Medicines & Healthcare products Regulatory Agency

MHRA

Public Assessment Report

National Procedure

Melatonin 3 mg film-coated tablets

(melatonin)

PL 39936/0006

Arriello s.r.o

LAY SUMMARY

Melatonin 3 mg film-coated tablets

(melatonin)

This is a summary of the Public Assessment Report (PAR) for Melatonin 3 mg film-coated tablets. 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.

This product will be referred to as Melatonin tablets in this lay summary for ease of reading.

For practical information about using Melatonin tablets, patients should read the package leaflet or contact their doctor or pharmacist.

What are Melatonin tablets and what are they 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 tablets can be used for treatment of jet lag in adults. Jet lag can be recognised by sleep disturbances, daytime tiredness, fatigue, mild mental impairment, irritability and digestive system disturbances experienced after flying.

How do Melatonin tablets work?
Melatonin is a hormone produced by the body that synchronises 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 tablets can help restore the normal day-and-night rhythm and reduce the symptoms.

The patient must talk to their doctor if they do not feel better or if they feel worse after 5 days.

How are Melatonin tablets used?
The pharmaceutical form of this medicine is a film-coated tablet and the route of administration is oral (taken by mouth). The tablets should be swallowed whole with water or other liquid *(e.g.* milk, fruit juice). Food should not be consumed 2 hours before or 2 hours after intake of Melatonin tablets.

The recommended dose for adults and elderly is 1 tablet daily for a maximum of 5 days. When the effect of Melatonin tablets is inadequate, 2 tablets can be taken simultaneously.

The first dose should be taken on arrival at destination at the patient's usual bedtime. Intake on the following days should also be at the patient's usual bedtime. The tablets should not be taken before 20:00 hour or after 04:00 hours.

The patient must talk to a doctor if they do not feel better or if they feel worse after 5 days.

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

Children and adolescents
This medicine should not be given to children and adolescents between 0 and 18 years as its safety and efficacy have not been established in this age group.

For further information on how Melatonin tablets are used, refer to the package leaflet and Summary of Product Characteristics 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 tablets 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 and two pharmacokinetic studies to show that Melatonin tablets are a safe and efficacious treatment for jet lag in adults.

What are the possible side effects of Melatonin tablets?
The most common side effects with Melatonin tablets (which may affect up to 1 in 10 people) are:
- -headache
- drowsiness.

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

Why were Melatonin tablets approved?
It was concluded that the data provided from literature references had shown that Melatonin tablets are 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 tablets?
A Risk Management Plan (RMP) has been developed to ensure that Melatonin tablets are 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 tablets
A Marketing Authorisation for Melatonin tablets was granted in the UK on 07 April 2020.

The full PAR for Melatonin tablets follows this summary.

This summary was last updated in June 2020.

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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 3 mg film-coated tablets (PL 39936/0006) 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 produced by the pineal gland and is structurally related to serotonin. Physiologically, melatonin secretion increases soon after the onset of darkness, peaks at 2 – 4 am and diminishes during the second half of the night. Melatonin is associated with the control of circadian rhythms and entrainment to the light-dark cycle. It is also associated with a hypnotic effect and increased propensity for sleep.

The activity of melatonin at the MT1, MT2 and MT3 receptors is believed to contribute to its sleep promoting properties, as these receptors (mainly MT1 and MT2) are involved in the regulation of circadian rhythms and sleep regulation.

This application was submitted under Article 10a of Directive 2001/83/EC, as amended, as a well-established use application. The active substance melatonin has been used in the EU for over 10 years for the indication jet lag.

With the exception of an *in vitro* Caco-2 permeability study, no new non-clinical data were submitted, as the data submitted for this application are 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. To address this requirement, the Applicant submitted two (one pilot and one pivotal) pharmacokinetic studies, comparing the Applicant's test product Melatonin 3 mg Hard Capsules with the Hungarian reference product Bio Melatonin 3 mg film-coated tablets.

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 07 April 2020.

II QUALITY ASPECTS

II.1 Introduction

Each film-coated tablet contains 3 mg of melatonin.

In addition to melatonin, this product also contains the excipients cellulose, microcrystalline; maltodextrin, silica, colloidal anhydrous and magnesium stearate.

The finished product is packaged in polyvinylchloride/polyvinylidene chloride/aluminium blisters. Each blister contains 7 or 10 film-coated tablets. The product is available in pack sizes of 10, 14, 28 and 30 film-coated tablets. Not all pack sizes may be marketed.

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.

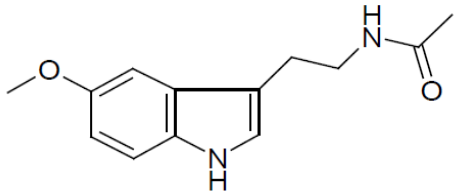
II.2 ACTIVE SUBSTANCE

rINN: Melatonin

Chemical Name: N-Acetyl-5-methoxytryptamine

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

Chemical Structure:



Molecular Weight: 232.27 g/mol

Appearance: Crystalline powder, ivory (off-white) 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.

Comparative *in vitro* dissolution profiles have been provided for the proposed product and comparator product Bio-Melatonin 3 mg film-coated tablets used in the bioequivalence study submitted to support bridging of the clinical bibliographic data to the proposed product.

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.

Confirmation has been given that the magnesium stearate used in the tablets is of vegetable origin.

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 below 25°C. Store in the original package in order to protect from light', is acceptable.

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. No new non-clinical studies were submitted, as the data submitted for this application are in the form of literature references. The literature review provided is satisfactory.

III.2 Pharmacology
Melatonin is an endogenous neurohormone that 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. In addition to the pineal gland, melatonin is synthesised in several other structures (retina, Harderian gland and gut) where the genetic expression and biochemical activity of the melatonin-synthesising enzymes have been detected. It has been proposed that melatonin plays an auto/paracrine role in these structures.

Extensive studies have been performed to understand the mechanisms of action of melatonin in the regulation of some seasonal and circadian functions and have demonstrated that the dynamic pattern of melatonin secretion is fundamental for its time-giving function. The rhythmic pattern of melatonin secretion is important because it provides information to the host about the concept and sense of time which, in turn, allows them to adapt some of their physiological functions to the daily and seasonal variations of their environment. Whilst this understanding of rhythm is useful it does not explain all of the different factors and its influence on the whole body. It is still poorly understood how the role of melatonin, from all the different sites of production, plays in regulating sleep and how this role is mediated. In addition, melatonin has a broader impact on other functions of the body mainly around the immune system.

Primary pharmacodynamics

***In vitro* studies**

Melatonin is described in the literature as acting at the central nervous system (CNS) level, modulating the synchronisation of the biological clock and promoting sleep through stabilisation and phase-shifting effects on the suprachiasmatic nucleus of the hypothalamus.

Melatonin shows its effects in mammals by four mechanisms:
1. Binding to melatonin receptors in plasma membrane
2. Binding to intracellular proteins such as calmoduline
3. Binding to Orphan nuclear receptors
4. Antioxidant effect.

Melatonin interacts with intracellular proteins named calmoduline, calreticulin and tubulin. Calmoduline is an intracellular secondary messenger. Melatonin directly antagonises binding of calcium to calmoduline. The anti-proliferative effect in cancer may be related to this. Retinoid-related Orphan nuclear hormone receptor family (RZR/ROR) is responsible for the immunomodulatory effects of melatonin. IL-2 and IL-6 are produced in mononuclear cells by this mechanism.

There are three different membrane receptors and one nuclear receptor:
1. Melatonin receptor type 1a: Mel 1a, ML1a, ML1, MT_1, MTNR1A. Encoded in human chromosome #4 and consists of 351 amino acids. MT1 receptor constitutes adenylate cyclase inhibition by binding to various G-proteins. MT1 receptors are commonly found in human skin . During aging process and Alzheimer's disease, the expression of MT1 receptor in suprachiasmatic nucleus (SCN) and cortex decreases. MT1 receptors reduce the neuronal discharge rate in SCN and suppress prolactin secretion.

2. Melatonin receptor type 1b: Mel 1b, ML1b, MT2, MTNR1B. Encoded in human chromosome #11 and consists of 363 amino acids. MT2 receptor creates adenylate cyclase inhibition by binding to various G -proteins. Additionally, it inhibits the soluble guanylyl cyclase pathway. Through melatonin receptor activation, adenylate cyclase inhibition occurs and the production of cyclic AMP (cAMP) is reduced.

In the skin, MT2 receptors are located within normal and malign melanocytes and eccrine sweat glands MT2 receptors inhibit GABA-A receptor-related functions in the hippocampus in rats. In Alzheimer's disease, MT2 receptor expression is reduced. MT2 receptors are involved in antidepressant activity. MT2 receptors contribute to the pathophysiology and pharmacology of sleep disorders, anxiety, depression, Alzheimer's disease and pain. MT2 receptors may be the new target for development of hypnotic agents.

