2.5 Clinical Overview

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2.5.1 PRODUCT DEVELOPMENT RATIONAL

2.5.1.1 PHARMACOLOGICAL CLASS

Melatonin (N-acetyl-5 methoxytryptamine) is a neurohormone that is primarily produced in the pineal gland, located behind the third ventricle in the brain with daily and seasonal rhythms mainly under the control of the circadian oscillator located in the suprachiasmatic nuclei of the hypothalamus (SCN) which have melatonin receptors. The pineal gland is a major component of the endocrine system that allows mammals to respond to the annual changes in photoperiod by adaptive alterations of their physiological state. 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. Melatonin is synthesized from tryptophan, which is taken up from the circulation and transformed into serotonin, which is then converted into melatonin by a two-step process involving the sequential activities of two enzymes, serotonin- N -acetyltransferase (NAT), the limiting enzyme in the synthesis of melatonin, and hydroxyindole- O -methyltransferase (HIOMT) [1]. The mRNAs encoding these enzymes are expressed in the pineal gland with a day/night rhythm.

Melatonin synthesis is initiated by the binding of norepinephrine to adrenergic β1-receptors, which leads to activation of pineal adenylate cyclase, an increase in cyclic AMP (cAMP) levels, and de novo synthesis of NAT. The cAMP-induced gene transcription repressor (ICER), an isoform of the cAMP-responsive element modulator (CREM), is activated in conjunction with NAT expression and represents a mechanism that limits the nocturnal production of melatonin [2]. Melatonin synthesis depends upon tryptophan availability, as it is reduced after acute tryptophan depletion [3]; other nutritional factors might also influence melatonin synthesis, for example, folate status [4] and levels of vitamin B6, a coenzyme in tryptophan decarboxylation that can stimulate melatonin production in prepubertal children, but not in adults [5, 6].

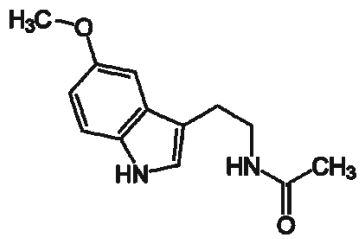

Figure 1 Chemical structure of Melatonin

Melatonin is involved in the entrainment (synchronization) of the circadian rhythms including sleep-wake timing, blood pressure regulation, seasonal reproduction, and many others. Many of its biological effects are produced through activation of melatonin receptors, while others are due to its role as an antioxidant, with a particular role in the protection of nuclear and mitochondrial DNA.

2.5.1.2 INFORMATION ABOUT THE CONDITION

The sleep- wake cycle may be pathologically affected in different ways. Furthermore, the sleep may also be disturbed by various processes. The disturbances of the sleep-wake cycle are called circadian rhythms disorders and include the jet lag (time zone change) syndrome, shift work sleep disorder, advanced sleep phase syndrome, non-24 h sleep-wake syndrome. In all these, insomnia might appear as a symptom. Chronic insomnia is itself a sleep disorder, in spite of being very complex. Transient difficulty in sleeping is a vastly more common phenomenon than is chronic insomnia. The diagnosis of

chronic insomnia is based on the subjective complaint of difficulty in initiating or maintaining sleep or of non- restorative sleep (not feeling well-rested after sleep that is apparently adequate in amount).

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 the following conditions under Circadian-Rhythm Sleep Disorders [7]:

- Jet Lag Disorder (ICD-10-CM code: G47.25)
- Shift Work Disorder (ICD-10-CM code: G47.26)
- Advanced Sleep–Wake Phase Disorder (ICD-10-CM code: G47.22)
- Delayed Sleep–Wake Phase Disorder (ICD-10-CM code: G47.21)
- Non-24-h Sleep–Wake Rhythm Disorder (ICD-10-CM code: G47.24)

All the indications are also included in the draft ICD-11, under section 7A6: Circadian rhythm sleep-wake disorder.

- Circadian rhythm sleep-wake disorder, jet lag type (ICD-11 code: 7A65)
- Circadian rhythm sleep-wake disorder, shift work type (ICD-11 code: 7A64)
- Circadian rhythm sleep-wake disorder, advanced type (ICD-11 code: 7A61)
- Circadian rhythm sleep-wake disorder, delayed type (ICD-11 code: 7A60)
- Circadian rhythm sleep-wake disorder, non-entrained disorder type (ICD-11 code: 7A63)

In all these indications insomnia might appear as a symptom.

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

The proposed indication for the product under assessment is the short term treatment of circadian rhythm disorders, such as jet lag and is presented in detail below in Section 2.5.1.2.1.

2.5.1.2.1 Jet Lag Disorder

Jet lag disorder, also known as time zone disorder, is a common complaint of travellers who fly across a number of time zones [8]. The symptoms of jet lag are primarily daytime fatigue and sleep disturbance, but also include loss of mental efficiency, weakness and irritability [9]. Jet lag is caused by desynchronization between the body's circadian system and the new day-night cycle at the traveller's destination. The sleep loss caused by the travel itself often contributes to jet lag. After a flight through six or more time zones most travellers will take 4 - 6 days to re-establish a normal sleeping pattern and not to feel tired during the day. The severity of jet lag symptoms largely depends on the number of time zones crossed and the direction of travel. They are worse the greater the number of zones crossed. Westbound travel generally causes less disruption, as it is easier to lengthen than to shorten the natural circadian cycle [10]. 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 [11].

2.5 Clinical overview

The disruptive effects of jet lag have been documented at the molecular level of clock genes present in the SCN and peripheral tissues [12]. Eastbound travel causes phase advances in the body's circadian rhythms, while westbound flight induces phase delays in circadian rhythms. As a consequence jet travellers are forced to synchronize their bodily rhythms; synchronization occurs at a speed of approximately 1.5 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 [13-15]. 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 [16, 17]. These factors aggravate the symptoms of jet lag.

A recent review clearly links jet lag with depression. Circadian rhythm disturbances evoked by jet lag are augmented via the intended 'sleep deprivation' the individual imposes upon himself in an attempt to adjust to the local time clock. Jet airline passengers who have travelled both eastward and westward also frequently report that they have experienced depressive symptoms [18]. The possible links between jet lag and major depressive disorder or psychotic disorder were evaluated based upon the following criteria: (a) absence of major mental problems before the flight, or good remission of existing disorders 1 year or more before the commencement of flight; and (b) the appearance of major affective syndromes or psychotic syndromes during first 7 days after landing. Evidence from epidemiological and electroencephalographic studies additionally implicate sleep disturbances as key factors in the pathogenesis of depressive illness [19]. The other evidence consistent with the circadian disruption hypothesis of depression comes from the observation that more than 80 % of depressed patients have complaints of sleep disturbances [20-22] and demonstrate a variety of polysomnographic abnormalities [23].

