limited field studies indicate that a combination of melatonin, exposure to outdoor light, and exercise have a potent ameliorative effect on jet lag symptoms.

Study 9
Sedentary volunteers (75 subjects crossing 13 time zones on an eastbound flight from Sydney to Buenos Aires, and 49 subjects on a westbound flight from Buenos Aires to Sydney, both by a transpolar route) were selected for investigation. Passengers on the eastbound flight received 3 mg of melatonin daily 30 minutes before their expected bedtime at Sydney, beginning on the day of the flight and continuing throughout the period of their trip. All subjects were advised to perform their normal routine and to walk outdoors for at least 30 minutes at two restricted times of the day. Passengers on the westbound flight took 3 mg melatonin on the day of their flight to Buenos Aires at the expected sleeping time at Buenos Aires and continued it for 8 days in Buenos Aires. On reaching Buenos Aires, all volunteers were advised to perform their normal routines and to walk outdoors for at least 30 minutes at the same two restricted periods of the day as in Sydney. Subjects were also advised to maintain sleep diaries throughout the period of study. The sleep log diaries included the evaluation of sleep quality, morning freshness, and daily alertness on a visual analogue scale. The mean resynchronisation rate was 2.27 ± 1.1 days during the eastbound flight and 2.54 ± 1.3 days for the westbound flight. These findings compared favourably to the expected minimal resynchronization rate after 13 hours of flight without any treatment, thus supporting the conclusion that jet lag symptoms can be significantly reduced by the carefully timed application of melatonin, light exposure, and physical activity.

Study 10
The efficacy of three melatonin formulations for circadian phase advance and delay: (a) 3 mg regular release (RR), (b) 3 mg sustained release (SR), and (c) 3 mg surge-sustained release (SSR; consisting of 1 mg RR and 2 mg SR) was evaluated. Circadian phase advances or delays were assessed in two separate experiments using plasma melatonin levels as a parameter. Thirteen normal healthy male subjects aged 26 to 53 years were chosen for experiment 1 (circadian phase advance) and nine normal healthy male subjects aged 26 to 54 years were included in experiment 2 (circadian phase delay). In both studies, a fast-release

melatonin preparation induced the expected phase changes. There were no differences in phase advance efficacy among the three melatonin release preparations, while in the phase-delay study, phase shifts for the sustained release preparations could not be determined due to persistent high melatonin levels during sampling times, however, a fast-release melatonin preparation is effective for reducing circadian misalignment for both eastward and westward travel.

Systematic review and meta- analysis (jet lag)
The objective of this systematic review and meta-analysis was to assess the effectiveness of oral melatonin taken in different dosage regimens for alleviating jet lag after air travel across several time zones.

Selection criteria were:

- Randomised trials in airline passengers, airline staff or military personnel given oral melatonin, compared with placebo or other medication
- Outcome measures should consist of subjective rating of jet lag or related components, such as subjective wellbeing, daytime tiredness, onset and quality of sleep, psychological functioning, duration of return to normal, or indicators of circadian rhythms.

Ten randomised, placebo-controlled trials were identified for the systematic review and meta-analysis. All compared melatonin with placebo; one in addition compared it with a hypnotic, zolpidem.

The meta-analysis concluded that melatonin, taken close to the target bedtime at the destination (10 pm to midnight), decreased jet lag from flights crossing five or more time zones. According to this meta-analysis, the daily doses of melatonin between 0.5 and 5 mg are similarly effective, except of people that fall asleep faster and sleep better after 5 mg than 0.5 mg. Doses above 5 mg appear to be no more effective. The relative ineffectiveness of 2 mg slow-release melatonin suggests that a short-lived higher peak concentration of melatonin works better. The benefit is likely to be greater the more time zones are crossed, and less for westward flights.

In summary, melatonin is effective in preventing or reducing jet lag, and occasional short-term use appears to be safe. It can be recommended to adult travellers flying across five or more time zones, particularly in an easterly direction, and especially if they have experienced jet lag on previous journeys. Travelers crossing 2 - 4 time zones can also use it if need be.

However, it should be noted that individuals differ greatly in the experience of jet lag, with some travellers extremely affected while others who may have flown the same route may report no jet lag symptoms. This suggests that individual differences may strongly influence the effectiveness of melatonin.

The findings for the meta-analysis concluded that the pharmacology and toxicology of melatonin needs systematic study, and the effects of melatonin in people with epilepsy, and a possible interaction with warfarin, need investigation.

To summarise, it is considered that 9 of the 10 studies demonstrated statistically significant effects on jet lag symptoms (*e.g.* mood, cognitive) or on sleep and that 2 of the 10 studies with results for responders analysis concerning global jet lag severity demonstrated a considerable difference (67% and 40%, respectively) in percentage responders. In addition, one of the studies included in the meta-analysis (study 4) and briefly described here showed that melatonin treated subjects took on average one day less to return back to normal sleep

(2.9 days compared to 4.2 days), which may be considered as clinically relevant and as tapping into the ability to return back to normal functioning (i.e. work).

The collective evidence of efficacy from ten placebo-controlled trials is persuasive that melatonin is effective in jet lag. Efficacy was shown in reducing key measurable effects of jet lag, in particular time to return to normal sleep. Furthermore, subjective reporting of global efficacy measured on a VAS score on severity of jet lag show clinically relevant superiority for melatonin compared to placebo.

A review of 11 randomised trials (refer to figure below), combined the evidence using meta-analysis and generated a summary of findings following the GRADE approach. It has been concluded that the use of oral melatonin reduces the symptoms associated with jet lag syndrome.

<table>
<tr><th colspan="5">Melatonin for Jet Lag syndrome</th></tr>
<tr><td>Population</td><td colspan="4">Healthy individuals traveling across more than five time zones</td></tr>
<tr><td>Intervention</td><td colspan="4">Melatonin</td></tr>
<tr><td>Comparison</td><td colspan="4">Placebo</td></tr>
<tr><td rowspan="3">Outcomes</td><td colspan="2">Absolute effect*</td><td rowspan="3">Relative effect (95 % CI)</td><td rowspan="3">Certainty of the evidence (GRADE)</td></tr>
<tr><td>Without melatonin</td><td>With melatonin</td></tr>
<tr><td colspan="2">Difference: patients per 1000</td></tr>
<tr><td rowspan="2">Global Jet Lag symptoms (0 to 100 scale)</td><td>45 points per 1000</td><td>27 points per 1000</td><td rowspan="2">MD-17.74 (-23.98 to -11.50)</td><td rowspan="2">+++
Moderate</td></tr>
<tr><td colspan="2">Difference: 18 points less (Margin error: 12 to 24 points less)</td></tr>
<tr><td colspan="5">MD: Mean Difference
Margin of error: 95 % Confidence Interval (CI)
Grade: Evidence grades of the GRADE Working Group

*The risk Without melatonin is based on the risk in the control group of the trials. The risk With melatonin (and its margin of error) is calculated from relative effect (and its margin of error).
[1]The certainty of the evidence was lowered one level due to the risk of bias because most studies did not adequately describe methods.</td></tr>
</table>

Other systematic reviews

In a review of a study it was concluded that melatonin is remarkably effective in preventing or reducing jet lag, and occasional short-term use appears to be safe. It should be recommended to adult travellers flying across five or more time zones, particularly in an easterly direction, and especially if they have experienced jet lag on previous journeys. Travellers crossing 2 - 4 time zones can also use it if need be.

The review of another study also assessed the effects of melatonin in sleep disorders including jet lag and concluded that melatonin decreases jet lag symptoms and quickens the return of normal alertness and energy levels. In most of the studies, polysomnography records were evaluated, along with wrist actigraphy, visual analogue scales and questionnaires.

Overall comments on the randomised controlled trials in jet lag

Study Participants

The 10 studies evaluated for efficacy in the meta-analysis recruited a diverse range of subjects, including visitors to a university travel clinic, university / hospital employees and their families, airline cabin crews, other airport staff, sports officials and scientists. 350 (39%) of the 892 participants in the 10 jet lag studies travelled in groups. Participant age

ranged from the mid-20s to mid-60s in most studies, 64% were men. The study populations in the presented published trials are considered reasonably representative of the population for which the treatment is intended. No concerns are raised.

Trial medication
The composition and formulation of the melatonin test treatments were described in 5 of the 10 study reports. Most were prepared specifically for the studies, either by the study investigators or by a hospital pharmacy. One of the studies used a commercial product, and this seems to be the case also for a number of other studies described in the meta- analysis. None of these formulations are likely to be related to the EU approved Bio-melatonin product.