MT2 receptors are responsible for anxiolytic effects of melatonin. Pharmacological studies have revealed that MT2 receptors regulate sleep, particularly NREMS. MT2 receptor ligands have more powerful hypnotic properties when compared to non-selective MT1/MT2 ligands. Mel1c, MTNR1C: It is not present in humans. It is found in fish, amphibians and birds. In chicken, the rhythm of MTNR1C receptor is the opposite of MT1 and MT2. Its level is highest at daytime and lowest at night-time. MT3, ML2= NQO2= Quinone reductase 2 enzyme= QR2. This enzyme belongs to the reductase group, which are involved in prevention from oxidative stress by inhibiting the electron transfer reactions of quinones. This enzyme (or MT3 receptor) is located in liver, kidney, heart, lung, intestine, muscle and brown fat tissue. It is a detoxification enzyme. There is evidence for its involvement in regulation of intra-ocular pressure.

3. RZR/RORα: With this receptor, melatonin binds to the transcription factors in nucleus which belong to retinoic acid receptor super-family. The following are described for retinoic acid receptor super-family variants RORα (retinoic acid receptor-related Orphan receptor-α; human gene ID: 6095): RORα isoform a (aka RORα1), RORα isoform b (aka RORα2) and RORα isoform d (also known as RZRα), and the product of another gene, RORβ (aka RZRβ; human gene ID: 6096).

4. GPR50: H9, ML1X: Melatonin-related Orphan receptor, X linked Orphan G-protein coupled. It is the orthologue of MEL1c, which is found in non-mammalian living creatures. Its gene is located on the X chromosome (Xq28) and consists of 618 amino acids. It is present in all mammalians including humans. It does not have the characteristics of binding to melatonin; however, it is effective in binding

of melatonin to MT1. GPR50 is not present in birds and fish. It is located in the brain and periphery. Its natural ligand has not been defined yet. It was reported that a deletion mutant in GPR50 might have been associated with bipolar disorder and major depression. GPR50 has no affinity to melatonin; however, when it dimerises with MT1, it inhibits the melatonin signal. GPR50 has other functions apart from melatonin. GPR50 interacts with neurite outgrow inhibitor (NOGO-A) and TIP60 (glucocorticoid receptor signal coactivator and histone acetyltransferase).

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 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 proteins, one of which mediates inhibition of adenylcyclase and the other activates phopsholipase 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.

Evidence suggests that melatonin can influence immune cells through nuclear and membrane melatonin receptors. 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, intraperitoneally (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 provided 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 (versus controls) for which a plausible explanation was not provided.

SECONDARY PHARMACODYNAMICS

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 (IFN-γ) was shown to increase the production of melatonin from *in vitro*-cultured rat pineal glands. Administration of recombinant IL-1β inhibited serum melatonin levels in rats through a receptor mediated mechanism, whereas granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF) 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 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 synthesise and release substantial amounts of melatonin, but that this melatonin modulates the IL-2/IL-2 receptor (IL-2R) 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 IgG1 and IgM 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 (MS), Systemic Lupus Erythematosus (SLE), Type 1 Diabetes (T1D), Irritable Bowel Syndrome/Inflammatory Bowel Disease (IBS/IBD), Breast Cancer and 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 CNS, 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 (12.8 mg/kg) increased serum prolactin levels. In adult males, subcutaneous (s.c.) 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 stimulation hormone (FSH) concentrations in treated rats suggests that melatonin interferes with the pubertal increase in GnRH secretion. The reversibility of the effects were also confirmed in another study.

A study confirmed that chronic melatonin administration (100 µg/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 s.c. 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 s.c. for 10 days decreases adrenal gland and serum corticosterone levels, and at 8mg/kg s.c. 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 s.c. 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
In one study, melatonin 30-60 mg/kg intravenously (i.v.) in rats, 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 i.v. 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; ED_{50} vs 3-MPA, 115 mg/kg; ED_{50} 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.

SAFETY PHARMACOLOGY
Central nervous system
Experiments have been carried out on the potential effects of melatonin on the nervous system. In one study, it was reported that melatonin, in physiological doses, causes vasoconstriction and also constricts cerebral arteries in rats. Although many compounds which prolong hypnotic activity can lower body temperature, melatonin (25 mg/kg) had no hypothermic action in the rabbit. Melatonin at doses of 10 mg/kg produced no change in post-synaptic spike potentials in the cat superior cervical ganglion and no change in the response in the nictitating membrane. In mice, 30 mg/kg of melatonin given intraperitoneally every 3 hours for 18 hours caused no change in gross behaviour or in the amount of noradrenaline in the brain or heart.

Cardiovascular system
Depending on the concentrations of melatonin or the preparation used, melatonin can exert either a vasoconstrictory effect at physiological concentrations (nanomolar) or a vasodilatory effect at higher concentrations (micromolar or millimolar), suggesting a biphasic pharmacology of melatonin. The subcellular mechanism of such an activity is as yet unknown despite the fact that melatonin receptors have been identified in different structures (arteries). Melatonin and its main target, the SCN, are able to modify cardiovascular rhythms (e.g. blood pressure, heart rate). Taken together, these data, among others, show that melatonin could modulate the rhythmicity of the cardiovascular system. Again, alterations of the circadian rhythmicity of melatonin could be deleterious from a long-term effect point of view.

The effects of melatonin on the circulatory system were investigated in one study. The results of experiments on the blood pressure of cats indicate that the administration of 10 mg of melatonin/kg did not alter the blood pressure. Melatonin (10 mg/kg) also failed to alter the contractile force or electrocardiogram of the dog. The inotropic and chronotropic actions of the isolated guinea pig and rat heart were unaltered when melatonin was perfused at a concentration of 10^{-4} mole/l.

Pharmacodynamic drug interactions
Melatonin has been shown to enhance tamoxifen's effects.

Additionally, in one study, the potential synergistic effect of melatonin on a classical drug, imipramine, was evaluated. To test this hypothesis, a porsolt swim test was conducted in mice. Imipramine at doses of 20 and 40 mg/kg caused no alteration and statistically significant reduction in the duration of immobility in forced swim test, respectively. While 5 mg/kg melatonin had no effect, 10 mg/kg melatonin slightly reduced the duration of immobility. When sub-effective doses of imipramine and melatonin (20 and 5 mg/kg, respectively) were co-administered, there was no alteration in responses compared

with those of each drug alone. Likewise, the effective dose of melatonin (10 mg/kg) did not cause any increase in responses to 20 mg/kg imipramine. Although combination of imipramine (40 mg/kg) and melatonin (5 mg/kg) did not exert an antidepressant effect above that of imipramine alone, co-administration of the effective doses (10 mg/kg and 40 mg/kg for melatonin and imipramine, respectively) displayed an additive effect. There were no significant differences between groups in relation with locomotor activity test. The results show that co-administration of imipramine and melatonin exhibits an additive effect and that there seems to be no interaction between the drugs.

Overall conclusions on pharmacology

The pharmacology of melatonin is well known and described in the literature. The non-clinical overview contains dedicated sections for primary and secondary pharmacodynamics, safety pharmacology and pharmacodynamic drug interactions. The non-clinical overview is acceptable.

III.3 Pharmacokinetics

Absorption

***In vivo* absorption**

In one study, 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. The authors commented that the endogenous serum levels of melatonin were low as compared to those obtained after oral administration of melatonin, which gave 104 to 106 times higher levels.

In another study, the 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 pharmacokinetics of melatonin. To probe the issue of nonlinear pharmacokinetics, oral bioavailability of a 1 mg/kg dose of melatonin was studied in dogs. The results indicate significant dose dependency in the pharmacokinetics, 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/hr/kg)	2.11	3.84		1.68
Half-life (hr)	0.33	0.31		0.57
Vd_{ss} (L/kg)	1.05	1.48		1.20
Oral dosing				
Dose (mg/kg)	10.00	0.98	1030	10.00
AUC (mg.hr/L)	2.49	0.05	3.44	8.85
Dose adjusted F (%)	53.5	16.9	>100	>100

In another study, the bioavailability after nasal application of 1.5 mg of melatonin in rabbits was found about 60% and C_{max}, T_{max} and $t_{1/2}$ were found 160 ng/ml, 5 min and 10 min respectively.

***In vitro* absorption**
The Applicant has performed an *in vitro* Caco-2 cell study to provide an estimate of the intestinal permeability of melatonin with the intention of bridging the bibliographic data with the product under assessment. The *in vitro* study assessed 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 (Papp) was 40.3 x 10-6 cm/s and 39.9 x 10-6 cm/s 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. These calculated permeability values are supported by the literature. The results of this study are discussed further under Clinical Aspects, Section IV.2 Pharmacokinetics.

Distribution
Melatonin readily penetrates biological membranes and thus appears in tissues or body fluids in concentration on the same order of magnitude as plasma.

In one study, the steady state volume of distribution of melatonin in different species (Sprague-Dawley [SD] rat, Beagle dog and Cyno monkey) ranged from 1.05 to 1.48 L/kg, indicating moderate tissue distribution of melatonin in these animals. In humans, authors have reported a steady state volume of distribution of 0.55 L/Kg, suggesting a significantly reduced distribution of melatonin in humans than in the animal models.

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 3H acetyl melatonin to SD rats on day 18 of gestation resulted in detection of radioactivity in whole foetuses and foetal tissues (brain, liver, heart, viscera, skin, muscle, and bone), with highest concentrations in foetal liver and lowest concentrations in foetal brain.