Recent important findings suggest also that jet lag is associated with increased breast cancer risk among female cabin crew [24]. Since several studies have indicated an increased risk of breast cancer (BC) among female flight attendants (FFAs), two meta-analyses have been performed. The meta-analysis of [redacted] suggest that FFAs have a higher risk of BC compared with the general population [25, 26]. The same meta-analysis reported that the development of breast cancer has also been linked to the disruption of circadian rhythms which is usually caused by shift work, short sleep duration and exposure to light at night.

These important findings, which link jet lag with breast cancer in female flight attendants and with depression of passengers after changing several time zones confirm that jet lag is associated with impaired functioning and there is a need of minimizing its effects.

A number of pharmacological interventions have been tried to minimize 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 [27].

The efficacy of melatonin in preventing or reducing jet lag has been reviewed in four systematic reviews [10, 28-30] that included numerous randomized controlled trials [31-39].

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 [10]. 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 need be.

2.5.1.3 SCIENTIFIC BACKGROUND

This application has been made under Article 10(a) of Directive 2001/83/EC Well Established Use.

Melatonin was first discovered in 1958 and has subsequently been widely available as both a nutritional supplement and an approved medicine for more than 10 years. Melatonin has been used as a medicinal product in the EU for over 10 years.

The current application is made under Article 10(a) of Directive 2001/83/EC, as amended for the short term treatment of circadian rhythm disorders, such as jet lag. The indication is the same as for the EU licensed product Bio-Melatonin 3 mg filmtabletta: treatment of jet lag in adults.

The application is not for indications such as sleep onset insomnia in children and adolescents with ADHD or other associated conditions. Any data submitted in this overview on alternative conditions to that applied for, is for supportive purposes only on the grounds of safety.

Bio-Melatonin 3 mg filmtabletta, was licensed under Article 10(a) as amended in 2003 for jet lag. This is clear evidence of well-established use in the EU for more than 10 years.

2.5.1.4 CLINICAL DEVELOPMENT PROGRAMME

Since Melatonin tablets is a medicinal product the active substance of which has a 'well-established medicinal use' within the Community for at least ten years, in the indications being applied for, with recognized efficacy and an acceptable level of safety it is possible to replace results of the pre-clinical and clinical trials by detailed references to published scientific literature (information available in the public domain). As a consequence, there is no special concern to be addressed and this clinical overview only mirrors and summarizes the toxicological and pharmacological well known properties of the active substance.

However, the applicant has performed two clinical studies, one pilot and one pivotal, single dose, crossover bioequivalence studies, comparing the rate and extend of the product under evaluation with the EU licensed product Bio-Melatonin 3 mg filmtabletta. These studies are described in the revised Modules 2.7 and 5.3.

2.5.1.5 SEARCH STRATEGY

[redacted].

2.5.2 OVERVIEW OF BIOPHARMACEUTICS

2.5.2.1 BIOEQUIVALENCE STUDIES

In order to support this application the applicant used the following bioequivalence studies which have been performed in the past for the proposed formulation.

2.5.2.1.1 Pilot Bioequivalence Study [redacted]

The title of the study was:

> *An open label, balanced, randomized, two-treatment, two-period, two-sequence, crossover, single oral dose, comparative bioavailability study of Melatonin 3 mg tablets [redacted] with Bio-Melatonin® 3 mg filmtabletta of Pharma Nord Aps, [redacted] in healthy, adult, human subjects under fasting condition.*

The Test product (T) Melatonin 3 mg Tablets [redacted] and the Reference product (R), Bio-Melatonin 3 mg filmtabletta marketed by Pharma Nord Aps.

The pilot study identification number is [redacted]

The purpose of the study was to compare and evaluate the bioavailability and characterize the pharmacokinetic profile of the Test product with respect to the Reference product in healthy, adult, human subjects under fasting conditions and to assess the bioequivalence.

It was an open label, balanced, randomized, two-treatment, two-period, two-sequence, single oral dose, crossover, comparative bioavailability study in healthy, adult, human subjects under fasting conditions.

The full details of the pilot study are presented in the revised modules 2.7 and 5 of the CTD. The following figures and tables summarize the pharmacokinetic parameters of the Test and the Reference products for the pilot study [redacted]

For baseline corrected data, primary pharmacokinetic parameters are C_{max} and AUC_{0-t} and secondary parameters are $AUC_{0-\infty}$, T_{max}, AUC_%Extrap_obs, λ_z and $t_{1/2}$.

The pharmacokinetic parameters for baseline corrected data of Melatonin for Test Product-T and Reference Product-R are summarized in Table I.

Table I Summary of pharmacokinetic data for Melatonin and Reference product (Baseline corrected data) (N=12)

Parameters (Units)	Mean ± SD (untransformed data)	
	Test Product-T	Reference Product-R
T_{max} (h)*	0.500 (0.333 - 0.667)	0.342 (0.250 - 1.500)
C_{max} (pg/mL)	5050.861 ± 4389.0470	4817.628 ± 3113.0194
AUC_{0-t} (pg.h/mL)	8136.771 ± 7751.6699	7247.183 ± 4840.2678
$AUC_{0-\infty}$ (pg.h/mL)	8226.750 ± 7823.3899	7347.669 ± 4889.1284
λ_z (1/h)	0.896 ± 0.1107	0.853 ± 0.0989
$t_{½}$ (h)	0.784 ± 0.0957	0.822 ± 0.0878
AUC_%Extrap_obs (%)	1.234 ± 0.6134	1.645 ± 1.1314

*T_{max} is represented in median (min-max) value

Table II Relative Bioavailability results for Melatonin (Baseline corrected data) (N=12)

Parameters	Geometric Least Squares Means			90 % CI	Intra Subject CV (%)	Power (%)
	Test Product-T	Reference Product-R	Ratio (T/R)%			
lnC_{max}	4161.316	4177.476	99.6	84.34 - 117.65	22.8	72.5
$lnAUC_{0-t}$	6435.533	6073.893	106.0	90.10 - 124.60	22.2	74.5
$lnAUC_{0-\infty}$	6516.085	6175.862	105.5	89.96 - 123.74	21.8	75.7