Regarding the conditions of dosing, melatonin was taken at destination for 3 days in 2 studies, for 4 days in 4, for 5 days in 3, and for 7 days in 1 study; as melatonin was also taken on the day of travel (pre-flight or in-flight) by at least 1 group in 7 of the studies, melatonin was typically taken for 4 – 5 days at destination and 5 – 6 days in total. The currently proposed posology and timing of dosing (at habitual bedtime at destination) is broadly in line with what was done in the clinical trials.

Efficacy endpoints
Primary endpoints were not defined *a priori* in most of the ten jet lag studies in the meta-analysis review. Outcome measures were somewhat diverse, including scale and item scores on daytime symptoms of jet lag, measures of sleep quality and latency, daytime sleepiness, and mood disturbances. Global jet lag symptom scores were also reported in most studies, typically using a 10cm visual analogue scale. The measure and reporting of participant-assessed global efficacy is considered important in the assessment of the efficacy of melatonin for jet lag as it is easy to understand in terms of clinical relevance and is perhaps less susceptible to data "cherry picking" in comparison with certain other measures. Evaluation of specific measures of jet lag such as sleep-latency, quality, and duration; daytime- tiredness, fatigue, alertness, and mood; appetite; and general well-being are important but not considered primary, not least because they were not all consistently assessed across the various trials.

Dose recommendations
Of the 10 key jet lag studies mentioned in the meta-analysis review, 7 investigated a dose of 5 mg once daily. One study found similar general efficacy for 0.5mg and 5 mg doses but greater improvements in sleep onset latency and sleep quality for the higher dose, indicating a greater hypnotic effect. Another study suggested that a dose of 0.5mg was not effective. A dose range of 3mg to 6mg is supported by the evidence.

The review stated that for many people, 5 mg may be a higher dose than necessary, and 2 or 3 mg may therefore be preferable to start with, but a dose of 6 mg may be required if the standard dose does not adequately alleviate symptoms. However, there are insufficient data to conclude this with confidence.

A counterargument to the review proposal would be that if there are grounds for advising a dose increase to 6mg to achieve added efficacy, at least one day might be lost in terms of achieving maximal efficacy. There seems to be no reason to think that efficacy might decrease with doses above 3mg (nothing to suggest a bell-shaped curve) and the safety profile is very benign so the posology as proposed is acceptable.

Timing of initiation of treatment

The review states that melatonin “is effective when taken at bedtime after darkness has fallen on the first day of travel; and again in the same way on the second (and any subsequent day) of travel, and at the destination on the following few days at the same time.” and that “taking melatonin before the day of travel does not hasten or improve adaptation to local time at destination and is not recommended.” This is agreed as available data does not support starting melatonin prior to the day of travel as there is no evidence of added benefit and there could be added problems with undesirable effects (excessive somnolence) before reaching the destination.

In the absence of data indicating otherwise, it is considered appropriate to advise that melatonin treatment should be initiated on arrival at destination, with the first dose taken at bedtime (or an hour or so before). This timing is consistent with the posology for the 3 mg Bio-melatonin tablet authorised in Hungary.

Duration of treatment
The 10 jet lag studies included in the review provide limited evidence for efficacy for periods over 4 days. However, a study mentioned in the review which involved travel over the greatest number of time zones (12) in both easterly and westerly directions, found overall jet lag scores to be elevated for 5 days after arrival (particularly following eastward travel), with evidence of benefit for this period in the melatonin group. Although there is not a great deal of data to support a treatment period of up to 5 days, this can be accepted as appropriate if symptoms of jet lag are persisting for this long. The dose that adequately alleviates symptoms should be taken for the shortest period.

Conclusion on clinical efficacy
To summarise, the efficacy has been demonstrated for the jet lag indication proposed for this application. The clinical overview depicts all the melatonin products which have been approved in the EU over the last 10 years since the first approval for the proposed indication of use in jet lag. Thus, the extent of use of melatonin on a geographical basis for over 10 years is approvable and the Applicant has successfully bridged the literature data to their product.

In conclusion, the submitted package of bibliographic data is considered sufficient to conclude that melatonin is effective in the treatment of jet lag in adults at the doses proposed.

IV.5 Clinical safety

Safety information summarised below indicates that short-term use (defined as 'days') and immediate-term use (defined as 'weeks-to-months') use of exogenous melatonin is generally safe in adult men and women. Safety data from studies of melatonin for jet lag, in other disorders, and in safety studies and reviews indicates that adverse events reported most commonly are sleepiness/drowsiness, headache, dizziness, and nausea, though their frequency is low and often comparable to that in subjects receiving placebo. No serious adverse effects were clearly associated with ingestion of typical therapeutic doses of melatonin in the individual studies, and this is supported by reviews addressing specific and general aspects of melatonin safety. A study considered short-term ingestion of even extreme doses of melatonin by adults in general to be safe, with the above adverse effects the most common signs and symptoms of overdose.

Data for long-term use (defined as 'years') of melatonin by adults are limited; such data is available for children and adolescents, though specifically designed long-term safety studies are lacking for adults and persons under 18 years.

Due to the potential concerns, and the limited information available to address them, the Applicant's product is not recommended in persons under 18 years or in pregnant or lactating women, or women or men planning a pregnancy.

Safety in jet lag
Most of the 10 jet lag studies did not collect / report adverse events in a systematic manner. Overall, only occasional serious or potentially serious adverse events were reported, though the causal role of melatonin was generally not assessed. The adverse events reported were generally comparable in nature and frequency in the melatonin and placebo groups, though transient drowsiness/sleepiness was more common with melatonin. In relation to the indication and recommended timing of intake (at bedtime) of the Applicant's product, transient drowsiness is considered a beneficial action rather than an adverse effect. The finding that drowsiness, and possibly also headache and dizziness/disorientation, are the most common (though still only occasional) adverse effects reported when melatonin is taken on a short-term basis to treat jet lag is in agreement with safety data for melatonin reported in other clinical studies, in safety studies and reviews addressing safety, and post-marketing data.

The adverse events (AE) most commonly reported in the published articles in jet lag included headache, nausea, dizziness and drowsiness. The incidence of AEs is low. There were no serious AEs or death reported.

As lack of reporting of AEs in published studies is well known and may not reflect actual safety. Other sources, in particular post marketing data from Hungary where Bio-Melatonin is approved, are considered important.

Safety in other clinical studies
Safety data for oral melatonin is available from a large number of published clinical studies. Studies in which a single daily dose of 3 – 6 mg IR melatonin was ingested for a minimum of 5 days by adult men and women (especially subjects with circadian rhythm disorders) are considered most relevant to the Applicant's product. A representative selection of clinical trials and focused reviews are summarised below:
Primary sleep disorder
A crossover study in which 14 adult men and women (mean age ~ 51 years) with reduced REM sleep duration ingested 3 mg IR melatonin or placebo at bedtime for 4 weeks found no adverse effect of melatonin on any efficacy parameter; no side effects were reported.

Another randomised control trial in which 62 children (6 – 12 years) with idiopathic chronic sleep-onset insomnia ingested 5 mg IR melatonin or placebo at 19:00 hr for 4 weeks found no adverse effect of melatonin on any efficacy parameter. No subjects withdrew from the study due to adverse events, and no serious adverse events were reported. After first intake of melatonin, 7 children reported one or more of the following symptoms: a cold feeling, decrease in appetite, dizziness, and impaired mood. After first intake of placebo, 3 children reported one or more of the following symptoms: headache, nausea, dizziness, and increased appetite. These possible adverse events subsided within 3 days of starting melatonin or placebo.

A retrospective study of longer-term treatment of 33 adolescents (10 – 18 years) with Delayed Sleep Phase Disorder (DSPD) with 3 – 6 mg (mean 5 mg) melatonin taken 2 h before desired bedtime for a mean of 6 months (range 1 – 16 months) found that melatonin had not adversely affected any efficacy parameter. No adverse effects had been reported spontaneously or at follow-up visits scheduled every 1 – 3 months. 15 of the subjects had

ADHD; the safety of melatonin in these patients was comparable to that in the subjects without ADHD.

A systematic review and meta-analysis that examined the efficacy and safety of exogenous melatonin for primary sleep disorders, primarily insomnia and DSPD included 10 randomised control trials rated of good quality and involving ~ 220 subjects in a safety analysis. 7 of the studies involved a dose of 4 or 5 mg, with melatonin administered for at least 3 weeks in 8 of the studies. There were few reports of adverse events during melatonin administration; the most commonly reported were headache (13 events), dizziness (10 events), nausea (3 events), and drowsiness (3 events). For each symptom, there was no significant difference in frequency between melatonin and placebo. This result did not change by type of sleep disorder, dose or formulation of melatonin, duration of treatment, gender, age, use of concurrent medication, study design or quality score, and allocation concealment score.