Melatonin seems to distribute fast through tissues in the rat after systemic injections, rapidly penetrates into brain and cerebrospinal fluid.

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.

As described in one study, when ^{14}C-melatonin was injected intracisternally into rats, 2.35% of the total radioactivity in the urine was recovered as compound II (N-acetyl-formyl-5-methoxykynurenamine). In the case of intravenous administrations of melatonin, 15% of the total radioactivity was recovered as compound (II). In either case, 65% of the radioactivity administered was recovered in the urine within 24 hours. These results taken together strongly indicate that the conversion of melatonin to compound II via compound I (after melatonin degradation) represents one of the major metabolic pathways of melatonin in the mammalian brain. One study demonstrated that melatonin has two principal metabolites, N-acetylserotonin as well as 6-Ha-melatonin after administration of various doses of melatonin in rats. The authors concluded that the conversion of melatonin to 6-Ha-melatonin and N-acetylserotonin resulted from two independent metabolic pathways.

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

In one study, following administration of different doses of melatonin, plasma hydroxymelatonin and melatonin concentrations increased in a dose-dependent manner (R = 0.99). Plasma 6-hydroxymelatonin represented approximately 1% of plasma melatonin, irrespectively of the dose of melatonin administered.

Excretion
Following IV administration of 5 mg/kg, the apparent elimination half-life of melatonin in rats was 19.8 minutes. 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.

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. Fluvoxamine is a potent inhibitor CYP1A2, and there is potential for interaction with drugs that are metabolised by this isoenzyme.

Authors 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 µM for the formation of 6-hydroxymelatonin and 0.05 µM for the formation of N-acetylserotonin. Other than fluvoxamine, fluoxetine, paroxetine, citalopram, imipramine, and desipramine were also tested at 2 and 20 µM. Among the other antidepressants, only paroxetine was able to affect the metabolism of melatonin at supratherapeutic concentrations of 20 µM, 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-ethinyloestradiol 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 and quinolones, all potentially increasing melatonin levels. CYP1A2 inducers such as carbamazepine and rifampicin theoretically could reduce the plasma concentrations of melatonin.

Other pharmacokinetic studies
It has been shown that the distribution and metabolism of exogenous melatonin in neonatal rats is similar to that in adult rats. Neonatal rats showed rapid absorption (~ 90%) of total dose within 45 minutes) and metabolism (~ 60% of total dose within 60 minutes) following incubation 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-hydroxymelatonin.

One study reported that the oral transmucosal route demonstrated higher C_{max} values of melatonin with similar T_{max} values compared to oral melatonin. On the other hand, the possibility of direct transport of melatonin from nasal cavity into the cerebrospinal fluid (CSF) after nasal administration (40 µg/rat) in rats has been also investigated. Melatonin quickly absorbed in plasma (T_{max} = 2.5 min) and showed a delayed uptake into the CSF (T_{max} =15 min) after nasal administration. The melatonin concentration-time profiles in plasma and CSF were comparable to those after intravenous delivery. The AUC_{CSF}/AUC_{plasma} ratio after nasal delivery (32.7 ± 6.3%) did not differ from the one after intravenous injection (46.0 ± 10.4%), which indicates that melatonin enters the CSF via the blood circulation across the blood-brain barrier. The result demonstrated that there is no additional transport via the nose-CSF pathway.

Additionally, melatonin supplementation in diabetes and acute exercise significantly changes the element metabolism of the liver tissue in adult male rats. As demonstrated in one study, prevention of the decrease in liver zinc in diabetes by melatonin supplementation in particular suggests that melatonin treatment can be beneficial in diabetes.

The effect of melatonin on cholesterol absorption in rats has been investigated in another study. Melatonin suspension (10 mg/kg) was administered to SD rats. Treatment with melatonin inhibited cholesterol absorption in intestine of rats fed on high cholesterol diet and consequently positively modified lipoprotein cholesterol profile in plasma and the content of lipids (cholesterol, TG) in the liver.

A study in rats, demonstrated that prolonged constant light exposure modified the distribution (reduced Vss) and elimination (reduced CLs) of a bolus injection of 1 mg/kg melatonin, without modifying its elimination half-life. Only the administration of low doses (0.01 mg/kg/day) resulted in both a circadian pattern for 6-sulfatoxymelatonin excretion and normal physiological values during the infusion free intervals.

Tissue distribution of ^{125}I-thyroxine (T4) and 3H-melatonin and the effect of each hormone on the tissue content of the other were studied in one study. Late pre- to prometamorphic *Rana catesbeiana* tadpoles on an 18 light: 6 dark cycle were used for injection of hormones in vivo or to supply tissues for in vitro hormone administration. Labelled melatonin uptake was highest in intestine, ventral skin and pituitary; lowest in thyroid and brain and intermediate in hindlimb, tail and gills. The tissue content of labelled T4 was distributed in nearly the same way, except that the thyroid level was relatively higher, and pituitary lower, than that of labelled melatonin. The pineal, studied only in the tracer T4, experiments, had the highest content of labelled T4 of all tissues. Simultaneous injection of either 0.007 or 0.2 pg T4, increased 3H-melatonin uptake into peripheral tissues that undergo major metamorphic changes but not into neural or endocrine organs. In contrast, 0.033, 3.75 or 15 pg melatonin had no significant influence on the content of ^{125}I-T4 in any tissue studied *in vivo*. Results of *in vitro* labelling of selected tissues were generally in agreement with the *in vivo* work except that the ^{125}I-T4 content of intestinal segments from late prometamorphic larvae was lower in melatonin-treated than in control groups. The results suggest that peripheral tissues are a major site for T4-melatonin interactions and that T4, may modulate its own action through influencing melatonin levels in target tissues and perhaps in the thyroid. Because melatonin had no effect on tissue T4 content in young tadpoles, retardation of metamorphic events by melatonin does not seem to involve modulation of T4 availability to the tissues.

Overall conclusions on pharmacokinetics

The non-clinical overview discusses published sources of information on the pharmacokinetics of melatonin, addressing absorption, distribution, metabolism, and excretion, as well as pharmacokinetic drug interactions. 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 pharmacokinetics section provides a brief but adequate review of the pharmacokinetics of melatonin.

III.4 Toxicology

The acute toxicity of melatonin was studied in mice and rats, by oral (p.o.), intravenous (i.v.), intraperitoneal (i.p.) and subcutaneous (s.c.) administration. The LD_{50} of melatonin was determined in both species. At high doses (400 mg/kg), vasodilatation of the extremities indicated by a reddening of the ears and feet, piloerection and ptosis were common. In addition, muscle relaxation, a marked lack of motor activity flexor reflexes, a marked reduction in body temperature and slow, laboured respiration preceded death. Values were similar for both species except that oral administration of melatonin had less behavioural effect and was considerably less toxic in the rat than the mouse.

Importantly, the LD_{50} by the oral route was shown to be approximately 1250 mg/kg in mice and > 3200 mg/kg in rats, which is greatly in excess of the maximum envisaged 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)

Organism	Test Type	Route	Reported Dose (Normalized Dose)	Source
mouse	LD_{50}	intraperitoneal	1375 mg/kg (1375 mg/kg)	
mouse	LD_{50}	intravenous	180 mg/kg (180 mg/kg)	
mouse	LD_{50}	oral	1250 mg/kg (1250 mg/kg)	[88]
mouse	LD_{50}	subcutaneous	> 1600 mg/kg (1600 mg/kg)	[88]
rat	LD_{50}	intraperitoneal	1131 mg/kg (1131 mg/kg)	[88]
rat	LD_{50}	intravenous	356 mg/kg (356 mg/kg)	[88]
rat	LD_{50}	oral	> 3200 mg/kg (3200 mg/kg)	[88]
rat	LD_{50}	subcutaneous	> 1600 mg/kg (1600 mg/kg)	[88]

In the same study, melatonin was also found to produce considerable motor incoordination in mice at high doses. By both routes (p.o. and i.p.) melatonin was most potent 15 to 30 minutes after dosing. A rapid decline in potency was seen after this presumably due to the rapid metabolism of melatonin.

IV.2 Repeated-dose toxicity

Rats

Melatonin was administered by gavage to Long-Evans and Fischer 344 rats in a 90-day toxicity study. The dose levels administered were 0, 0.005, 0.05, 5.0, 50 or 200 mg/kg bw/day. Doses were administered daily for 90 days, excluding weekends and holidays, for a total of 17 dosing days and 68 dosing days, for the Special Study Group and Core Groups, respectively.

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 their control. Also, in the Fischer rats, a reduction in body weight gain was observed, 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 measurements have been declared as 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 study, it was shown that, at doses of 15 mg/kg/day iv for 6 days, there was no change in blood pressure, heart rate or body temperature. At intravenous 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, SD rats received 60 µl/day of vehicle (PEG 400) containing 0.03%, 0.3% or 3% melatonin s.c., 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.