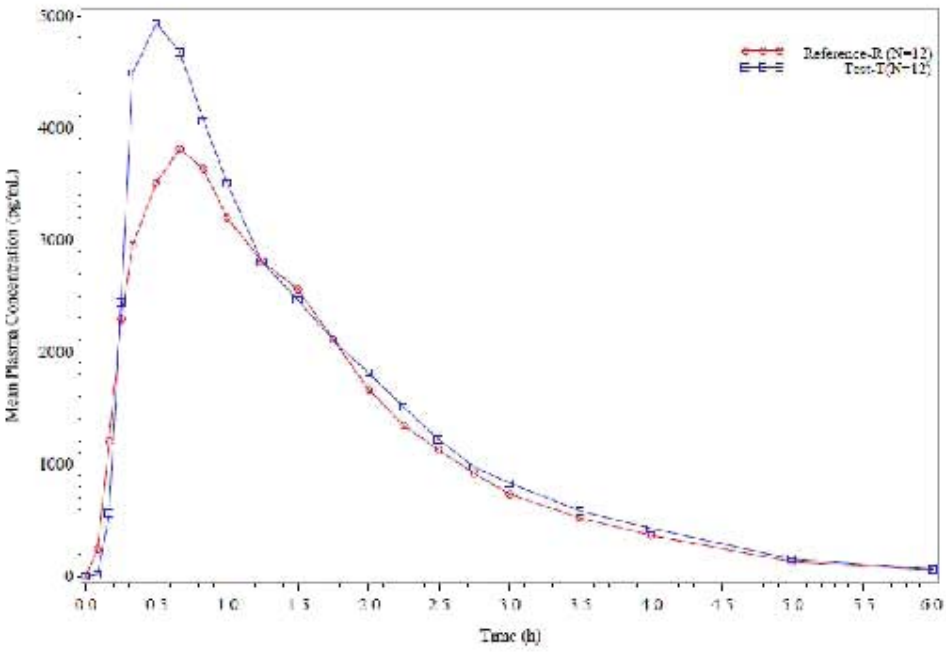

Figure 2 Mean plasma concentration vs time curve for Melatonin (Baseline corrected data)-Linear plot

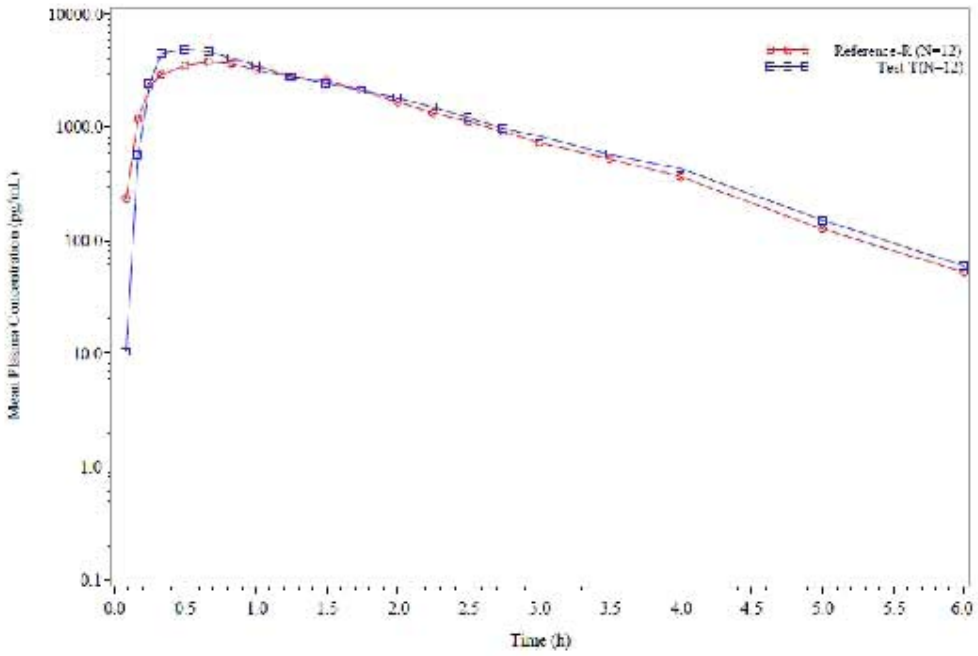

Figure 3 Mean plasma concentration vs time curve for Melatonin (Baseline corrected data)-Semilog plot

The results of the pilot study showed that the bioavailability of the Test product with that of the Reference product is bioequivalent with respect to C_{max} and AUC_{0-t} for baseline corrected data of Melatonin. Data from this study demonstrated that the test and the reference products were well tolerated.

2.5.2.1.2 Pivotal Bioequivalence Study [redacted]

The title of the study was:

> *An open label, balanced, randomized, two-treatment, three-period, three-sequence, partial replicate, crossover, single oral dose, comparative bioavailability study of Melatonin 3 mg Tablets [redacted] with Bio-Melatonin® 3 mg filmtabletta of Pharma Nord Aps, [redacted] in healthy, adult, human subjects under fasting condition.*

The Test product (T) Melatonin 3 mg Tablets [redacted] and the Reference product (R), Bio-Melatonin 3 mg filmtabletta marketed by Pharma Nord Aps.

The pivotal study identification number is [redacted]

The purpose of the study was to compare the bioavailability and characterize the pharmacokinetic profile of the Test product with respect to the Reference product in healthy, adult, human subjects under fasting conditions and to assess the bioequivalence.

It was an open label, balanced, randomized, two-treatment, three-period, three-sequence, single oral dose, partial replicate crossover, comparative bioavailability study in healthy, adult, human subjects under fasting conditions.

2.5 Clinical overview

The full details of the pivotal study are presented in the revised modules 2.7 and 5 of the CTD. The following figures and tables summarize the pharmacokinetic parameters of the Test and the Reference products for the pivotal study [redacted]

For baseline corrected data, primary pharmacokinetic parameters are C_{max} and AUC_{0-t} and secondary parameters are $AUC_{0-\infty}$, T_{max}, AUC_%Extrap_obs, λ_z and $t_{1/2}$.

The pharmacokinetic parameters for baseline corrected data of Melatonin for Test Product-T and Reference Product-R are summarized in Table III.