A meta-analysis of the efficacy and safety of exogenous melatonin in DSPD that included 5 randomised control trials including 91 adults, and 4 randomised control trials including 226 children noted that 4 of the 9 studies did not report any adverse events. In 4 of the studies, ~ 7% of subjects experienced headaches during melatonin treatment, though in 1 study headache was reported in the placebo group only. Other adverse events during melatonin treatment, but not placebo treatment, were feeling cold, dizziness, mood dip, and decreased appetite, though these events were primarily reported in a single study that did not state their frequency. Elevated alkaline phosphatase level was observed in 1 subject on melatonin, thought the elevation was almost reversed after 20 weeks of continued melatonin treatment. 1 subject developed a mild case of generalised epilepsy 4 months after having started melatonin (the potential for melatonin to exacerbate or initiate epilepsy is addressed in section *Epilepsy* (Safety in special groups and situations).

Secondary sleep disorders

A randomised control trial in which 157 patients (mean ± SD age 77.4 ± 8.9 years; 56.1% women) with Alzheimer's disease and sleep disturbances received 2.5 mg melatonin (slow release), 10 mg melatonin (probably IR) or placebo 1 h before habitual bedtime for 2 months found that melatonin did not adversely affect any efficacy parameter. There were no differences in the mean number, severity, seriousness, or relatedness ratings of spontaneously reported adverse events in the 3 treatment groups. None of the adverse events reported were considered definitely related to the study medication. All the named groupings of adverse events reported in the 2 melatonin groups were also reported in the placebo group.

A crossover randomised control trial in which 23 children and adolescents (6 – 14 years) with ADHD and initial insomnia ingested 5 mg melatonin ('short-acting') or placebo 20 minutes before bedtime for 30 days found no adverse effect of melatonin on any efficacy parameter. 20% and 23% of the adverse events (no further details) reported occurred during melatonin or placebo treatment, respectively. 1 event (a migraine) was categorised as severe. There were no serious adverse events. None of the adverse events were considered causally related to the study interventions.

A systematic review and meta-analysis that examined the efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction included 7 studies of secondary sleep disorders rated of good quality and involving 164 subjects, and 10 studies of sleep restriction rated of good quality and involving 487 subjects. The most commonly reported adverse events were headache, dizziness, nausea, and drowsiness; the occurrence of each symptom did not differ significantly for melatonin or placebo. Thus, 17 clinical trials involving 651 participants showed no evidence of adverse

effects of melatonin with short-term (< 3 months) use.

A systematic review and meta-analysis that examined the efficacy and safety of exogenous melatonin for treating sleep problems in individuals with intellectual disabilities included 9 randomised control trials involving 183 subjects. Typically, melatonin doses were 2.5 – 5 mg (though up to 9 mg was given) administered for 2 – 4 weeks. Specific reports on adverse effects were given in 4 of the 9 studies; they were considered minor, and their incidence comparable in the melatonin and placebo groups. 2 of the 9 studies reported that no significant adverse effects were observed, while adverse effects were not addressed in the 3 remaining studies, suggesting serious affects were not observed.

Other disorders and situations

A crossover randomised control trial that studied the effect of 3 mg IR melatonin or placebo taken before bedtime for 11 – 12 days by 45 paediatric residents (29 women, 16 men; mean age 29 years) after working a night-shift found that melatonin did not adversely affect any efficacy parameter. Subjects were required to complete (daily) an adverse events log that covered general symptoms, gastrointestinal symptoms, excessive sleepiness, and other symptoms. The number of subjects reporting symptoms while on melatonin or placebo were comparable. There were no significant differences in the number of days with each symptom for melatonin compared to placebo. None of the adverse effects were described as serious. Melatonin was considered well-tolerated.

A randomised controlled trial in which 121 patients scheduled for elective ambulatory laparoscopic cholecystectomy ingested 5 mg IR melatonin or placebo for 3 nights after surgery found nausea, tiredness, and dizziness to be the most commonly reported side effects in the 2 treatment groups. The number and type of side effects reported was not considered to differ in the 2 groups.

A randomised control trial in which the effect of 3 mg melatonin or placebo ingested 2 h before bedtime for 4 weeks on sleep and asthma were examined in 22 women (mean ± SD) age 29.7 ± 7.7 years) with asthma found no adverse effect of melatonin on any efficacy parameter.

In summary, the representative selection of studies of melatonin for disorders other than jet lag summarised above have not observed unexpected adverse effects on efficacy parameters and have not reported serious adverse effects. The nature and frequency of adverse events in the melatonin and placebo groups of the individual studies were generally comparable, and pooling data for adverse events in reviews and meta-analyses did not identify any adverse events clearly related to typical therapeutic doses of oral melatonin.

Sleepiness/drowsiness, headache, dizziness, and nausea were the most common adverse events reported with melatonin and placebo treatment; these and other adverse events were generally reported with comparable frequency by subjects receiving melatonin or placebo, a finding consistent with a recent review of the safety of melatonin. It is likely that sleepiness/drowsiness is associated with melatonin treatment, particularly in younger children, though this effect is not considered adverse in relation to the proposed indication. Overall, safety data from studies other than jet lag are not considered to raise any new safety concerns. The safety profile of melatonin in other disorders seems comparable to that seen in treatment of jet lag.

Safety studies and reviews addressing safety

Safety studies

While safety data for oral melatonin have been collected from a large number of clinical

studies, only 2 published studies were identified that were specifically designed as safety studies. One of the studies is considered the most relevant as it involved intake of 10 mg melatonin for 28 days, though it involved men only. The second study involved ingestion of a single, very high dose (50 mg/kg; equivalent to 3000 mg for a 60 kg person) prior to an operation.

A study which depicts randomised male volunteers (29 ± 1 years) to 10 mg melatonin (probably IR) (n = 30) or placebo (n = 10) taken at 22:00 hr for 28 days in a double-blind trial. Laboratory measurements included blood chemistry, complete blood count, urinalysis, selected hormones (T3, T4, TSH, LH/FSH, cortisol), and melatonin concentration. Polysomnography (PSG) was carried out, and the Epworth Somnolence Scale (ESS) and a sleep diary were applied (in additional to the routine laboratory parameters referred to above) at each of 5, weekly visits. None of the above parameters were adversely affected in the melatonin group. The subjects were also asked about possible side effects that appeared during treatment. The number of subjects reporting somnolence (57% vs. 60%) or headache (47% vs. 30%) did not differ significantly between the melatonin group and control group, respectively. The following side effects were reported by the melatonin and placebo subjects, respectively: fatigue (3 vs.1), tremor (2 vs. 0), cognitive alteration (2 vs. 2), pyrosis (1 vs. 0), depression (1 vs. 1), irritability (1 vs. 0), illness (2 vs. 0), torpor (0 vs. 1), tinnitus (0 vs.1). Taking into account the number of subjects in each group, the number of subjects reporting each symptom is considered comparable in the melatonin and control groups.

A pilot randomised control trial evaluated the safety of single oral dose of 50 mg/kg IR melatonin or placebo ingested before applying general anaesthesia to 48 patients (20 women, 28 men; 57± 11 years) undergoing major liver resection. Biochemical panel, hematology, and liver tests were measured on the day of operation and daily during the postoperative study period of 7 days. No statistically significant difference was observed between the treatment groups for any of the laboratory parameters at any given time point. Melatonin treatment tended to lower postoperative transaminases over the study period. 11 adverse events (non-serious) occurred in 8 patients receiving melatonin, while 18 adverse events occurred in 7 patients receiving placebo; none were considered treatment-related and all patients with adverse events completed the study.

Although not specifically designed as a safety study, a randomised controlled trial that measured routine blood chemistry and haematology parameters, and selected hormones in 22 subjects (60.1± 9.5 years; 16 women) before and after 6 months intake of 3 mg of melatonin (probably IR) 30 minutes before bedtime did not observe any adverse changes to these parameters, and no adverse effects attributed to melatonin were reported.

Reviews addressing melatonin safety

A review of adverse effects reported by 2337 adults taking 1 – 10 mg oral melatonin, or placebo, for a minimum of 3 days in 50 RCTs found little evidence of adverse effects, and essentially no evidence of serious adverse effects in healthy persons and in patients with a range of medical conditions. The review addressed both objective and subjective effects. The studies generally reported data for safety parameters such as haematology and blood chemistry as group means, though it is normal practice for any significant deviations from the reference range observed in individual subjects to be mentioned in study reports.