In rats, the toxicological profile of melatonin after 90-day period of administration was low, but very low doses were used in the study (0.3, 1.2 and 6 mg/kg/day). Data showed that plasma concentrations were up to 40 pg/ml, which are lower than those expected to be reached in humans, but the time of sampling is not specified. The only melatonin-related effect reported was a decreased body weight gain of the animals at mid (males) and high doses (males and females). Also decreased testis and increased kidney relative weights were 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-weeks group.

Dogs
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. Based on toxicokinetic data the C_{max} values obtained with the mid and high doses were high compared to the levels that ca be reached in humans.

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.

In another study, the effect of melatonin on the initiation of N nitroso N methylurea (NMU)-induced carcinogenesis in rats and mutagenesis was investigated, *in vitro*. Within the *in vitro* tests performed for the mutagenesis studies an Ames test was conducted using strains TA 100 and TA 102 of *Salmonella typhimurium*. Melatonin itself revealed no genotoxic effect. No protective action of melatonin (at doses of up to 2 micromol/plate) towards NMU was found in the Ames test.

***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 most 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 4 and more tumours was reduced in the group with constant melatonin treatment. Interrupted treatment with melatonin promote 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.

In a second study, 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 year). 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 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary adenocarcinoma in SD 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.

Another 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 SD 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 the promotion rather than the initiation phase and that melatonin appears to have antiestrogenic properties.

Further short-term studies in mice (10 µg topical administration for 14 days) and rats (20 mg/L in water for 3 days; 100 µg/mL in water for 28 weeks) showed further evidence of the protective effect of melatonin against known carcinogens.

Reproductive and developmental toxicity
Fertility and Early Embryonic Development
In a study in female CD-1 mice (16/group) melatonin was administered, 100 µg [~3-4 mg/kg] i.p.) 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 s.c. for 30 days (at 1700 hours) 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 [3H]dihydrotestosterone in prostate but caused a significant decrease in activity of acid phosphatase and uptake of [3H]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 s.c. for 30 days at 1700 hours) in comparison to vehicle-treated and untreated pinealectomised 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 pinealectomised groups, respectively).

Embryo-foetal Development
Melatonin was administered to SD derived rats on gestation days 6 - 19. Melatonin treated groups received 1-, 10-, 100-, 150-, or 200 mg/kg body weight/day in the screening study and 50, 100, or 200 mg/kg/day in the definite study. No maternal morbidity/mortality was found in either study. Melatonin had no effect on prenatal survival, foetal body weight, or incidences of foetal malformations/variations. Thus, in the definitive study, the maternal toxicity No Observed Adverse Effect Level) (NOAEL) and Lowest Observed Adverse Effect Level (LOAEL) were 100 and 200 mg/kg/day, respectively and the developmental toxicity NOAEL was ≥ 200 mg/kg/day.

Melatonin was administered by gavage to 25 timed-mated SD 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.

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.

One study investigated the effect of exogenous melatonin on embryo viability in undernourished ewes study. The data demonstrated that the treatment with melatonin implants at lambing improves the viability of embryos of undernourished ewes during the reproductive season, although the effect of melatonin seems not to be mediated at the oocyte competence level. Moreover, melatonin induces changes in the endometrial sensitivity of steroids in undernourished ewes. Neither nutrition and melatonin nor their interaction had a significant effect on the *in vitro* oocyte development. Melatonin treatment tended to increase the percentage of positive cells to progesterone receptors (PR) in deep glandular epithelium, independently of diet ($P = 0.09$), and the greatest staining intensity of PR was observed in the luminal and superficial glandular epithelia ($P < 0.0001$). Thus, the use of melatonin implants at parturition, even during the breeding season could be helpful tool, particularly when embryo development is affected by negative factors as undernutrition or the post-partum period.

Prenatal and Postnatal Development

A study in female Wistar rats (19-20/group) administered melatonin (2.5 mg/kg/d s.c.) 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 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 24:00 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 versus 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 another 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 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.

Although melatonin may exert inhibitory effects on puberty, its continuous administration is only capable to delay of 20 to 30 days, but not to block pubertal development.

Local tolerance
No specific local tolerance studies were researched.

Other toxicity studies
In rats, melatonin (5 mg/kg bw) was injected prior to a single dose of 10 mg/kg bw LPS and thereafter at 6-hours intervals up to 72 hours. The number of micronucleated polychromatic erythrocytes MN-PCE) 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 hours after LPS administration and continuing to the end of the study (72 hours). In blood, the increase in micronuclei formation was time-dependent in LPS-treated rats with peak values being reached at 36 – 48 hours. 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-hours interval) and thereafter at 6-hours intervals to the end of the study (72 hour). Paraquat treatment increased the number of MN-PCE at 24, 48 and 72 hours, both in peripheral blood and bone marrow cells, while no differences were observed in the polychromatic erythrocytes/normochromatic erythrocytes (PCE/NCE) ratio. Melatonin inhibited the paraquat-induced increase in MN-PCE by more than 50% at 48 and 72 hours. 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 i.p. to mice 30 min prior to an i.p. injection of paraquat (20 mg/kg x2), and thereafter at 6-hours intervals until the conclusion of the study (72 hours). 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 hours after paraquat administration. The induction of micronuclei was time-dependent with peak values occurring at 24 and 48 hours. 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 minutes were observed.

Melatonin was able to further depress the weight of testes and ventral prostates in rats after hypophysectomy. Melatonin inhibited testosterone production by rat testicular tissue *in vitro*, but exerted no effect on cAMP level. Guanylate cyclase activity and cGMP level, on the other hand, increased.

Exogenous administration of melatonin has no specific use during breastfeeding and no data exist on the safety of maternal use of melatonin during breastfeeding. However, doses higher than those expected in breastmilk after maternal supplementation have been used safely in infants. It is unlikely that short-term use of usual doses of melatonin in the evening by a nursing mother would adversely affect her breastfed infant, although some authors recommend against its use in breastfeeding because of the lack of data and a relatively long half-life in preterm neonates.

Regarding the drug substance and final drug product, the residual solvents and excipients in the formulation are discussed and raise no toxicological concerns.

Conclusion on toxicology
Single dose toxicology has been adequately addressed. 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.

Adequate discussion on the potential for genotoxicity of melatonin concluding that melatonin is not mutagenic or clastogenic has been provided. 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 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

The Applicant has provided a partial Phase I assessment with a calculated $PEC_{SURFACEWATER}$ of 0.0075 µg/L using a refined Fpen. However, in addition, the Applicant has submitted recorded sales of melatonin in the EU for years 2014-2017, which do not indicate an increase in use.

Suitable justification has been provided for non-submission of a complete Environmental Risk Assessment, based on the expectation that introduction of this product containing an active substance of well-established use 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 the environment to melatonin.

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. The literature review provided is satisfactory.

To bridge the literature reviews to the proposed product, the Applicant submitted two ((one pilot and one pivotal) pharmacokinetic studies using the well-acknowledged Hungarian reference product, Bio-Melatonin 3mg film-coated tablets, as comparator versus the proposed product. The bridging pharmacokinetic studies were conducted in-line with current Good Clinical Practice (GCP).

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. 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.

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 desynchronisation 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 synchronise their bodily rhythms; synchronisation occurs at a speed of approximately 1.5 hour a day after westward flights and approximately 1 hour 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.

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 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. 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, 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 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. Travelers crossing 2-4 time zones can also use it, if needed.

IV. 2 Pharmacokinetics

An adequate discussion on the absorption, distribution, metabolism and elimination, pharmacokinetics in special populations and interactions of melatonin has been provided in the Applicant's clinical overview. To further support the application, the Applicant has provided suitable bridging data that demonstrate comparability between their product and the bibliography. With the exception of the pharmacokinetic bridging studies, no new pharmacokinetic data have been submitted for this application and none were required.

The pharmacokinetics of melatonin have been reviewed recently. In clinical studies, melatonin was typically administered orally, sublingually, or intravenously. Until now, the pharmacokinetics 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-acety-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 decreases 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.

A summary of the literature data is provided below:

Absorption
In humans, intravenous melatonin exhibits linear pharmacokinetics over a dosage range of 0.01 to 5.0 µg/kg. However, few studies have examined absolute bioavailability of melatonin in humans. One study reported significant rises in plasma melatonin over a 3-hour period following a 50 mg oral dose, with plasma concentrations returning to baseline after 3 hours. In another study, wide ranges in melatonin peak serum concentrations and in the bioavailability of oral melatonin have been reported (mean: 33%; range: 10% - 56%) in 4 normal male volunteers given 20 µg intravenously and 500 µg oral melatonin. Oral bioavailability in humans with doses of melatonin 80 mg has been also examined in one study. However, the investigators did not administer an intravenous dose and thus could not determine the absolute bioavailability.

Pharmacokinetic parameters of melatonin after i.v. infusion, i.v. bolus or oral administrations, is described in the published literature.

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 immediate release forms, with T_{max} usually achieved in 60 minutes (normal range: 20 - 90 minutes). A recent study has shown oral melatonin to have a $T_{1/2}$ as low as 6 minutes.

After taking 3 - 6 mg melatonin, the serum C_{max} value is usually at least 10 times higher than the serum concentration of endogenous night-time melatonin.