Table III Summary of pharmacokinetic data for Melatonin and Reference product (Baseline corrected data)

Parameters (Units)	Mean ± SD (untransformed data)	
	Test Product-T (N = 56)	Reference Product-R (N = 110)
T_{max} (h)*	0.500 (0.250 – 1.250)	0.500 (0.167 – 1.500)
C_{max} (pg/mL)	5745.167 ± 6367.5185	6315.524 ± 5808.9297
AUC_{0-t} (pg.h/mL)	7890.877 ± 7898.3660	8398.405 ± 7610.6031
$AUC_{0-\infty}$ (pg.h/mL)	7966.324 ± 7937.1334	8469.917 ± 7639.4738
λ_z (1/h)	0.890 ± 0.1232	0.937 ± 0.1811
$t_{½}$ (h)	0.794 ± 0.1188	0.764 ± 0.1332
AUC_%Extrap_obs (%)	1.896 ± 2.6242	1.424 ± 1.3947

*T_{max} is represented in median (min-max) value

Table IV Bioavailability results for Melatonin (Baseline Corrected Data)

Parameters	Geometric Least Squares Means			90% CI	Acceptance Criteria	Intra Subject CV of Reference Product-R (%)	Power (%)
	Test Product-T (N=56)	Reference Product-R (N=110)	Ratio (T/R) %				
lnC_{max}	3427.123	4149.518	82.6	73.15 - 93.25	72.97 – 137.04	43.3	99.5
lnAUC_{0-t}	4929.624	5529.972	89.1	79.97 - 99.37	80.00 – 125.00	36.7	95.9
ln$AUC_{0-\infty}$	5025.701	5610.593	89.6	80.46 - 99.73	N/AP	36.1	96.2

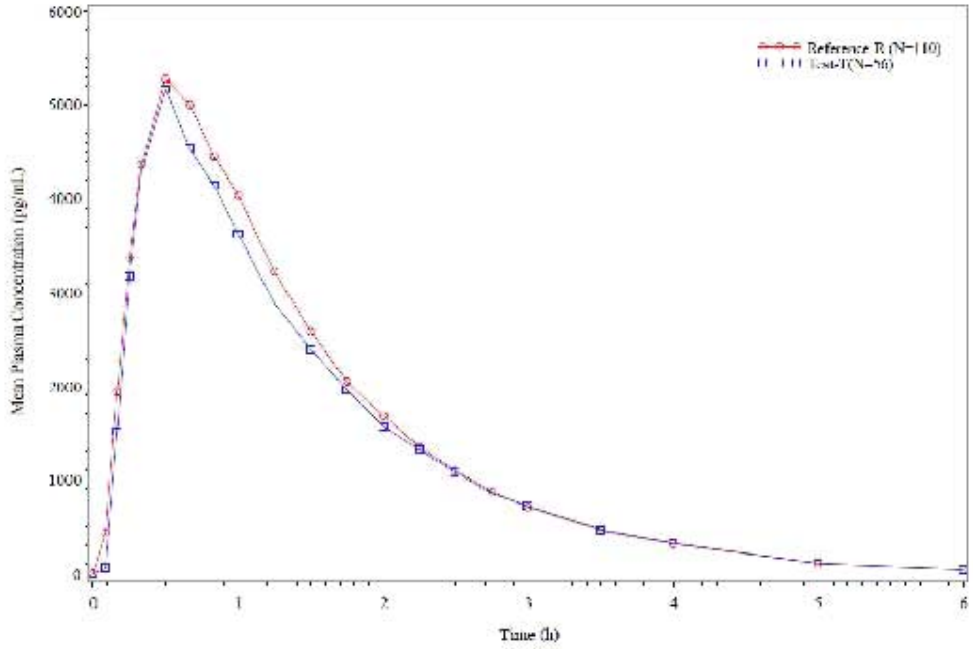

Figure 4 Combined mean plasma concentrations vs time curve for Melatonin (Baseline Corrected Data)-Linear plot

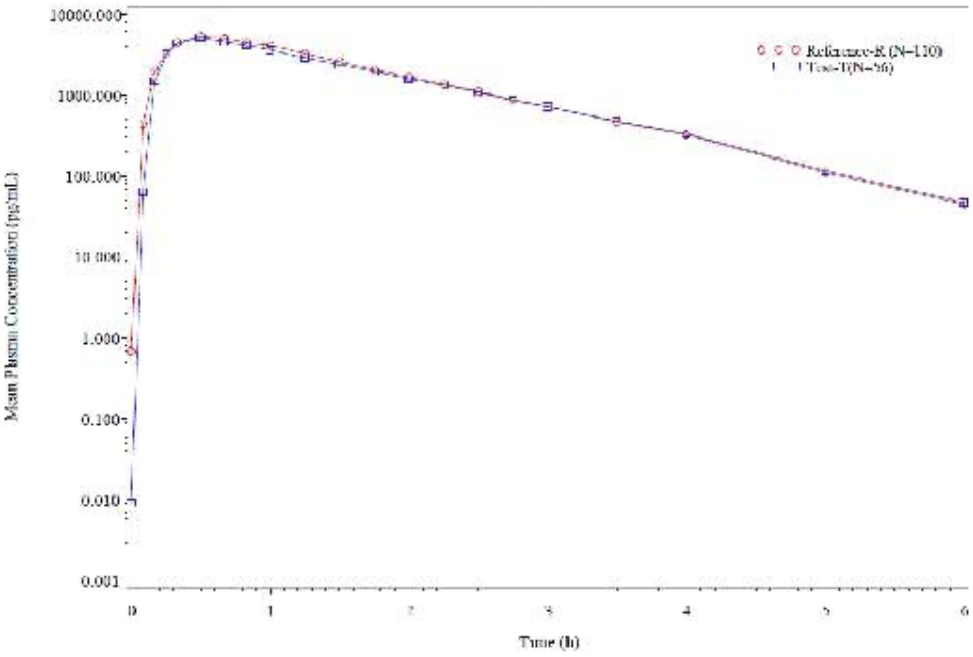

Figure 5 Combined mean plasma concentrations vs time curve for Melatonin (Baseline Corrected Data)-Semilog plot

The data of the pivotal trial show that the intrasubject variability of the reference product was high for both the C_{max} (43.3 %) and the AUC_{0-t} (36.7 %). Since the study was designed as a semi-replicate, widening of the acceptance range for the C_{max} parameter is allowed.

The bioavailability of the Test Product with that of the Reference Product is comparable with respect to C_{max}; however, it is not comparable with respect to AUC_{0-t} for baseline corrected data of Melatonin under fasting condition as per criteria set in the protocol. More specifically, the study failed to show bioequivalence for the AUC_{0-t} parameter by 0.03 % (90 % CI: 79.97 % - 99.37 %). The high variability of the molecule, as evidenced by the variability of the reference product, is the main reason of this borderline result. Although the mean plasma concentrations of Test and Reference products practically superimpose (Figure 4) the 90 % Confidence Intervals.

2.5.2.1.3 Pooled Analysis [redacted]

The first study demonstrated bioequivalence [redacted] and the second did not [redacted] In order to assess the totality of evidence a pooled analysis of both studies has been performed, as suggested in the Guideline on the Investigation of Bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1/Corr**), section 4.1.8 Evaluation, subsection Presentation of Data (page 16/27). There is a least one successful study and both studies fulfill the criteria of the Guideline.