A review of 35 clinical trials of the effectiveness of melatonin for promoting sleep noted that 15 of the trial reports included information on adverse events, with no serious adverse events reported. An additional study report stated that adverse events occurred, but did not describe them, and a further 2 study reports stated that no adverse events occurred. The most common

adverse events were headache and somnolence. Palpitations and abdominal pain were each reported in 2 studies. The remaining adverse events were reported infrequently, each occurring in only 1 of the multiple studies, and comprised nasopharyngitis, arthralgia, tachycardia, dizziness, nausea, vomiting, nightmares, difficulty swallowing and breathing, hypnotic activity, heavy head, heartburn, flatulence, swelling of arms / legs, sweating / hot flush, exanthema, sleeping difficulties, depression, and sleep walking.

A recent review of the safety of melatonin concluded that, in general, clinical data (supported by animal data) indicate that short-term (defined as 'days') use of melatonin is safe, even in extreme doses, with only mild adverse effects such as sleepiness, headache, dizziness, and nausea having been reported. Immediate-term and long-term use of melatonin (defined as 'weeks-to-months' and 'years', respectively) was also only associated with minor adverse effects. It was considered that no studies have indicated that melatonin induces serious adverse effects, but that long-term safety in children and adolescents requires further investigation. Due to lack of human studies, it was recommended that pregnant and breast-feeding women should not take exogenous melatonin.

The British National Formulary of June 2016 (**BNF 2016**) lists (alphabetically) the following adverse effects for melatonin:

Frequency	Adverse effects
Uncommon	abdominal pain, abnormal dreams, anxiety, chest pain, dizziness, dry mouth, dry skin, dyspepsia, glycosuria, headache, hypertension, irritability, malaise, mouth ulceration, nausea, nervousness, proteinuria, pruritus, rash, restlessness, weight gain
Rare	aggression, arthritis, electrolyte disturbances, flatulence, gastritis, haematuria, halitosis, hot flushes, hypertriglyceridaemia, impaired memory, increased libido, lacrimation, leukopenia, mood changes, muscle spasm, nail disorder, palpitation, paraesthesia, polyuria, priapism, prostatitis, restless legs syndrome, salivation, syncope, thirst, thrombocytopenia, visual disturbances, vomiting
Not known	galactorrhoea, mouth oedema, tongue oedema

Long-term safety

A study found 3 mg melatonin (probably IR) ingested 30 minutes before bedtime for 6 months to have no effect on routine blood biochemical tests (including measures of glycaemic control, and liver and kidney function) or differential WBC counts in 22 subjects (60.1 ± 9.5 years; 16 women) with insomnia (20 taking a benzodiazepine). Serum concentrations of oestradiol and FSH in the women, and serum concentrations of prolactin and TSH in the same 16 women plus 6 men were unchanged. No side effects attributed to melatonin were reported. These data are in good agreement with those of another study following ingestion of 10 mg melatonin (probably IR) daily for 28 days to healthy younger men.

Several clinical studies that administered oral melatonin to children and/or adolescents for periods of up to 3 years have reported safety data, though none were specifically designed as safety studies. Serious adverse effects or other important safety signals were not observed, though the safety data comprised primarily adverse events reported by patients or their parents / caregivers or collected on questioning by the investigators. None of the studies systematically measured routine blood chemistry or haematology parameters. The lack of data for endocrine parameters, and for reproductive issues in general, is of concern considering the apparent role of endogenous melatonin – more specifically the gradual fall in melatonin level around 9 – 10 years of age – in the cascade of events preceding the

awakening of the hypothalamic-pituitary-gonadal axis at puberty.

A recent review of the safety of melatonin considered intermediate use (defined as weeks-to-months) to be associated only with minor adverse effects in children and adults, but that its long-term safety requires further investigation. It is also considered relevant to mention that a study of the melatonin-receptor agonist, ramelteon, in which 122 subjects (69 women, 52 men; mean age 34 years) with chronic insomnia were randomised to ramelteon 16 mg, or placebo, nightly for 6 months found no consistent statistically significant effects on measures of thyroid function (TSH, total T3, total- and free T4), adrenal function (cortisol, ACTH), or reproductive endocrine function (LH, FSH, estradiol (women), total- and free testosterone (men)); prolactin concentration in women showed a mild, transient increase though no clinical effects of elevated prolactin were reported. Average menstrual cycle length, duration of menses, and ovulation probability were unaffected.

In summary, published data on the long-term safety of oral melatonin is limited. While no safety concerns related to long-term use are considered evident, studies specifically designed to examine the long-term (≥ 1 year) safety of typical therapeutic doses of oral melatonin have not been published. Lack of systematic studies of longer-term use of melatonin on the onset of puberty, and on the gonadotrophic system in general in children, adolescents, and women and men of reproductive age is a concern, despite the fact that humans, as non-seasonal breeders, may have reproductive endocrinology and physiology less sensitive to melatonin that than of the seasonally breeding species in which the majority of animal studies have been made (see the relevant sections of the non-clinical section).

Melatonin 1mg/ml oral solution is intended for short-term, periodic use, no specific warnings regarding long-term use are included in the SmPC for this product, though text under the *Posology* heading of Section 4 notes that the product may be taken for a maximum of 16 treatment periods per year, that the maximum duration of each treatment period is 5 days, and that the dose that adequately alleviates symptoms should be taken for the shortest period. Text is also included in Sections 4.2, 4.4 and 5.1 of the SmPC noting that as the safety (and efficacy) of the product have not been established in children and adolescents aged 0 – 18 years it should not be used in children and adolescents.

Specific safety issues

Immune system adverse effects

Studies involving ingestion of doses of melatonin comparable to or greater than the recommended doses for the Applicant's product for periods of up to 6 months were not found to have adverse effects on white blood cells counts or other immune system parameters, nor to be associated with increased incidences of adverse effects involving the immune system.

A study reported a temporal relationship between melatonin use and the development of autoimmune hepatitis. Another study described exacerbation of symptoms of Crohn’s disease when melatonin was taken in addition to corticosteroids and salicylazosulfapyridine. An author described exacerbation of well-controlled ulcerative colitis following 2 months intake of an unidentified melatonin 3 mg product at bedtime that resolved 24 – 48 h after seponation of melatonin. The role melatonin in the events described in the case reports is generally uncertain. A case report of autoimmune hepatitis that developed in association with use of the melatonin agonist, ramelteon is also considered relevant

Knowledge on the capacity of melatonin to modulate immune system function, have led to the inclusion of text in Section 4.4 of the proposed SmPC stating that occasional case reports have described exacerbation of an autoimmune disease in patients taking melatonin, that

there are no data regarding use of the Applicant's product in patients with autoimmune diseases, and that its use is not recommended in patients with autoimmune disease. This is endorsed.

Sexual development adverse effects
Information presented in the clinical overview addresses the role of endogenous melatonin in modulation of sexual development, and the potential for exogenous melatonin to adversely affect sexual development. Data from 1 long-term study in pre-pubertal / pubertal children were presented, though this study was not specifically designed as a safety study. Relevant published data are limited.

No relevant case reports were identified in the literature. Exposure to a 3 mg melatonin tablet (IR) equivalent to ~ 820,100 7-day (10-tablet) treatment periods did not result in receipt or identification of a case report involving sexual development.

Although available data have not confirmed any safety concerns regarding the potential for oral melatonin to adversely affect sexual development, the information available is limited and specifically designed safety studies have not been performed. Based on the above information, text included under the heading *Paediatric population* in Sections 4.2 and 4.4 of the proposed SmPC for the Applicant's product notes that as the safety (and efficacy) of the product have not been established in children and adolescents aged 0 – 18 years it should not be used in children and adolescents. Both sections refer to text in Section 5.1 which, under the heading *Paediatric population*, includes comparable text, plus additional text noting that the recommendation against use in persons under 18 years is based on the fact that interference with the function of endogenous melatonin on the development of the hypothalamic-pituitary-gonadal axis cannot be excluded.

Adverse effects on glycaemic control
Published data for oral melatonin were not considered to suggest that the Applicant's product is likely to have any long-term adverse effects glycaemic control, though limited data indicate that melatonin taken in close proximity to meals might cause transient impairment.

No relevant case reports were identified in the literature.