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 minutes) with peak serum levels 350 - 10,000 times higher than the endogenous night-time peak within 60 – 150 minutes of dosing. 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 in exposure parameters have 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 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 AUC values ranged from 2.640 to 39.735 ng·h/mL and 3.072 to 53.132 ng·h/mL for pre- and post-menopausal women, respectively. In this study, high plasma melatonin concentrations were also determined for melatonin in 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). In a retrospective analysis on multiple studies that used intravenous melatonin or oral preparations (but not both in the same subjects) the estimated oral bioavailability ranges 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 healthy young adults (mean age 29.2 ± 6.5 years) and 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 D_7 melatonin. All subjects participated in two experiments: one with 250 µg of oral D_7 melatonin at midday and, after a washout period of 1 week, one with 250 µg of oral D_7 melatonin at midnight. In addition, the young subjects participated in a third study, involving a 23 mg D_7 melatonin infusion. Significant gender differences and between subject variability in exposure parameters were reported. Following oral dosing with 250 µg of D_7 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 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. It was concluded that the bioavailability of oral melatonin was only 3%.

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, resulted in supraphysiological and sustained concentrations of serum melatonin during 12 hours overnight in subjects (critically ill patients). These findings support the fact 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.

Melatonin soft capsules showed similar pharmacokinetic parameters compared with the highest dose of melatonin in powder form, but its bioavailability was improved. Results evidenced that 3 mg of melatonin powder and 1 mg of melatonin soft gel had the same pharmacokinetics, but comparing the absorption, 1 mg melatonin soft gel capsules was faster absorbed than 3 mg melatonin powder. 1 mg of melatonin powder had a low pharmacokinetic profile and was not well absorbed.

In one study, the bioavailability of a new oral spray of melatonin emulsion was compared with a standard oral formulation in healthy subjects. Data obtained in this study showed that the extent of melatonin absorption after oral spray delivery was 1.8 times that observed after administration of the standard oral tablet; the peak concentration was also significantly higher, 1.5 times the corresponding oral tablet value. The absorption rate expressed as T_{max} and K_a was comparable between the two products.

The bioavailability of long acting melatonin has also been investigated. After 12 hours overnight fast,

subjects received a single 5 mg long acting capsule dose. The pharmacokinetic values were C_{max} of 8.768 ± 7.043 ng/mL, T_{max} of 2.7 ± 0.77 h, AUC_{0-t} of 29.814 ± 24.931 h.ng/ mL, $AUC_{0-\infty}$ of 38.537 ± 24.658 h.ng/mL, Cl of 185.293 ±121.806 L/h, Vd of 451.370 ± 510.039 L and $t_{½}$ of 1.509 ± 0.768 h.

Ingestion of 3 mg melatonin caused a marked increase in serum melatonin (3561 ± 1201 pg/ml) within 20 minutes, followed by gradual decrease, but the level still remained higher than the basal level at 240 minutes after ingestion. The saliva melatonin 60 minutes after the ingestion showed the highest level (1177 ± 403 pg/ml) which was one-third the plasma level. The high saliva melatonin level indicated that its measurement may be a suitable indicator for the melatonin secretion into general circulation.

Elevated melatonin concentrations were observed with peak values of 435 nmol/l in serum and 241 nmol/l in saliva at 60 minutes, after administration of high doses of melatonin (100 mg). Elimination was monophasic following first-order kinetics. The half-lives for serum and saliva melatonin were 41 and 38 minutes, respectively. These results suggested that melatonin is passively secreted into saliva which reflects closely the changes in serum melatonin.

According to literature data, the permeability of melatonin is high. Available literature data from renal excretion support high absorption, and reports 'over 90 % of the administered radioactivity ([β-^{14}C melatonin) recovered in the first 24 h urine sample and the remainder in the next 24 hours.' An *in-vitro* study to assess the extent of permeability of melatonin across Caco-2 monolayers and efflux from Caco-2 cells was also performed by the Applicant (see Section III.3 Non-Clinical Aspects, Pharmacokinetics).

To bridge the bibliographic data to the proposed product, the following two (one pilot and one pivotal) pharmacokinetic studies were submitted:

Pilot study
This was an open label, balanced, randomised, two-treatment, two-period, two-sequence, crossover, single oral dose, comparative bioavailability study comparing the test product Melatonin 3 mg tablets versus the reference product Bio-Melatonin 3 mg film-coated tablets in healthy, adult, human subjects under fasted conditions.

Subjects were administered a single dose (3 mg; 1 tablet) of the test or reference product with 240 ml of water, following an overnight fast of at least 10 hours. Blood samples were taken pre-dose and up to 6 hours post dose, with a washout period of 6 days between the treatment periods.

Results
As melatonin is an endogenous substance a baseline correction was applied. Data of baseline uncorrected melatonin were supportive only. A summary of the pharmacokinetic results for baseline corrected data is presented below.

Table 4: Comparative bioavailability evaluation of melatonin (Baseline corrected data, transformed values)

Pharmacokinetic parameter	Geometric Mean Ratio Test/Reference	90 % Confidence Intervals	CV %[1]
$AUC_{(0-t)}$	106.0	90.10 - 124.60	22.2
C_{max}	99.6	84.34 - 117.65	22.8

[1]Estimated from the Residual Mean Squares.

In line with the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev 1/Corr**), the Test/Reference ratios and their 90% confidence intervals were within the specified limits to show bioequivalence between the test and reference products.

Pivotal study

This was an open label, balanced, randomised, two-treatment, three-period, three-sequence, partial replicate, crossover, single oral dose, comparative bioavailability study comparing the test product Melatonin 3 mg tablets versus the reference product Bio-Melatonin 3 mg film-coated tablets in healthy, adult, human subjects under fasted conditions.

Subjects were administered a single dose (3 mg; 1 tablet) of the test or reference product with 240 ml of water, following an overnight fast of at least 10 hours. Blood samples were taken pre-dose and up to 6 hours post dose, with a washout period of 6 days between the treatment periods.

Results

As for the pilot study, a baseline correction for melatonin was applied. Data of baseline uncorrected melatonin were supportive only. A summary of the pharmacokinetic results for baseline corrected data is presented below.

Table 5: Comparative bioavailability evaluation of melatonin for melatonin (Baseline corrected data, transformed values)

Pharmacokinetic parameter	Geometric Mean Ratio Test/Reference	90 % Confidence Intervals	CV %[1]
$AUC_{(0-t)}$	89.1	79.97 - 99.37	36.7
C_{max}	82.6	73.15 - 93.25	43.3

[1]Estimated from the residual mean squares using only the reference product data

The protocol defined bioequivalence as 90% confidence intervals (CI) of 80.00-125.00% for AUC_{0-t} and a widened criteria of 72.97-137.04 % for C_{max} on the basis of C_{max} variability (intra-subject CV) of the reference product was calculated at 43.3%).

The 90% CI of the test/reference ratio for C_{max} lie within the acceptable pre-specified limits. However, the 90% CI lower value for AUC_{0-t} for melatonin lies outside the specified limit. Thus, the data from this study failed to demonstrate bioequivalence between the applicant's test product and the reference product Bio-Melatonin 3 mg film-coated tablets under fasted conditions.

Pooling of data:

As the pilot study demonstrated bioequivalence, whilst the pivotal study failed to demonstrate bioequivalence between the proposed and referenced products, the Applicant performed a pooled analysis of the data from both studies to assess the totality of the evidence, in accordance with the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**).

The pharmacokinetic parameters of the pooled data from two different studies were derived individually for each analysed subject from the plasma concentration versus time profiles for baseline corrected and uncorrected data of melatonin. A summary of the pharmacokinetics parameters for pooled data is presented in the following table.

Table 6 Relative bioavailability results for melatonin [Pooled data, baseline corrected, transformed values]

Parameters	Ratio (T/R) %	90 % Confidence Interval	Acceptance Criteria
lnC_{max}	84.8	76.04 – 94.53	72.97 – 137.04
$lnAUC_{0-t}$	91.3	82.83 – 100.68	80.00 – 125.00
$lnAUC_{0-\infty}$	91.6	83.22 – 100.93	N/A

Conclusion of the pooling of the data from the pharmacokinetic studies
In line with the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev 1/Corr**), the Test/Reference ratios and their 90% confidence intervals were within the specified limits to show bioequivalence between the test and reference products.

Overall conclusion of the pharmacokinetic studies.
The pharmacokinetic results showed that the test product can be considered similar to the reference product. A bridge to the supporting literature has been established.

Distribution
A study has reported that melatonin reaches all tissues of the body within a very short period.