The pharmacokinetic parameters of the pooled data from two different studies are derived individually for each analysed subject from the plasma concentration vs. time profiles for baseline corrected and uncorrected data of Melatonin. The individual PK parameters along with descriptive statistics on pooled data are tabulated and presented on the following tables.

Table V Descriptive statistics of formulation means for Melatonin (Baseline corrected data, N=68)

Parameters	Mean ± SD (untransformed data)	
	Test Product-T (N = 68)	Reference Product-R (N = 122)
T_{max} (h)*	0.500 (0.250 - 1.250)	0.500 (0.167 - 1.500)
C_{max} (pg/mL)	5622.642 ± 6042.9508	6168.190 ± 5610.5974
AUC_{0-t} (pg.h/mL)	7934.270 ± 7815.6941	8285.170 ± 7377.3548
$AUC_{0-\infty}$ (pg.h/mL)	8012.282 ± 7859.6121	8359.532 ± 7406.7069

*T_{max} is represented in median (min-max) value

Table VI Relative Bioavailability results for Melatonin (Baseline corrected data, N=68)

Parameters	Geometric Least Squares Means			90 % CI	Acceptance Criteria	Intra Subject CV	Power (%)
	Test Product-T (N = 68)	Reference Product-R (N = 122)	Ratio (T/R) %				
lnC_{max}	3736.535	4409.339	84.7	75.82 - 94.71	72.97 – 137.04	46.2	99.9
lnAUC_{0-t}	5550.488	6080.898	91.3	82.56 - 100.91	80.00 – 125.00	41.3	97.8
ln$AUC_{0-\infty}$	5645.804	6163.253	91.6	82.95 - 101.16	N/AP	40.8	98.0

Based on the data analysed from the pooled data of total 68 subjects (N = 56 subjects from Project No. [redacted] and N = 12 subjects from [redacted]) for baseline corrected of Melatonin, the pharmacokinetics were assessed for the comparison of Test Product-T vs. Reference Product-R. The 90 % CI of geometric least square means ratio of Test to Reference was within the acceptance range of 80.00 to 125.00 % for ln-transformed pharmacokinetic parameter AUC_{0-t} for baseline corrected data of Melatonin and

the 90 % CI for ln-transformed pharmacokinetic parameter C_{max} was within the newly widened range of 72.97 to 137.04 % (Project No. [redacted]) for baseline corrected data of Melatonin.

It can be concluded that the bioavailability of the Test Product with that of the Reference Product is comparable with respect to C_{max} and AUC_{0-t} after pooling of the data of baseline corrected Melatonin under fasting condition.

The full details of pooled analysis for studies [redacted] are presented in the revised modules 2.7 and 5 of the CTD.

2.5.2.2 DOSAGE FORM/POSOLOGY AND STRENGTH PROPORTIONALITY

The proposed product is available in a single strength of 3 mg as tablets.

The posology of the proposed product is 3 mg (1 tablet) for a maximum of 5 days. The dose may be increased to 6 mg (2 tablets) if the standard dose does not adequately alleviate symptoms. The first dose should be taken on arrival at destination at the habitual bed-time. Due to the potential for incorrectly timed intake of melatonin to have no effect, or to cause an adverse effect, on re-synchronization following jet lag, Melatonin should not be taken before 20:00 hr or after 04:00 hr at destination.

There are only two published trials, which examine the dose proportionality of oral melatonin and deserve further analysis.

[redacted] studied the absolute bioavailability of oral melatonin tablets in 12 normal healthy volunteers. Subjects were administered, in a randomized crossover fashion, melatonin 2 mg intravenously and 2 mg and 4 mg orally. Blood was sampled over approximately eight (estimated) half-lives. Both the 2 and the 4 mg oral dosages showed an absolute bioavailability of approximately 15 %. No difference in serum half-life was seen in any of the study phases. Oral melatonin tablets in dosages of 2 and 4 mg showed poor absolute bioavailability, either due to poor oral absorption, large first-pass metabolism, or a combination of both.

[redacted] examined the effects of very low doses of melatonin (0.1 mg - 10 mg, orally) or placebo, administered at 11:45 h, on sleep latency and duration, mood, performance, oral temperature, and changes in serum melatonin levels in 20 healthy male volunteers. Areas under the time-melatonin concentration curve varied in proportion to the different melatonin doses ingested, and the 0.1- and 0.3-mg doses generated peak serum melatonin levels that were within the normal range of nocturnal melatonin levels in untreated people.

According to the Guideline on the Investigation of Bioequivalence [42]:

> *[...] pharmacokinetics is considered to be linear if the difference in dose-adjusted mean AUCs is no more than 25 % when comparing the studied strength (or strength in the planned bioequivalence study) and the strength(s) for which a waiver is considered. In order to assess linearity, the applicant should consider all data available in the public domain with regard to the dose proportionality and review the data critically. Assessment of linearity will consider whether differences in dose-adjusted AUC meet a criterion of ± 25 %.*

By applying the above methodology and using the AUC data from the study [redacted] a linear relationship is obtained over the 2 - 4 mg range (Table VII). Although there is a trend for a more

than proportional increase by increasing the dose, the deviation from linearity does not exceed the ± 25 % criterion. In a similar manner, the same methodology in the AUC data from the study [redacted] can be applied [41]. There is a trend for a more than proportional increase by increasing the dose; however, it does not exceed the threshold of non-proportionality between the usual therapeutic ranges of 1 mg to 10 mg. For lower doses (0.3 mg) there is a borderline non-proportionality.

Table VII Assessment of Pharmacokinetic dose proportionality of Melatonin

[redacted]				
Dose (mg)	**AUC (ng × min/mL)**	**Dose normalised AUC (ng × min/mL × mg)**	**Ratio**	**Linear**
4	530.57	132.64	N/A	N/A
2	237.77	118.89	89.63 %	Yes
[redacted]				
Dose (mg)	**AUC (pg × h/mL)**	**Dose normalised AUC (pg × h/mL × mg)**	**Ratio**	**Linear**
10	21000.4	2100.04	N/A	N/A
1	1599	1599.00	76.14 %	Yes
0.3	459.9	1533.00	73.00 %	No

The data [redacted] suggest that melatonin has linear kinetics over the range of 1 - 10 mg. This finding is consistent with the SmPC of the centrally approved product, Circadin, which states that kinetics are linear over the range 2 - 8 mg [43].

The linear pharmacokinetics of melatonin have also been shown in a study [redacted] Additionally, [redacted] showed linear pharmacokinetics between 0.4 mg and 4 mg following two different oral surge-sustained release doses in older adults [45].