Although the available data are limited, the finding that oral melatonin can transiently impair glycaemic control when taken immediately prior to an oral glucose tolerance test has led to the inclusion of text in Sections 4.2 and 4.4 of the proposed SmPC noting that intake of melatonin with carbohydrate-rich meals may impair blood glucose control for several hours, and text in Section 4.4 recommending that the product should be taken at least 2 h before and at least 2 h after a meal, and ideally at least 3 h after a meal by persons with significantly impaired glucose tolerance or diabetes. Section 4.8 of SmPC for Melatonin 1mg/ml oral solution lists *hyperglycaemia* (SOC *Metabolism and nutrition disorders*; Frequency *not known*).

Endogenous melatonin synthesis
Regarding the potential for exogenous melatonin to affect the quantity of endogenous melatonin synthesised, there is no evidence of adverse effects.

Safety in special populations
Elderly
The PK of oral melatonin are generally comparable in younger and older adults. In particular, rapid plasma elimination half-life ($T_{½}$ ~ 45 minutes) is not prolonged in the elderly in

general, limiting the potential for adverse effects due to accumulation of melatonin. Thus, the PK of melatonin in the elderly is not considered to have implications for the safety of the Applicant's product in this patient group.

The available data do not warrant dose adjustment or warnings in elderly.

Hepatic impairment
Hepatic impairment can reduce the clearance of exogenous melatonin, though the relationship between the nature / degree of hepatic impairment and the extent of the reduction in melatonin metabolism is unclear. Consequently, there is considered to be insufficient data to permit alternative dosing recommendations for any degree of hepatic impairment. The available data is, however, considered sufficient to support inclusion of text in Sections 4.2 and 4.4 in the SmPC for Melatonin 1mg/ml oral solution, recommending that the product is not used by patients with severe hepatic impairment, with background information included under the heading *Hepatic impairment* (Special populations) of Section 5.2.

Case reports in which adverse effects were linked to hepatic impairment have not been identified in the literature.

In summary, there are no reported adverse events related to melatonin use in patients with hepatic impairment available. The text regarding hepatic impairment included in sections 4.2 and 4.4 of the SmPC for Melatonin 1mg/ml oral solution are primarily based on the potential safety implications of the available (limited) PK data, though the text in section 4.4 notes that only limited data are available on the safety of melatonin in patients with hepatic impairment. The proposed SmPC text is endorsed.

Renal impairment
As less than ~ 1% of exogenous melatonin is excreted untransformed in the urine, renal impairment is not expected to significantly increase absolute plasma melatonin concentration. However, supporting data are limited and indirect, and do not address the relationship between the nature / degree of renal impairment and the extent of the reduction in melatonin excretion, and there is no relevant data for the Applicant's product. Consequently, there is considered to be insufficient data to permit alternative dosing recommendations for any degree of renal impairment. The available data is, however, considered sufficient to support inclusion of text in sections 4.2 and 4.4 of the proposed SmPC recommending that the product is not used by patients with severe renal impairment, with background information included under the heading *Renal impairment* (Special populations) of Section 5.2.

In summary, there are no reported adverse events related to melatonin use in patients with renal impairment available. The text regarding renal impairment included in sections 4.2 and 4.4 of the SmPC for Melatonin 1mg/ml oral solution is primarily based on the potential safety implications of the available (limited) PK data, though the text in section 4.4 notes that only limited data are available on the safety of melatonin in patients with renal impairment. The proposed SmPC text is endorsed.

Genetic factors
The degree of impairment of melatonin metabolism due to race or genotype is considered to be of limited clinical significance. In summary, no concerns regarding race genotype are considered evident, and no related safety information is included in the SmPC for Melatonin 1mg/ml oral solution.

Epilepsy
A number of clinical trials have examined the efficacy of oral melatonin for sleep disorders

associated with neurological and psychiatric disorders, with the majority involving children and adolescents. As such conditions can be associated with an increased risk of seizures, the studies in question have typically monitored the potential for melatonin to exacerbate seizures or to induce seizures in those with no previous history. Several relevant trials and reviews are summarised below.

A study found no suggestion that long-term intake of melatonin activated an epileptic event in 19 paediatric patients with a seizure disorder.

A study in which 51 children and young adults with intellectual disability were treated with melatonin or placebo for 4 weeks observed no changes in seizure frequency.

A 3.7-year follow-up study of 101 children with ADHD who had previously been given 3 – 6 mg melatonin for 4 weeks found none to have developed epilepsy, a finding considered consistent with literature data indicating no clear pro-convulsive action of melatonin.

A review of melatonin for sleep disorders associated with intellectual disability noted that while a few studies have reported worsening of or development of seizures after initiation of melatonin, a number of others have not found melatonin to have such effects and that it may actually have beneficial effects on seizure.

A recent review of the potential for melatonin to affect epileptic seizures identified 3 randomised clinical trials of which 2 showed no overall worsening or improvement in seizures, and the third a statistically significant reduction in seizures. The open studies identified reported conflicting results. The available data were considered limited.

A single relevant case report for a 3 mg melatonin tablet (IR) in Hungary described reduced seizure activity in a male child and a female adolescent with early onset epilepsy 1 month after starting melatonin.

Based on the above, section 4.4 of the SmPC for Melatonin 1mg/ml oral solution notes that melatonin may increase seizure frequency in patients experiencing seizures (*e.g.* epileptic patients), and that patients suffering from seizures must be informed about this possibility before using the product. Although the product is not recommended in persons under 18 years of age, Section 4.4 also notes that melatonin may promote or increase the incidence of seizures in children and adolescents with multiple neurological defects. The proposed SmPC text is endorsed.

Pregnancy

The clinical overview notes that exogenous melatonin readily crosses the human placenta and enters the foetal circulation, though there are no published data on the effect of *in utero* exposure to melatonin on pre- and post-natal development in humans. No safety data (including case reports) for clinical exposure to exogenous melatonin during pregnancy were identified in the literature. Exposure to a 3 mg melatonin tablet (IR) equivalent to ~ 820,100 7-day (10-tablet) treatment periods did not result in receipt or identification of a case report involving pregnancy.

Based on the above information the *Pregnancy* heading of section 4.6 of the proposed SmPC includes text noting that there are no or limited amount or data from the use of melatonin in pregnant women, that animal studies are insufficient with respect to reproductive toxicity (with reference to Section 5.3), and that the product is not recommended during pregnancy. Section 5.3 includes text noting that after oral and subcutaneous administration of large doses

of melatonin to pregnant rats, foetal body weight and length tended to be lower, possibly due to maternal toxicity. It also notes that delay in sexual maturation in male and female off-spring of rats and ground squirrels occurred on exposure to melatonin during pregnancy, indicating that exogenous melatonin crosses the placenta and that it may influence the ontogeny and activation of the hypothalamic pituitary- gonadal axis, though as the wild rat and ground squirrel are seasonal breeders, the implications of these findings for humans are uncertain. Text in section 5.2 of the proposed SmPC notes that melatonin readily crosses the placenta, and that the melatonin level in umbilical blood of full-term babies closely correlates with and is only slightly lower (~ 15 – 35%) than, that of their mothers following ingestion of a 3 mg dose.

In summary, no safety data concerning melatonin use and pregnancy are available. However, hormonal effects have been observed, including enhancement of luteinizing hormone levels in women during the follicular phase of the menstrual cycle, and of cortisol levels in older women, as well as enhancing prolactin secretion, and decreasing plasma progesterone and oestradiol levels in healthy women. Melatonin may have an effect on fertility in women and men, on pregnancy, and on breast feeding. The proposed SmPC text is endorsed.

Lactation

Based on the literature review submitted, text included under the heading *Lactation* in section 4.6 of the SmPC for Melatonin 1mg/ml oral solution notes that there is insufficient data on the excretion of melatonin or its metabolites in human milk though it is known that endogenous melatonin is excreted in human milk, that available PD/toxicological data in animals have shown excretion of melatonin / metabolites milk (with reference to Section 5.3), that a risk to the breastmilk feeding child cannot be excluded, and that the product should not be used during breastfeeding. Section 5.3 includes text noting that delay in sexual maturation in male and female off-spring of rats and ground squirrels occurred on exposure to melatonin postpartum, indicating that exogenous melatonin is secreted in milk and that it may influence the ontogeny and activation of the hypothalamic-pituitary-gonadal axis, though as the rat and ground squirrel are seasonal breeders the implications of these findings for humans are uncertain.

No safety data concerning melatonin use and lactation are available. However, due to the observed hormonal effects, the proposed SmPC text is endorsed.