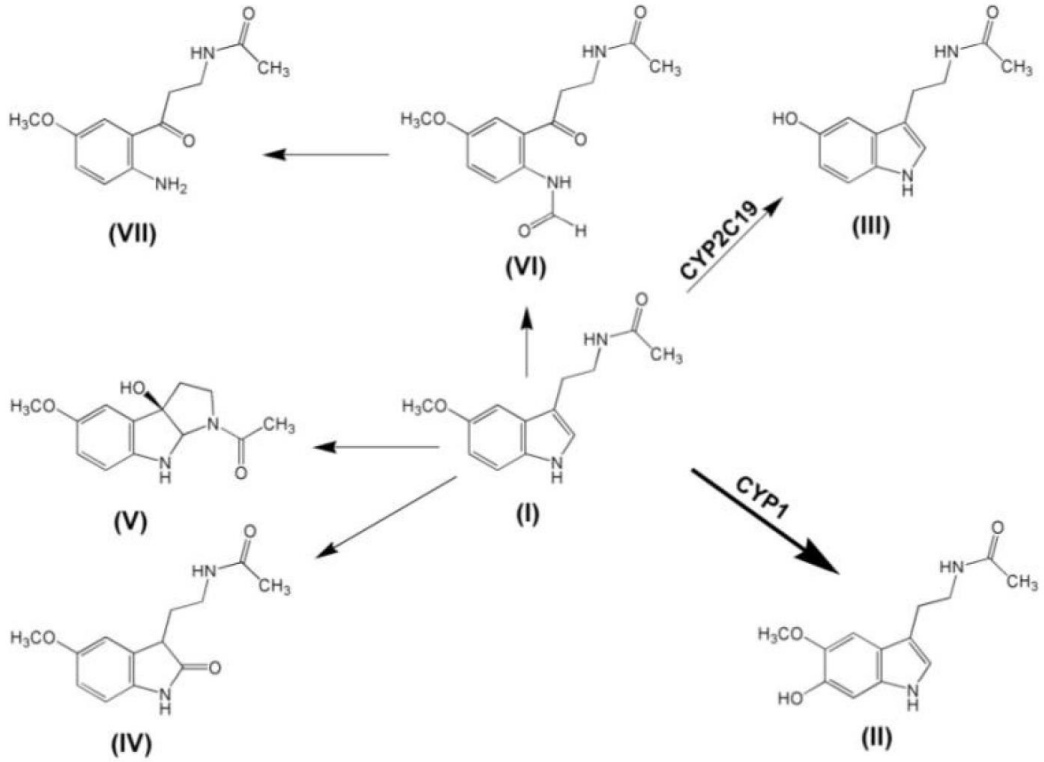
Melatonin half-life is bi-exponential, with a first distribution half-life of 1.4 minutes and a second of 28.4 minutes. Melatonin released to the cerebrospinal fluid via the pineal recess attains, in the third ventricle, concentrations up to 20 – 30 times higher than in the blood. These concentrations, 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 ^{14}C-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.

The mean steady state volume of distribution (Vdss) in healthy adult volunteers, following an intravenous infusion of D_7 melatonin, is estimated to be 0.98 L/kg distribution. No gender difference in the Vdss normalised 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 60%. Melatonin is mainly bound to albumin, alpha1-acid glycoprotein and high-density lipoprotein. The level of melatonin binding appears to be constant over a 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 melatonin pharmacokinetics are not expected to be significant.

Metabolism
The literature provides information regarding the metabolic fate of melatonin. The metabolic pathway of melatonin is presented in the figure below:



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 demethylates melatonin to N-acetylserotonin, being otherwise its precursor.

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 metabolised non-enzymatically in all cells, and also 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.

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.

Excretion and elimination

The primary metabolite of melatonin, 6-sulphatoxymelatonin, accounts for 90% of the dose excreted in the urine. The other main metabolite results from melatonin O-demethylation, yielding N-acetylserotonin. Approximately 2% of the exogenous metabolite is excreted in an unchanged form. No figures are provided as to the extent of urine excretion of the secondary metabolite, mainly the glucuronide conjugate of 6-hydroxymelatonin. A $T_{1/2}$ elimination of approximately 45 minutes has been documented in several studies in a wide range of doses, up to 100 mg intravenously. This parameter may also be described by first-order elimination kinetics and is independent of dose and route of administration. Additionally, according to one study, approximately 1% of blood melatonin is excreted in the urine without being metabolised. The authors concluded a positive correlation between AUC_{MLT} and 6 OH MLT-S in the urine. In another study, it is reported that 6-Sulphatoxymelatonin (aMT6s) is the

major urinary metabolite of melatonin and its measurement in urine appears to provide a robust, simple and reliable assessment of melatonin secretion. Over 90% of the administered radioactivity ([β-^{14}C-melatonin) was recovered in the first 24 hours urine sample and the remainder in the next 24 hours.

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 minutes, 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 hours, longer than 0.5 to 1.0 hour 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
Adequate data have been provided covering special populations including paediatrics, young adults and the elderly, impaired renal function, impaired hepatic function and pregnancy.

IV.3 Pharmacodynamics
The clinical pharmacodynamic properties of melatonin is well established. No new pharmacodynamic data have been submitted for this application and none were required. The bibliographic data provided to support the pharmacodynamic properties of melatonin is considered sufficient. A summary of the submitted pharmacodynamic data is presented below.

Melatonin binds to two receptors co-localised in the SCN, MT1 and MT2. While the specific functional roles of each receptor are not yet defined, there is some evidence that the MT2 receptor may be more important in the phase-shifting actions of melatonin and the MT1 receptor in sleep-related actions. The MT_1 receptor is coupled to different G proteins that mediate adenylyl-cyclase inhibition and phospholipase Cβ activation, while the MT2 receptor is also coupled to inhibition of adenylyl-cyclase and, additionally, inhibits the soluble guanylyl cyclase pathway. MT1 and MT2 polymorphisms have been found in humans and may be associated with sleep disorders. A binding protein originally thought to represent a third membrane receptor (MT3) turned out to be the primarily cytosolic enzyme quinone reductase 2 (QR2).

Melatonin may also promote sleepiness via its effects on peripheral vessels. It induces a vasodilatation itself leading in turn to an increase of skin temperature which constitutes an effective signal for sleepiness. This last effect may be the prominent mechanism of action of exogenous melatonin.

Circadian Regulation of Sleep and Mechanism of Action
The neurons of the major circadian clock, the SCN of the hypothalamus, are normally active during the day and slow down at night. The activation of SCN neurons has an inhibitory effect on the pineal gland, defining a nocturnal pattern of melatonin secretion. If SCN neurons are activated at night, *e.g.* by environmental light perceived by the retina, melatonin production declines. Melatonin, in turn, can acutely attenuate the activity of SCN. This melatonin action is likely to support a normal decline in the activity of the SCN at night, further promoting melatonin secretion and contributing to an overall increase in the amplitude of circadian body rhythms. A temporal and functional interplay between melatonin and SCN, and their response to environmental light, promote a temporal alignment of multiple circadian body rhythms with each other (internal synchronisation) and with the periodic changes in the environment (external synchronisation).

Different mechanisms of action have been reported for melatonin. Its antioxidant and radical scavenging properties are well known and widely described in the scientific literature. Melatonin is also the endogenous agonist of two G-protein-coupled membrane receptors, named MT1 and MT2, displaying binding affinities in the nanomolar range. Its lipophilic nature favours membrane crossing and interaction with intracellular targets. The so-called MT3 binding site is an enzyme belonging to the quinone reductase family; it has lower affinity for melatonin than MT1 and MT2 receptors and may contribute to the antioxidant properties of melatonin. Moreover, melatonin has been found to interact with other cellular targets, such as calmodulin. MT1 and MT2 receptors are negatively coupled to adenylate cyclase, and they can also interact with other intracellular second messengers. In humans, they are expressed in different areas of the CNS, such as the SCN, cerebellum, hippocampus, substantia nigra and nucleus accumbens. They have also been found in peripheral tissues, such as retina, coronary arteries, immune cells, intestine and epithelial cells. The differential role of the two receptor subtypes has been elucidated only partially. Activation of MT1 receptors inhibits neuronal firing within the SCN and inhibits the release of hormones, such as prolactin, while activation of MT2 receptors induces splenocyte proliferation, vasodilatation of coronary arteries and inhibits dopamine release in the retina. Experiments on rat caudal artery demonstrated the involvement of the MT1 receptor in the vasoconstrictive effect of melatonin and of the MT2 receptor in vasodilatation.

In addition to an acute inhibition of SCN activity, melatonin administration can also produce a shift in the circadian phase of SCN activity, either advancing or delaying its onset. The direction of the phase shift depends on the time of melatonin treatment, i.e., administration of melatonin in the late afternoon can advance the circadian clock, while early-morning treatment can cause a phase delay. Studies conducted *in vitro* suggest that a chronobiological effect of melatonin, i.e., the induction of circadian phase shift, is likely to be explained by its direct effect on SCN neurons via specific, most likely, MT2 receptor. Although the magnitude of the melatonin-induced phase shifts can vary between the species, the overall phenomenon appears to be well conserved. Such phase shifts in the circadian oscillation of SCN activity may change the physiological and behavioural rhythmicity of the entire organism, including the sleep-wake cycle, and can significantly affect the sleep quality in both nocturnal and diurnal species. In humans suffering from circadian sleep disorders, daily melatonin treatment can help to reinforce the circadian synchronisation with the environment and entrain the physiological rhythms to a 24-hour cycle.