These data suggest, also, that a saturable first-pass hepatic metabolism may be responsible for the apparent dose-dependent oral bioavailability.

The existence of an extensive first pass effect for melatonin has been proposed in a study [redacted] where the authors found a markedly increased AUC for the ratio of 6-sulfatoxymelatonin to melatonin in plasma after oral as compared with intravenous administration (13 ± 13 vs. 1 ± 1), which can be explained only if one assumes that there was considerable first-pass hepatic extraction after oral administration, giving rise to the conversion of melatonin to 6-sulfatoxymelatonin and thereby decreasing the bioavailability of melatonin. Obviously, the trend towards a more than proportional increase in the AUC, by increasing the dose, can be explained by the saturation of the first pass metabolism. As discussed previously, melatonin is mainly metabolized by the CYP1A2 isoenzyme. For CYP1A2, metabolism of most substrates can be described using the Michaelis-Menten equation, demonstrating saturation kinetics.

The saturable metabolism and subsequent non-linearity is more obvious in high doses of melatonin. For example, some authors have commented that there is non-linearity in the pharmacokinetics of oral melatonin with the calculated plasma AUC following a 2.5 mg dose being 0.0014 µg.hr/mL, while that following an 80 mg dose was 0.465 µg.hr/mL, i.e., a 332-fold difference in AUC corresponding to a 32-fold difference in dose [47]. However, the administration of 80 mg dose falls outside the scope of the proposed product.

A comparison of Literature pharmacokinetic data between different immediate release dosage forms of melatonin for doses up to 10 mg does not reveal any differences (Table VIII and Figure 6).

The superimposed linear regression of melatonin is presented in Figure 6.

Table VIII: AUC values of solid and liquid immediate release dosage forms of melatonin for doses up to 10 mg

Reference	Dose (mg)	Form	AUC (pg × h/mL)	Comments
[48]	0.25	Liquid	14,160.00	Male
	0.25	Liquid	42,084.00	Female
[49]	2	Liquid	237,180.00	Fasting condition
	2	Liquid	349,560.00	Fed condition
[50]	10	Liquid	1,800,000.00	-
[41]	0.3	Solid	27,594.00	-
[51]	0.3	Solid	26,514.00	20 – 43 years
	0.3	Solid	35,748.00	49 – 73 years
[41]	1	Solid	95,940.00	-
[52]	1	Solid	283,200.00	-
	1	Solid	90,516.00	-
[49]	2	Solid	222,720.00	Fasting condition
	2	Solid	482,160.00	Fed condition
[40]	2	Solid	237,770.00	-
	3	Solid	530,570.00	-
[53]	3	Solid	1,690,000.00	-
[52]	3	Solid	26,911.00	-
[54]	5	Solid	1,179,230.00	-
[55]	5	Solid	372,000.00	(–) fluvaxamine
[56]	6	Solid	1,180,000.00	premenopausal women
	6	Solid	1,240,000.00	postmenopausal women
[44]	10	Solid	1,260,024.00	-

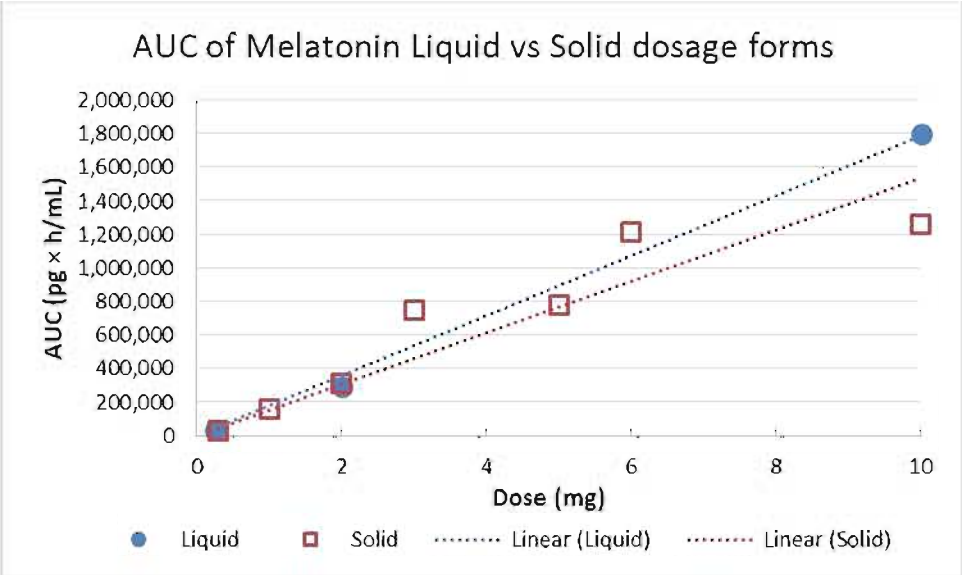

Figure 6: Dose proportionality of solid and liquid immediate release dosage forms of melatonin (Mean AUC values calculated from data of Table VIII

2.5.2.3 Differences Between The To-Be-Marketed Formulation And The Formulation(S) Used In Clinical Trials

The current application is supported by two comparative bioavailability studies, which bridge the proposed formulation with a commercially available melatonin product. The results of the studies show that the proposed formulation has comparable absorption to the marketed product.

In addition, literature data support the similarity of the pharmacokinetic profiles of different melatonin dosage forms.

Different oral dosage forms of melatonin have been used in the cited literature articles and clinical trials and it is not expected that there are differences between the to-be-marketed formulation and the formulations used in the literature articles. Melatonin tablets have been studied in several studies [redacted] and a number of trials have been conducted with immediate release tablets [redacted]

It should be taken into consideration that melatonin has good solubility (aqueous solubility in room temperature is 0.4344 mg/ml) [59], high permeability (in vitro literature permeability values 105 - 125.5 nm/s and Caco-2 permeability study performed by the applicant, Module 4.2.2.2) [43, 47, 60] and linear pharmacokinetics over the 1 mg to 10 mg dosage range [41, 43]. Therefore, melatonin is a BCS I active substance with linear pharmacokinetics. Data from the permeability study and literature data are presented in Table X of Section 0.

The pharmacokinetic profiles of immediate release oral dosage form, such as capsules, should be comparable. Data from studies conducted at other strengths can be extrapolated to the 3 mg strength, since the pharmacokinetics are linear (as presented in Table VIII and Figure 6). The variable

bioavailability of oral melatonin is therefore not related to the differing instant release formulations but is due to the variability in the high first pass metabolism of melatonin.