Fertility

As melatonin modulates annual gonadal hypertrophy in seasonally-breeding animals, a potential adverse effect of exogenous melatonin on fertility is possible. Humans, unlike most mammals, are not seasonal breeders, and thus may be less susceptible to effects of exogenous melatonin. A study considered the balance of evidence from clinical studies to suggest that the effect of melatonin on human reproductive processes such as ovulation and fertility is attenuated compared to that in animals. Section *Reproductive function* (in Secondary PD and safety pharmacology) notes that information on the effects of exogenous melatonin on human ova, sperm, and fertility are limited, but that no clear adverse effects of doses comparable to those recommended for the Applicant's product have been seen with short term (days-to-weeks) intake, and that beneficial effects of such doses have been reported. No case reports involving fertility (including effects on ova or sperm) were identified in the literature. Exposure to a 3 mg melatonin tablet (IR) equivalent to ~ 820,100 7-day (10- tablet) treatment periods did not result in receipt or identification of a case report involving fertility.

Based on the above information the *Fertility* heading of section 4.6 of the SmPC for Melatonin 1mg/ml oral solution includes text noting that high doses of melatonin and use for

longer periods than indicated may compromise fertility in humans, that animal studies are insufficient with respect to effects on fertility (with reference to section 5.3), and that the product is not recommended in men and women planning a pregnancy. Section 5.3 does not provide any specific data on effects on fertility.

Children and adolescents
The safety of oral melatonin in children and adolescents and in particular its potential to adversely affect sexual development, is highly relevant generally, however, the Applicant is seeking an indication only in patients aged 18 years or older. A recent article strongly criticises the increasing prescription of melatonin for sleep disorders in children. Use in children and adolescents is not recommended.

Lack of evidence in children and the potential of melatonin effects on the reproductive organs in non-clinical studies as well as potential long-term endocrine effects on cardiovascular, immune and metabolic systems when administered to children and adolescents warrant SmPC limitations and warnings on paediatric use.

Food (type and timing of meals)
The ~ 2-fold greater increase in plasma melatonin level expected if the recommended dose of the Applicant's product is taken with food (as opposed to on an empty stomach) is not considered a safety concern. That food appears to have a limited effect on T_{max} and plasma half-life for IR melatonin are also positive efficacy/safety findings, as the rapid rise in plasma melatonin level post-ingestion, and the subsequent efficient elimination of melatonin from the plasma, have implications for the efficacy of the single daily dose of melatonin to re-entrain circadian rhythmicity.

Text included under the heading *Absorption* in section 5.2 of the SmPC for Melatonin 1mg/ml oral solution notes that food is not expected to affect the safety or efficacy of the product, however it is recommended that food is not consumed 2 before or 2 h after intake of melatonin. Relevant text regarding the potential for food to increase plasma exogenous melatonin level, and the possibility that melatonin taken with carbohydrate-rich meals may impair blood glucose control, is included in sections 4.2 and 4.4.

Alcohol
No studies examining the influence on alcohol on the safety of oral melatonin in general, or its safety/efficacy for treatment of jet lag, were identified in the literature. Several of the 10 key jet lag studies in a published review of melatonin for jet lag required that alcohol (any alcohol in 2 studies, alcohol intake greater than normal in 2 studies, strong alcohol in 1 study) was avoided in-flight and/or post-flight. As alcohol can impair sleep, cause diuresis (also disturbing sleep due to the need for toilet visits), potentially worsen certain symptoms of jet lag (*e.g*. headache, morning fatigue, concentration), and have a sedative effect when consumed in large quantities, avoidance of alcohol during melatonin treatment is considered prudent. Relevant information is included under the *Posology* heading in section 4.2 of the SmPC for Melatonin 1mg/ml oral solution).

Tolerance
For short duration treatment as applicable for jet lag, development of tolerance is considered unlikely. Tolerance was not addressed in the vast majority of clinical studies involving adults. Tolerance has not been raised as a safety concern in reviews addressing melatonin safety.

Dependence

Clinical studies have not raised concerns regarding the potential for dependence. The brief duration of treatment applicable for jet lag contributes to a low risk of dependence in this indication but data regarding longer term use is lacking.

Withdrawal
Clinical studies summarised in this overview of safety have not raised concerns regarding the potential for withdrawal effects.

Abuse
Clinical studies and reviews of melatonin summarised in in this overview of safety have not raised concerns regarding the potential for abuse.

Overdose
Overdose may be considered in terms of acute accidental or deliberate overdose, or prolonged overdose (technically misuse). Data from published clinical trials and a cohort study specifically designed to assess the safety of oral melatonin (10 mg, probably IR), taken once daily at 22:00 hr for 28 days by 30 healthy men (an additional 10 men took placebo) did not observe differences between melatonin and placebo for a broad range of objective or subjective safety parameters nor for adverse effects.

A study reported that daily intake of 300 mg melatonin for 4 months by 8 younger women (19 – 37 years) reduced levels of luteinising hormone, estradiol, and progesterone, but did not affect menstrual bleeding patterns, or result in physical complaints or complaints about altered sleep or activity patterns, emotional well-being, or mood. The results of all haematological and biochemistry measurement during and after the treatment were normal.

A study in which patients undergoing liver resection were given a single oral dose of 50 mg/kg bw melatonin (equivalent to 3000 mg for a 60 kg person) pre-surgery reported no signs of adverse effects or post-operative complications associated with melatonin.

A study in which 4 women and 7 men (in and outpatients) with Parkinsonism were given increasing daily doses of melatonin with maximum doses in the range 3000 – 6600 mg/d for 15 – 35 days reported headache, diarrhoea, flushes, abdominal cramps, scotoma lucidum (flashes of light in the eyes); there were no clinically significant changes to haematology and blood chemistry.

Based on the submitted information, Section 4.9 of the SmPC for Melatonin 1mg/ml oral solution notes that drowsiness, headache, dizziness, and nausea are the most commonly reported signs and symptoms of overdose with oral melatonin, that ingestion of daily doses of up to 300 mg melatonin did not cause clinically significant adverse reactions, and that flushes, abdominal cramps, diarrhoea, headache and scotoma lucidum have been reported after ingestion of extremely high melatonin doses (3000 – 6600 mg) for several weeks. It is also noted that clearance of the active substance is expected within 12 h of ingestion.

Ability to drive or operate machines
A crossover study in which 5 mg IR melatonin was ingested at 12:30 hr by 16 healthy subjects (10 men, 6 women, mean age 22 years) observed impairment of task tracking and of response and reaction time scores for visual tasks, with the degree of impairment inversely correlated with saliva melatonin concentration (a good surrogate for plasma level). It was concluded that daytime administration of melatonin can impair neurobehavioral performance.

Another study found administration of 5 mg IR melatonin at 17:00 hr to 8 men (23 – 28

years) to reduce alertness over the subsequent 4 h by ~ 30%, with a comparable effect seen in each subject. When measured 1 h after ingestion of 10 mg melatonin by 14 healthy subjects (5 men, 9 women, mean age 28 years) in the late morning, posturographic performance (the contribution of visual information to balance) was decreased in all subjects, with 6 subjects showing impaired eye movements. Improvements in some parameters were also seen in some subjects. None of the subjects reported fatigue, drowsiness, dizziness or other side effects. It was concluded that melatonin interacts with vestibular reflexes, causing specific impairment of oculomotor and postural performance.

A crossover study specifically designed to examine the effect of melatonin on driving performance collected data via a clinical examination, body-sway test, and a standardised driving computer test battery (assessing attention, reaction time, concentration and sensomotor coordination), and for subjective sleepiness, for 20 healthy subjects (12 men and 8 women aged 21 – 57 years) starting 1 h after ingestion of 5 mg IR melatonin at 16:30 hr. Overall, the driving test battery showed no objective adverse impact of melatonin on driving performance, and the clinical examination results and body-sway test were unaffected. The subjective sleepiness expressed by the participants did not cause practically relevant impairment of driving performance, though the authors considered it grounds to recommended that caution should be exercised when driving under the influence of melatonin.

A study found 2 mg melatonin (prolonged release) to exacerbate the adverse effects of zolpidem on psychomotor function and driving performance. This study is addressed in Section *Pharmacodynamic drug interactions*. Section 4.7 of SmPC for Melatonin 1mg/ml oral solution notes that melatonin has moderate influence on the ability to drive and use machines; as melatonin may decrease alertness for several hours, use of the Applicant's product is not recommended prior to driving or using machines.

The above conclusions are endorsed by the MHRA assessor. Taking into account that melatonin may be used e.g. by flight crew it is important that the SmPC addresses the potential effects on alertness.