Depending on the tissue and species, melatonin can activate different second messenger cascades acting on the same receptor subtype. By using recombinant melatonin receptors, it has been shown that the predominant cellular effect of the melatonin is the inhibition of forskolin-stimulated cAMP accumulation in the SCN and PT. This effect of melatonin is pertussis toxin sensitive, indicating coupling of the receptor to a Gi protein. Thus, the classical effect of MT1 and MT2 receptors are primarily coupled, in an inhibitory manner, to the AC → cAMP → PKA signalling pathway, via a pertussis toxin sensitive Gi protein. The decrease in cAMP production reduces the uptake of linoelic acid, an essential and major fatty acid, by specific fatty acid transporters. Co-precipitation experiments showed that the MT1 receptor is coupled to different G proteins that mediate AC inhibition and phospholipase Cβ activation. Thus, MT1 receptor activation leads to activation of a large variety of G proteins including Giα2, Giα3, and Gαq/11 proteins, and Giαs, Gαz, and Gα16. Moreover, activation of MT1 receptors leads to activation of phospholipase Cβ (PLC-β), with a concomitant increase of inositol--(1,4,5)-triphosphate (IP3), cytosolic Ca2+ and 1,2-diacylglycerol. Activated MT1 receptors inhibit cAMP responsive element binding protein (CREB) phosphorylation, a nuclear transcriptional activator of cAMP-sensitive gene factor, and also inhibit the formation of immediate early gene products, c-Fos and Jun B.

The functional significance of this differential G protein coupling has further deciphered that Gi2 and Gi3 proteins mediate AC inhibition through a pertussis toxin– insensitive Gq/11 protein and are coupled to phospholipase Cβ activity in cell lines (HEK293, Cos-7, CHO cells) through stably expressing MT1 receptors. Parallel signalling processes are observed through other G proteins, including G0, Gz, or G16. This stimulatory effect is independent of an interaction with Gi or Gs proteins and associated with a calcium–calmodulin (CaM) signal transduction pathway and c-Jun N-terminal kinase activation. Stimulation of recombinant human MT1 receptors potentiates also the prostaglandin F2a-induced release of arachidonate and hydrolysis of phosphoinositide. MT_1 receptors induce a transient elevation in cytosolic calcium ion concentration and in inositol phosphate accumulation, are associated further

with increased phosphorylation of mitogen-activated protein kinase (MEK1/2) and extracellular signal regulated kinase (ERK1/2) and regulate other ion fluxes and specific ion channels, such as increase in potassium conductance by activating inward rectifier potassium channels (Kir3/GIRK or Ca^{2+} activated K+ channel, BKca), and potentiate prostaglandin F2α- and ATP-mediated stimulation of PLC activity.

Expression of human MT1 and MT2 receptors in COS-7 cells demonstrates that activation of these receptors stimulates c-Jun N-terminal Kinase (JNK) activity via pertussis toxin–sensitive and insensitive G proteins.

The expression of MT1 receptors in the human SCN decreases with advancing age as well as in the late stages of Alzheimer's disease. Sleep disruptions, nightly restlessness and circadian rhythm disturbances seen in the elderly and in patients with Alzheimer's disease may be due to alterations of MT_1 receptor expression found in the SCN.

A third mechanism of the biological effects of melatonin is through MT3 receptor, which is identified with lower melatonin affinity, very rapid ligand association/dissociation kinetics and widely distributed in various tissues of the body.

Melatonin and Jet Lag – Mechanism of Action
The mechanism of melatonin action is not known for certain. As described, melatonin can advance or delay the body clock according to when it is ingested. However, it may also have a hypnotic action due to its temperature lowering abilities that may be attributed to its actions on peripheral blood vessels, i.e. melatonin acts separately as a chronobiotic and hypnotic. If melatonin is to be used for its temperature lowering, hypnotic effect it should be taken at approximately 20:00 hours at the new local time, irrespective of the flight that had been undertaken. However, if melatonin is to be used as a chronobiotic it should be taken at a certain time (according to the direction of travel and number of time zones crossed) to phase advance or phase delay endogenous rhythms. The usefulness of melatonin in jet lag could be due to either its chronobiotic effects, its hypnotic effects or both.

Effect of administered melatonin on suppression of endogenous melatonin secretion
Melatonin is used for short term administration in cases of jet lag. Thus, there is no effect on endogenous melatonin secretion. There are studies that confirm the safety of melatonin administration, even in long-term administration cases and no effect on endogenous melatonin secretion has been observed.

It has been considered that endogenous melatonin was not modified by administration of D_7 melatonin, as it was previously demonstrated that the amplitude of melatonin production is not affected by melatonin administration and that melatonin only has an influence on circadian timing when administered several hours before onset time. One study demonstrated that the low basal concentrations of melatonin in the blood are not affected by an increased melatonin supply up to a certain critical threshold, that the pineal gland would have to release all its melatonin content almost every 10 sec in order to sustain the elevated steady-state level of melatonin in the circulation during the dark period and that significant day/night differences exist in the disposition of circulating melatonin if administered in near physiological amounts and under near physiological conditions.

Another study measured the endogenous melatonin profiles after administration of a physiological dose of melatonin (0.5 mg) or placebo at bedtime to night shift workers (n = 21) for seven days. The amplitude of endogenous melatonin secretion was unchanged by treatment. Additionally, a melatonin treatment trial using a 50 mg daily bedtime dose for 37 days to a blind subject resulted in no change in the endogenous melatonin profile.

Exogenous melatonin did not affect the production of endogenous melatonin in terms of secretion rate, amplitude and duration. One study investigated the effects of an artificially prolonged melatonin (1.5 mg) profile on endogenous melatonin and cortisol rhythms, wrist actigraphy, and reproductive hormones in humans. Compared with placebo, melatonin administration advanced the timing of endogenous melatonin and cortisol rhythms. They concluded that melatonin treatment did not affect the endogenous melatonin profile duration, pituitary/gonadal hormone levels (24 hours), or sleepiness and mood levels on the subsequent day.

Effect of Melatonin on Cardiovascular System
One study evaluated the effect of 2 mg melatonin or placebo on the Heart Rate Variability (HRV) of 26 healthy men. Compared with placebo, melatonin administration within 60 minutes increased R-R interval, the square root of the mean of the squared differences between adjacent normal R-R intervals, high-frequency power, and low-frequency power of HRV and decreased the low frequency to high-frequency ratio and blood pressure in the supine position (all $P < 0.01$). Plasma norepinephrine and dopamine levels in the supine position 60 minutes after melatonin administration were lower compared with placebo ($P < 0.05$ and $P < 0.01$, respectively). Standing up resulted in the decrease of HRV and the increase of blood pressure and plasma catecholamine levels in both administration groups, and the differences between the groups found in the supine position disappeared. Melatonin administration also may exert suppressive effects on sympathetic tone.

Pharmacodynamic drug interactions
In humans, co-administration of melatonin and zolpidem showed pharmacodynamic interaction (increased sedation). The effects of therapeutic oral doses of prolonged-release melatonin (2 mg), zolpidem (10 mg) and their combination administered at bedtime in cognitive functions in healthy subjects were assessed in a randomised, double-blind, placebo-controlled and four-way crossover study. A new pharmacodynamic interaction between melatonin and zolpidem at 1 hour following co-dosing was observed, which was partly attenuated by 4 hours. Melatonin concentrations after administration of melatonin and melatonin + zolpidem were comparable with a peak at 1 – 2 hours. The same scheme was observed also for zolpidem concentration and thus a pharmacokinetic interaction can be discarded. Melatonin was not found associated with impairment of psychomotor functions, memory recall and driving skills and point to a pharmacodynamic interaction between melatonin and GABA-A modulators.

Concomitant administration of melatonin and drugs that affect the CNS may result in pharmacodynamic drug interactions. For example, relative to monotherapy with the CNS-active drug, patients receiving melatonin prolonged release and imipramine had increased feelings of tranquillity and difficulty in performing tasks, and those receiving melatonin prolonged-release plus thioridazine had increased feelings of ‘muzzy-headedness’. Combination of melatonin and imipramine did not exert an antidepressant effect that of imipramine alone, co-administration of the effective dose displayed an additive effect and that there seems to be no interaction between the two compounds.

In one study, Alzheimer’s Disease patients with sleep disturbances were treated with melatonin 3 mg capsules for 21 days. Patients who received 25 mg/day thioridazine because of their behavioural and sleep disorder interrupted thioridazine treatment after 5 and 24 months of starting melatonin treatment, respectively.

Alcohol should not be taken with melatonin, because it reduces the effectiveness of melatonin on sleep.

According to literature data, melatonin has the potential to interact with warfarin for many reasons. The potential interaction between melatonin and warfarin was recently evaluated in one study. Bleeding events, INR (International Normalised Ratio), PT (Prothrombin Time), albumin, and LFTs (Liver Function Tests) were recorded for each patient. Melatonin dose was stable in all 10 patients while warfarin dose had changed (increased/ decreased) in some patients. Both INR and PT increased in most patients during concurrent administration of melatonin with warfarin and no bleeding events were noted. These results demonstrate that concurrent use of melatonin and warfarin may affect coagulation activity and monitoring of INR and PT is suggested. The results of this study are consistent with previous cases reported in a meta-analysis.