Additionally, and in order to bridge the proposed product with literature data, dissolution profiles in the physiological pH range versus one of the EU-licensed products have been performed. The report is presented in Module 3 and a brief presentation of the result is given in Module 2.7.1. Since melatonin is a BCS I active substance (high solubility and high permeability) and the dissolution profiles are similar, the proposed product and the EU-licensed product are expected to behave similarly *in vivo*. It should be noted that the EU-licensed product is also used in the UK for over 10 years through the parallel import scheme. The details are presented in Module 1.5.1.

2.5.2.4 INFLUENCE OF FOOD ON EXPOSURE

Food deprivation resulted in an increase of tissue and plasma concentrations of Melatonin. Melatonin also acts as an autocrine and paracrine hormone affecting not only epithelium and immune system but also smooth muscle of the digestive tract. Low doses of melatonin improve gastrointestinal transit and affect MMC (Myoelectric Migrating Complex). Pharmacological doses of melatonin delay gastric emptying via mechanisms that involve CCK2 and 5HT3 receptors [61]. It appears that exogenous melatonin inhibits gastric motility in part by activating sympathetic neurons [62].

2.5.2.5 INFLUENCE OF METHOD OF ADMINISTRATION

The proposed product is intended to be administered orally as tablets only.

2.5.3 OVERVIEW OF CLINICAL PHARMACOLOGY

2.5.3.1 PHARMACOKINETICS

The pharmacokinetics of melatonin have been reviewed recently [63]. In clinical studies, melatonin has 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 [40, 46]. Melatonin is synthesised in the pineal gland during the dark phase of the light/dark cycle and is rapidly delivered to the body via the systemic circulation. Tryptophan is converted to serotonin (5-hydroxytryptamine), then acetylated (N-acetylserotonin) and finally converted to melatonin, which is an indole (N-acetyl-5 methoxytryptamine). Several studies have been performed and have demonstrated that the dynamic pattern of melatonin secretion is fundamental for its time-giving function. The peak of melatonin levels is reached in the middle of the night (between 2 - 4 a.m.) and decrease to low levels in the second half of the night. In young adults, the average daytime levels of melatonin are 10 pg/ml and the peak night time level is 60 pg/ml. Endogenous production of melatonin is reduced in the elderly. The rhythmic pattern of melatonin secretion is important because it brings to organisms information about time that allows them to adapt some of their physiological functions to the daily and seasonal variations of their environment [64].

In humans, intravenous melatonin exhibits linear pharmacokinetics over a dosage range of 0.01 to 5.0 µg/kg [65]. However, few studies have examined absolute bioavailability of melatonin in humans. [redacted] 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 [46]. Oral bioavailability in humans with doses of melatonin 80 mg has been also examined by [redacted] However, these authors did not administer an intravenous dose and thus could not determine the absolute bioavailability. 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 % [68]. Sex differences in the oral variability of a 0.25 mg dose of melatonin was examined and determined 9 % in men and17 % in women [48].

Pharmacokinetic parameters of melatonin after i.v. infusion, i.v. bolus or oral administrations are presented in Table IX, as described [redacted] supplemented with other clinical studies from the public domain.

Table IX Pharmacokinetic variables after melatonin administration

Study	Dose (mg)	Dosage form	Conditions	C_{max} (pg/ml)	T_{max} (min)	$T_{1/2}$ (min)	AUC (pg × min/ml)	% BA
[48]	0.25	Oral solution		244.00	23.00	36.00	14160.00	9
	0.25	Oral solution		624.00	23.00	45.00	42084.00	17
[51]	0.3	IR Capsule		170.00	48.00	–	26514.00	–
	0.3	IR Capsule		255.00	45.00	–	35748.00	
[45]	0.4	Tablets (IR+CR)		405.00	78.00	108.00	95700.00	–
[46]	0.5	IR		–	–	47.00	–	33
[52]	1	Powder		799.00	60.00	–	90516.00	–
	1	IR Capsule		2620.00	60.00	–	283200.00	–
[49]	2	IR Capsule	Fasting	2800.00	15.00	32.00	222720.00	–
	2	IR Capsule	Fed	6800.00	30.00	–	482160.00	–
	2	MR tablet		–	–	–	–	–
	2	Oral solution	Fasting	3500.00	30.00	–	237180.00	–
	2	Oral solution	Fed	4400.00	30.00	40.00	349560.00	–
[40]	2	IR Tablet		2175.00	52.00	61.00	237770.00	14
[69]	3	IR Tablet		5766.00	60.00	65.00	530570.00	16
	3	IR Tablet		11040.00	16.00	94.00	1690000.00	–
	3	Powder		2405.00	40.00	–	26911.00	–
[70]	3	IR		3561.00	20.00	–	–	–
	4	Tablets (IR+CR)		3999.00	90.00	126.00	727400.00	–
[55]	5	IR Tablet		25100.00	–	804.00	8480000.00	–
	5	IR Tablet		2180.00	–	564.00	372000.00	–
[63]	5	IR Tablet		4823.00	30.00	38.00	256885.00	–
[71]	5	MR tablet		8770.00	167.00	91.00	2300000.00	–
[72]	6	IR Tablet	Fasting	10618.00	30.00	113.00	–	–
	6	IR Tablet	Fasting	4480.00	60.00	106.00	–	–
[73]	6	IR Tablet		7900.00	60.00	36.00	684000.00	–
	6	IR Tablet		1800.00	60.00	37.00	138000.00	–
	6	IR Tablet		7200.00	60.00	38.00	654000.00	–
	6	IR Tablet		1700.00	45.00	49.00	144000.00	–
[56]	6	IR Tablet		16756.00	30.00	46.00	1180000.00	–
	6	IR Tablet		16438.00	53.00	52.00	1240000.00	–
[50]	10	Oral solution		14974.00	30.00	88.00	1800000.00	–
	10	MR tablet		3820.00	45.00	48.00	507911.00	–
	10	MR tablet		4072.00	210.00	50.00	595400.00	–
[74]	25	IR Capsule		640.00	90.00	–	102419.00	
	25	IR Capsule		1858.00	90.00		294002.00	–
[67]	80	IR Capsule		–	–	48.00	27870000.00	–
[75]	100	IR Capsule		101163.00	60.00	41.00	–	–
	240	IR Capsule		–	–	–	31300000.00	–

Study	Dose (mg)	Dosage form	Conditions	C_{max} (pg/ml)	T_{max} (min)	$T_{1/2}$ (min)	AUC (pg × min/ml)	% BA
[65]	0.0005 mg/kg	IV		–	–	40.00	15054.00	–
	0.0005 mg/kg	IV		–	–	47.00	18006.00	–
	0.0005 mg/kg	IV		–	–	47.00	22614.00	–
	2	IV		96850.00	–	60.00	1630000.00	–
	0.023 mg (250 ml/h)/	IV		124.80	113.40	36.00	15288.00	–
	0.023 mg (250 ml/h)	IV		169.00	110.40	41.00	21846.00	–
[76]	-	IV		–	4.00	44.00	–	–
[77]	0.005 mg	IV		–	–	28.00	5400.00	–
	0.02 mg (10 ml/h)	IV		72.10	–	45.00	–	–

2.5.3.1.1 Absorption

According to literature data, the permeability of melatonin is high (Table X).