Safety related to drug-drug interactions and other interactions

Pharmacodynamic drug interactions

Zolpidem (and related drugs)

A crossover study investigating the efficacy for treatment of jet lag of a 5 mg oral dose of IR melatonin, a 10 mg dose of zolpidem, and their combination, taken in-flight (17:00 – 21:00 hr) and at bedtime on 4 consecutive days after arrival found the combination to result in a significantly higher incidence of morning sleepiness, nausea, and confusion (particularly on the first day of arrival), and to reduce activity during the first hour after getting up, compared to melatonin or zolpidem alone when symptoms of jet lag were graded by the participants (137 men and women, 18 – 68 years) each evening.

Adverse events (including nausea and confusion) were generally comparable in nature and frequency in the zolpidem and combination groups, and as essentially no adverse events were reported by the melatonin group, the adverse events data do not suggest that melatonin exacerbated adverse events due to zolpidem. However, drop-out rate was higher in the combination group (11 of 40) compared to the melatonin group (5 of 40) and the zolpidem group (6 of 40).

Co-administration of a single oral dose of 2 mg melatonin (prolonged release) with zolpidem 10 mg at ~ 20:00 hr to 12 men and 4 women (55 – 65 years) exacerbated the adverse effects of zolpidem on psychomotor function, driving performance 1 h and 4 h post-dosing, and

early memory recall. As plasma C_{max}, T_{max}, and AUC for each drug alone did not differ significantly from those measured for the drugs when given in combination, the authors suggested a PD interaction between melatonin and zolpidem (involving GABA-A receptors) that resulted in enhancement of the acute sedative effects of zolpidem by melatonin. The effects were short-lived, and the study did not expect any hangover effects on cognitive function next morning that would impair the ability to conduct the activities of normal living. The findings of the 2 studies differ in relation to the potential for the interaction between zolpidem and melatonin to affect next-morning status however the studies did not examine the same parameters, and the subjects in a study were also affected by jet lag. Although zolpidem (an imidazopyridine) is structurally unrelated to benzodiazepines, it binds the same receptor (i.e. GABA-A). The presence of GABA-A receptors in the SCN raises the possibility that its function may be influenced by zolpidem, which may in turn modulate the action of melatonin on the SCN.

Text under the *Pharmacodynamic interactions* heading of section 4.5 of the SmPC for Melatonin 1mg/ml oral solution notes that melatonin may enhance the sedative effect of benzodiazepines (*e.g.* midazolam, temazepam) and non-benzodiazepine hypnotics (*e.g.* zaleplon, zolpidem, zopiclone), and that in a study of jet lag therapy the combination of melatonin and zolpidem resulted in a higher incidence of morning sleepiness, nausea, and confusion, and reduced activity during the first hour after getting up, compared to zolpidem alone.

Opioidergic drugs (morphine, fentanyl) and diclofenac

The clinical overview summarised information on the apparent analgesic action of exogenous melatonin. The underlying physiologic mechanisms were considered unclear, though the involvement of GABA-B receptors was named as one of several possibilities. This raises the possibility for PD interaction with GABAergic drugs such as opioidergic drugs (morphine, fentanyl) and diclofenac, and the potential for excessive analgesia. Several of the studies included in the reviews noted that peri-operative intake of melatonin was associated with reduced post-operative intake of the above drugs, though the issue of whether this apparent PD drug interaction could have adverse effects, such as excessive analgesia, was not addressed. Safety reviews of melatonin have not raised relevant concerns. The above data suggest that oral doses of melatonin comparable to the recommended dose for the Applicant's product have an analgesic action, though analgesic, sedative, and eventual anxiolytic effects may, in fact, contribute to the efficacy of melatonin taken at bedtime for treatment of jet lag. Considering the posology and brief duration of treatment period proposed for the SmPC for Melatonin 1mg/ml oral solution, the apparent analgesic effect of melatonin is not considered a safety concern.

Medication frequently used by diabetics

A study in which 2 mg melatonin (prolonged release) was taken has been reported. 36 Type 2 diabetics (25 women, 11 men; 46 – 77 years; 16 on oral hypoglycaemic agents, 20 on insulin) for 3 weeks in a controlled phase, and for a further 5 months in an open phase, stated that no interaction with any of the medications frequently used by diabetic patients was observed (*i.e.* metformin, sulfonylureas, thiazolidinediones, peroxisome-proliferator activated receptors agonists, insulin, fibrates and other lipid-lowering agents, angiotensin-converting inhibitors, calcium antagonists, beta-blockers, anticoagulants, and serotonin-reuptake inhibitors).

The potential for oral melatonin to transiently impair glycaemic control when taken in close proximity to a meal, its implications for diabetics, and relevant safety information included in the proposed SmPC is addressed in section A*dverse effects on glycaemic control* (in Specific

safety issues).

The submitted evidence suggest that melatonin can increase plasma glucose in healthy persons and there is a potential for interaction with food, but no interactions with commonly used medication in diabetes have been reported. The SmPC text addressing glycaemic control in section 4.2 is endorsed.

Warfarin
No potential safety issues have been identified on the concomitant use with warfarin in the literature review submitted and no warning in the SmPC for the proposed product is considered necessary.

Conclusion on clinical safety
The most common AEs reported in the published articles included headache, nausea, drowsiness and sedation. The incidence of AEs is low. There were no serious AEs or death reported.

PD studies and animal studies show that melatonin has strong effects on several hormones involved in the sexual maturation of pubertal female and male rats. In addition, effects of melatonin on the level of several hormones involved in reproduction have been found in mature rats and dogs. In addition, PD studies show effects on reducing glucose tolerance and insulin sensitivity. Likewise, the analgesic effect of melatonin may point to the possibility of interactions with opioidergic and GABAergic medications.

The SmPC wording on immune system adverse events and the potential of melatonin to modulate immune system function is endorsed.

The fact that no cases were identified in the safety database is reassuring and support the contention that melatonin has a benign safety profile.

In addition, the evidence submitted suggest that melatonin may have an effect on fertility in women or men, on pregnancy, and on breast feeding. Although the data are sparse a warning about these effects are included in the relevant SmPC sections.

In addition, the submitted evidence also suggest that melatonin can increase plasma glucose in healthy persons and there is a potential for interaction with food. The SmPC warning indicating that intake of melatonin with carbohydrate-rich meals may impair blood glucose control and should therefore be avoided for 2h before and 2h after intake of melatonin is endorsed.

Although melatonin may have analgesic effects, no evidence was identified to show a potential for PD interactions with analgesics. Therefore, no warning is deemed necessary in connection with the analgesic effect of melatonin.

An important potential risk of melatonin is its co-administration with other medications or substances that are also involved with CYP1A metabolism such as fluvoxamine, zolpidem or related drugs (zopiclone, zaleplon), thioridazine and imipramine, which may increase the plasma concentration of either products. A warning against such co-administration is included in the SmPC of and therefore this risk is considered addressed. Likewise, the risk of high exposure to melatonin in persons with renal or hepatic dysfunction is mentioned in the SmPC, and therefore this risk is considered addressed as well.

Lack of evidence in children and the potential of melatonin effects on the reproductive organs in preclinical studies as well as potential long-term endocrine effects on cardiovascular, immune and metabolic systems when administered to children and adolescents warrant SmPC limitations and there are warnings on paediatric use in sections 4.2 and 4.4 with additional text in section 5.1.

Altogether, the (limited) available evidence from clinical studies and post-marketing data suggests that melatonin is generally safe and well tolerated in the short term, with headache, nausea and drowsiness as the most frequent side effects. There seem to be no important identified safety issues and there is a very long and extensive history of the use of melatonin from the US in particular where it is available as a food supplement. There is good agreement between the tabulated adverse reactions presented in the bibliographic dossier and in section 4.8 of the SmPC for Melatonin 1mg/ml oral solution.

There is a lack of medium to long term safety data to support more than occasional short-term use.

IV.6 Risk Management Plan (RMP)

The Applicant has submitted an RMP, in accordance with the requirements of Directive 2001/83/EC, as amended. The Applicant proposes only routine pharmacovigilance and routine risk minimisation measures for all safety concerns. This is acceptable.

IV.7 Discussion on the clinical aspects

It is agreed that melatonin has demonstrated PD effects that can be anticipated to be of clinical value in reducing symptoms of sleep-wake disorders such as jet-lag disorder, in which the primary issue is a phase shift in circadian rhythm. Melatonin has been shown to be effective in preventing or reducing jet-lag, based on the cumulative data from many studies. The timing of the melatonin dose is important.