IV.4 Clinical efficacy
No new efficacy data have been submitted for this application and none were required. The Applicant has provided an appropriate package of information to justify well established use of melatonin in the EU in the claimed indication. The clinical efficacy of melatonin is well known and adequately discussed in the clinical overview. The bibliographic dossier is considered to be sufficiently comprehensive and its content adequately justified. The literature reviews have adequately demonstrated the efficacy for the jet lag indication.

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 a Cochrane Review of melatonin for the prevention and treatment of jet lag. 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.

Key randomised controlled trials in jet lag

Study 1

The impact of various dosage forms of melatonin and placebo on jet lag symptoms was evaluated 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, the melatonin immediate release formulations 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, sleep quality and sleep, were significantly greater with melatonin 5.0 mg.

Study 2

Investigators 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 (16:00), and from D1 to D3 (23:00), 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 flights 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 included a large number of time-zone changes over 1 week or more, melatonin taken a few days prior to returning home resulted in a worse adjustment. One explanation for this finding is that it may be caused by the natural circadian rhythm being so disrupted at the end of 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 authors as Study 3, subjects taking melatonin reported less jet lag and took less time to recover from their shift across 12 time zones. Subjects also reported 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 gelatin 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) 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 maximising 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 hypothesised 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 authors, 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 SRC 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 resynchronisation 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 a Cochrane 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. A number of the ten studies included in the meta-analysis are also discussed above.

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 ages 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 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 10 cm 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 in the meta-analysis review, 7 investigated a dose of 5 mg once daily. One study found similar general efficacy for 0.5 mg 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 0.5 mg was not effective. A dose range of 3 mg to 6 mg is supported by the evidence.

The review also stated that, for many people, 5 mg may be a higher dose than necessary, and 2 – 3 mg may be a preferred starting dose, 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 counter argument to the review proposal would be that if there are grounds for advising a dose increase to 6 mg 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 3 mg (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 meta-analysis 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." It is agreed that the available data do 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 meta-analysis 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
Altogether, it is considered that nine of the 10 studies demonstrated statistically significant effects on jet lag symptoms (*e.g*. mood, cognitive) or on sleep. Two of the studies, which conducted a responders analysis concerning global jet lag symptoms of self-assessed jet lag severity, demonstrated a considerable difference (67% and 40%, respectively) in percentage responders. In addition, one study 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 visual analogue score on severity of jet lag show clinically relevant superiority for melatonin compared to placebo.

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

Other systematic reviews
A review of 11 randomised trials (refer to the 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.

Melatonin for Jet Lag syndrome				
Population	Healthy individuals traveling across more than five time zones			
Intervention	Melatonin			
Comparison	Placebo			
Outcomes	Absolute effect*		Relative effect (95 % CI)	Certainty of the evidence (GRADE)
	Without melatonin	With melatonin		
	Difference: patients per 1000			
Global Jet Lag symptoms (0 to 100 scale)	45 points per 1000	27 points per 1000	MD-17.74 (-23.98 to -11.50)	+++ Moderate
	Difference: 18 points less (Margin error: 12 to 24 points less)			

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.

In a review of one study, it was concluded that melatonin is remarkably effective in preventing or reducing jet lag, and occasional short-term use appears to be safe. Further it was concluded that melatonin 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 melatonin, if needed.

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.

Open studies
The process of re-entrainment of circadian melatonin rhythm was investigated in six subjects. Except during 24-hour blood sampling, the subjects were exposed to natural zeitgeber (time giver) outdoors and given 3 mg melatonin at 23:00 hours. The subjects were exposed to bright sunlight from 3000 to 12000 lux. All of them showed orthodromic re-entrainment without taking melatonin. Melatonin accelerated the rate of the re-entrainment of the circadian melatonin rhythm and was useful to jet travel from Tokyo to Los Angeles. In a later study of the same author, the effect of 3 mg of melatonin on the rate of re-entrainment of plasma melatonin rhythm after an 11-hour eastward flight was assessed. Subjects were exposed to natural zeitgeber outdoors and took 3 mg of melatonin at 20:00 hours local time on the days when no blood sampling was done. Antidromic re-entrainment was dominant whereby melatonin administration in the evening promoted re-entrainment. Melatonin accelerated the rate of re-entrainment by 15 minutes per day and alleviated the jet lag symptoms.

Conclusion on clinical efficacy (jet lag)
To summarise, efficacy has been demonstrated for the jet lag indication proposed for this application. 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

Satisfactory safety information of short or long-term use of melatonin are presented in the clinical overview. Safety data indicate that melatonin is well tolerated, in most of the studies, with mild adverse events reported. The most common adverse reactions after oral administration of melatonin are headache, hyperactivity, dizziness, drowsiness, abdominal pain, nausea and diarrhoea. The number of adverse events in most of the clinical studies do not differ significantly between melatonin and placebo groups and none of the adverse events require treatment.

Taking into consideration the safety profile of melatonin, as presented in the dossier, it can be stated that melatonin is effective on the treatment of jet lag disorder with a good safety profile and absence of withdrawal symptoms or addiction compared to other sleep-inducing agents.

No new or unexpected safety concerns were raised from the safety data submitted with the bridging clinical pharmacokinetic studies.

IV.6 Risk Management Plan (RMP)

The Applicant has submitted a 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

The clinical overview has provided adequate evidence of efficacy and safety of Melatonin 3 mg film-coated tablets in the proposed indication. A bridge between the proposed product and the bibliographic dossier has been established via bioavailability studies comparing the proposed product with the comparator EU product, Bio-Melatonin 3 mg film-coated tablets.

It is agreed that melatonin has demonstrated pharmacodynamic 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.

The benefit/risk is considered positive for occasional short-term management of jet lag.

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

V USER CONSULTATION

A user consultation with target patient groups on the Patient Information Leaflet (PIL) has been performed on the basis of a bridging report making reference to Melatonin 1mg/ml oral solution (PL 41344/0050; Colonis Pharma Limited). The bridging report submitted is acceptable.

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. Extensive clinical experience with melatonin is considered to have demonstrated the therapeutic value of the compound. To further support the application, the Applicant

has provided suitable bridging data demonstrating comparability between their product and the bibliography. Based on extensive analysis of submitted 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 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 SmPC and PIL for this product are available on the MHRA website.

The following text is the currently approved label text. No label mock-ups have been provided for this product. In accordance with medicines legislation, this product shall not be marketed in the UK until approval of the label mock-ups has been obtained.

PARTICULARS TO APPEAR ON THE OUTER PACKAGING

CARTON

1. NAME OF THE MEDICINAL PRODUCT

Melatonin 3 mg film-coated tablets
Melatonin

2. STATEMENT OF ACTIVE SUBSTANCE(S)

Each film-coated tablet contains 3 mg melatonin.

3. LIST OF EXCIPIENTS

The product also contains lactose monohydrate. See the leaflet for further information.

4. PHARMACEUTICAL FORM AND CONTENTS

Film-coated tablets

10 film-coated tablets
14 film-coated tablets
28 film-coated tablets
30 film-coated tablets

5. METHOD AND ROUTE(S) OF ADMINISTRATION

For oral use.
Read the package leaflet before use.

6. SPECIAL WARNING THAT THE MEDICINAL PRODUCT MUST BE STORED OUT OF THE REACH AND SIGHT OF CHILDREN

Keep out of the sight and reach of children.

7. OTHER SPECIAL WARNING(S), IF NECESSARY

Not applicable.

8. EXPIRY DATE

EXP:

9. SPECIAL STORAGE CONDITIONS

Store below 25°C. Store in the original package in order to protect from light.

10. SPECIAL PRECAUTIONS FOR DISPOSAL OF UNUSED MEDICINAL PRODUCTS OR WASTE MATERIALS DERIVED FROM SUCH MEDICINAL PRODUCTS, IF APPROPRIATE

Not applicable.

11. NAME AND ADDRESS OF THE MARKETING AUTHORISATION HOLDER

Arriello s.r.o
Olivova 2096/4,
Prague, 110 00,
Czech Republic

12. MARKETING AUTHORISATION NUMBER(S)

PL 39936/0006

13. BATCH NUMBER

Lot:

14. GENERAL CLASSIFICATION FOR SUPPLY

POM

15. INSTRUCTIONS ON USE

Not applicable.

16. INFORMATION IN BRAILLE

Melatonin 3 mg film-coated tablets

17. UNIQUE IDENTIFIER – 2D BARCODE

2D barcode carrying the unique identifier included.

18. UNIQUE IDENTIFIER – HUMAN READABLE DATA

PC: <to be completed nationally>

SN: <to be completed nationally>

NN: <to be completed nationally>

MINIMUM PARTICULARS TO APPEAR ON BLISTERS OR STRIPS

Blister

1. NAME OF THE MEDICINAL PRODUCT

Melatonin 3 mg film-coated tablets
Melatonin

2. NAME OF THE MARKETING AUTHORISATION HOLDER

Arriello s.r.o

3. EXPIRY DATE

EXP

4. BATCH NUMBER<, DONATION AND PRODUCT CODES>

Lot

5. OTHER

PL 39936/0006

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**