Melatonin is generally well tolerated although headaches, dizziness nausea and drowsiness are reported with short term melatonin administration. There is lack of medium to long-term safety data to support more than occasional use.

The benefit/risk is considered positive for occasional short-term management of jet lag.
Based on extensive analysis of literature data, it can be stated that the therapeutic benefit clearly outweighs the possible risk associated with the use of melatonin as recommended by the Applicant.

The grant of a marketing authorisation is recommended for this application.

V USER CONSULTATION

The Patient Information Leaflet (PIL) has been evaluated via a user consultation study in accordance with the requirements of Articles 59(3) and 61(1) of Directive 2001/83/EC. The results show that the PIL meets the criteria for readability as set out in the guideline on the readability of the label and package leaflet of medicinal products for human use.

VI OVERALL CONCLUSION, BENEFIT/RISK ASSESSMENT AND RECOMMENDATION

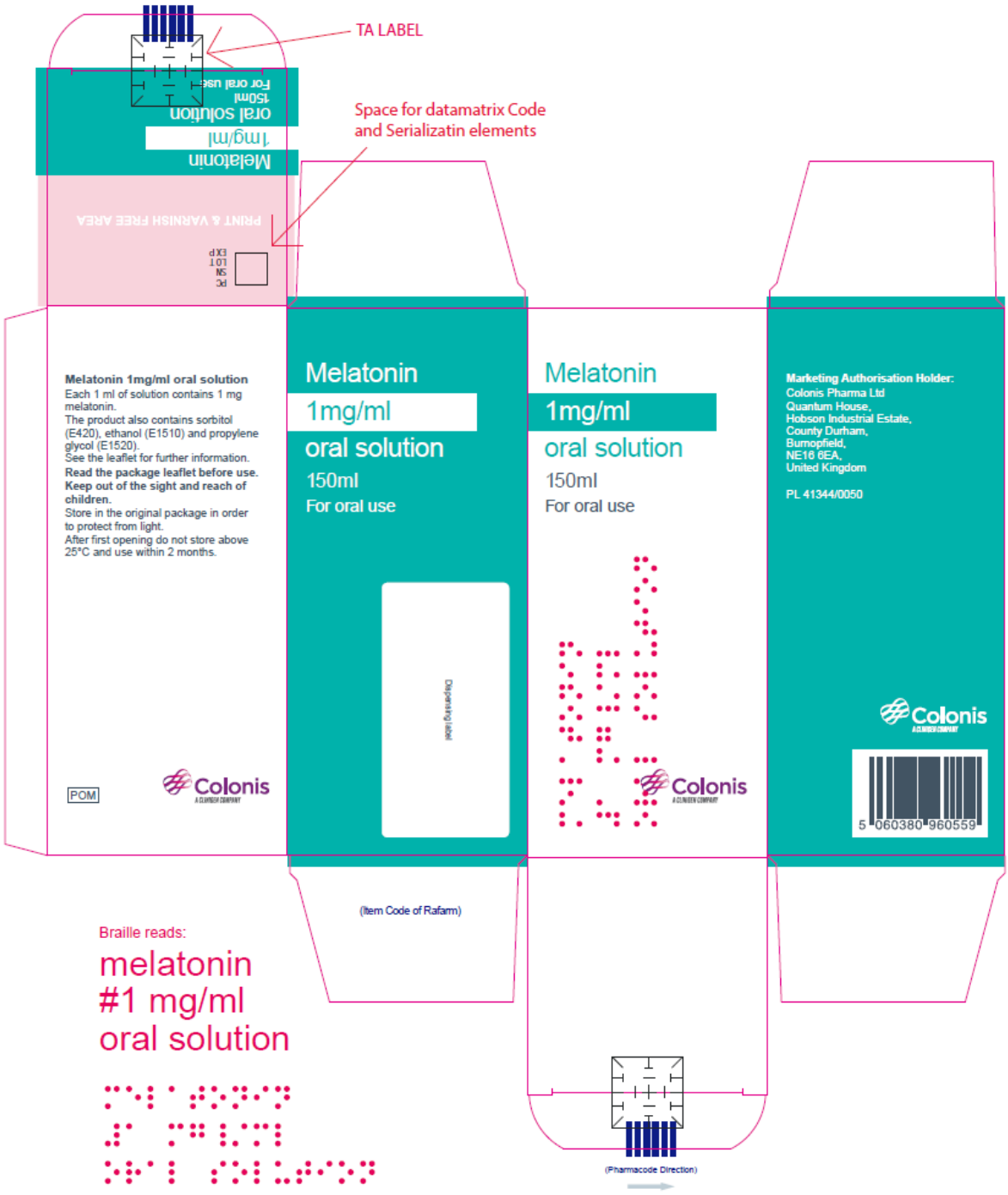
The quality of the product is acceptable, and no new non-clinical or clinical safety concerns have been identified from the literature. Based on extensive analysis of literature data, it can be stated that the therapeutic benefit outweighs the possible risks associated with the use of melatonin as recommended by the Applicant.

The benefit/risk is, therefore, considered to be positive.

The Summary of Product Characteristics (SmPC), Patient Information Leaflet (PIL) and labelling are satisfactory, and in line with current guidelines.

In accordance with Directive 2012/84/EU, the current approved UK versions of the SmPCs and PILs for these products are available on the MHRA website.

Representative copies of the labels at the time of UK licensing are provided below.



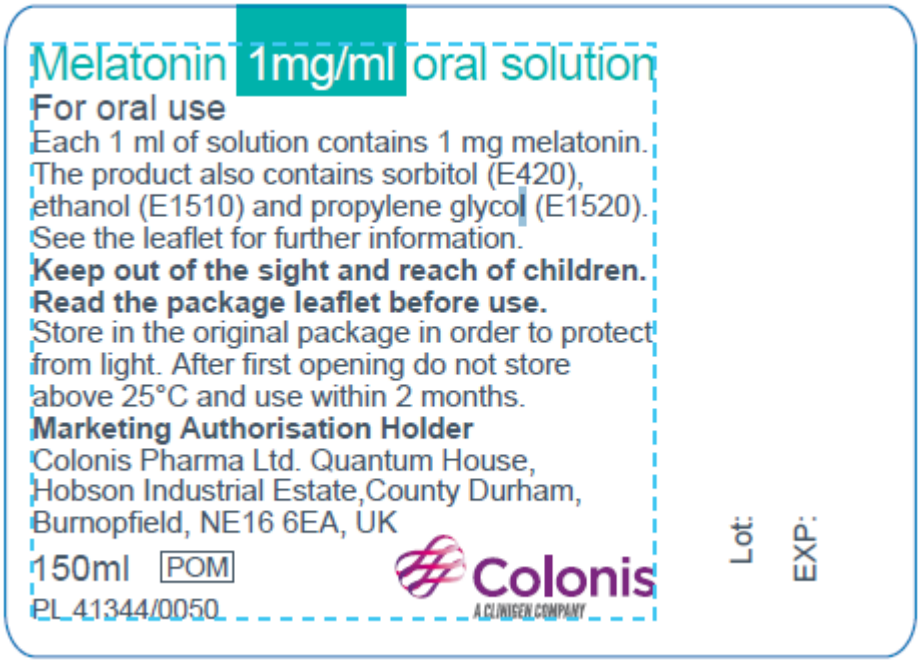

Melatonin 1mg/ml oral solution
For oral use
Each 1 ml of solution contains 1 mg melatonin.
The product also contains sorbitol (E420), ethanol (E1510) and propylene glycol (E1520).
See the leaflet for further information.
Keep out of the sight and reach of children.
Read the package leaflet before use.
Store in the original package in order to protect from light. After first opening do not store above 25°C and use within 2 months.
Marketing Authorisation Holder
Colonis Pharma Ltd. Quantum House, Hobson Industrial Estate,County Durham, Burnopfield, NE16 6EA, UK
150ml POM
PL 41344/0050
Colonis
A CLINIGEN COMPANY
Lot:
EXP:

TABLE OF CONTENT OF THE PAR UPDATE

Steps taken after the initial procedure with an influence on the Public Assessment Report (non-safety variations of clinical significance).

Please note that only non-safety variations of clinical significance are recorded below and in the annexes to this PAR. The assessment of safety variations where significant changes are made are recorded on the MHRA website or European Medicines Agency (EMA) website. Minor changes to the marketing authorisation are recorded in the current SmPC and/or PIL available on the MHRA website.

Application type	**Scope**	**Product information affected**	**Date of grant**	**Outcome**	**Assessment report attached Y/N**