Additionally, the applicant undertook a validated in-vitro study to assess the extent of permeability of melatonin across Caco-2 monolayers ad efflux from Caco-2 cells. The data showed that Melatonin is highly permeable in the apical to basolateral direction within the Caco-2 cells and when compared with the reference propranolol, it suggests that human intestinal absorption of melatonin would be greater than 90 % (P_{app} 40.3 × 10^{-6} cms^{-1} for A2B, P_{app} 39.9 × 10^{-6} cms^{-1} for B2A). As such, no additional clinical work was performed across different dosage forms, including capsules, due to the high absorption of the melatonin, as the molecule could be considered potentially suitable for a biowaiver under the BCS classification due to it being highly absorbed

The results of the permeability study are presented in Table X along with the literature data and the report of the permeability study is located in Module 4.2.2.2.

Table X Reported permeability values of Melatonin

Reference	Dose of Melatonin	$P_{app\ A-B}$	$P_{app\ B-A}$	Efflux ratio ($P_{app\ B-A}/P_{ap\ A-B}$)
[redacted]	5 µM	11.56 ± 2.00 × 10^{-6} cm/s	11.58 ± 1.01 × 10^{-6} cm/s	1.0
[redacted]	6.5 µM	12.5 × 10^{-6} cm/s	-	-
[redacted]	10 µM	40.3 ± 4.74 × 10^{-6} cm/s	39.9 ± 1.30 × 10^{-6} cm/s	0.99
[redacted]	50 µM	~ 11.0 × 10^{-6} cm/s	-	-

Usually, molecules with permeability > 10 nm/s in the Caco-2 system (1 × 10^{-6} cm/s) are classified as highly permeable and have intestinal absorption >90 % [79]. Other authors suggest a cut-off value of 100 nm/s (or 10 × 10^{-6} cm/s) [80]. It is reported that the overall ranking of compounds with P_{app} < 1 × 10^{-6} cm/sec, between 1 - 10 ×10^{-6} cm/sec and > 10 ×10^{-6} cm/sec can be classified as poorly (0 – 20 %), moderately (20 – 70 %) and well (70 – 100 %) absorbed compounds, respectively [80]. According to the table above (Table X), all reported values from literature are > 10 × 10^{-6} cm/sec for Melatonin indicating that the proposed product has high permeability and therefore all instant release dosage forms would be expected to act in a similar manner.

The absorption and bioavailability of orally administered (exogenous) melatonin in humans has been extensively reported in the literature. Melatonin is rapidly absorbed following oral administration of instant release forms, with T_{max} usually achieved in 60 minutes (normal range: 20 - 90 minutes [40, 48]. A recent study has shown oral melatonin to have a $T_{1/2}$ as low as 6 minutes [81].

After taking 3 - 6 mg melatonin, 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) with peak serum levels 350 - 10,000 times higher than the endogenous night-time peak within 60 - 150 minutes of dosing was observed [67]. 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 [82]. However, significant intra -subject variability in exposure parameters has been reported within the literature [48, 49, 56]. In the case where 2 × 3 mg immediate release tablets were evaluated in pre and post-menopausal healthy female volunteers, the rate of absorption, C_{max}, ranged from 2.827 to 29.289 ng/mL in premenopausal women and from 1.892 to 40.488 ng/mL in postmenopausal women, whereas AUC all values ranged from 2.640 to 39.735 ng·h/mL and 3.072 to 53.132 ng·h/mL for pre- and postmenopausal women, respectively. In this study of [redacted] high plasma melatonin concentrations were determined also in the case of melatonin formed in oral solution [49].

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 [40].

On the other hand, it has been reported in another study that there was significant between subject and gender variability following an oral melatonin solution of 250 µg [48]. 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) [83]. From a retrospective analysis on multiple studies that used intravenous melatonin or oral preparations (but not both in the same subjects) and estimated oral bioavailability to range from 3 to 76 % [40].

Basal serum melatonin levels were studied in conjunction with the administration of a low oral dose of melatonin (0.3 mg) in a group of healthy young adults (mean age 29.2 ± 6.5 years) and in a group of older adults (mean age 60 ± 8.8 years). Serum melatonin levels were measured at 30 minute 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 [51].

A comparison of the endogenous and exogenous melatonin levels was conducted in 23 healthy subjects, 12 young and 11 older adults, of both genders. In the same blood sample, they were able to distinguish endogenous melatonin from exogenously administered D7 melatonin, a molecule in which seven deuterium atoms replace seven hydrogen atoms. All subjects participated in two experiments: one with 250 µg of oral D7 melatonin at midday and, after a washout period of 1 week, one with 250 µg of oral D7 melatonin at midnight. In addition, the young subjects participated in a third study, involving a 23- mg D7 melatonin infusion. Significant gender differences and between subject variability in exposure parameters were reported. Following oral dosing with 250 µg of D7 melatonin, mean ± sd C_{max} was 243.7 ± 24.6 pg/ml and 623.6 ± 575.1 pg/ml, whereas AUC was 236 ± 07 pg.h/ml and 701 ± 45 pg.h/ml, in males and females respectively. However, there were no significant differences in total body clearance normalised to body weight: 1.27 ± 0.20 L/h/kg and 1.18 ± 0.22 L/h/kg for males and females respectively [48, 83].

In a cohort crossover study, the pharmacokinetic parameters of oral and intravenous (i.v.) melatonin in healthy volunteers were investigated. The volunteers received either 10 mg oral melatonin or 10 mg intravenous melatonin on two separate study days. Blood samples were collected at different time points following oral administration and short i.v. infusion, respectively. Mean ± SD $t_{1/2}$ absorption of oral melatonin was 6.0 ± 3.1 min. Mean T_{max} was 40.8 ± 17.8 min with a median (IQR) C_{max} of 3550.5 (2500.5 – 8057.5) pg/ml. Mean $T_{1/2}$ elimination was 53.7 ± 7.0 min. Median absolute bioavailability was 2.5 (1.7 – 4.7 %). Median C_{max} after short i.v. infusion of melatonin was 389,875.0 (174,775.0 – 440,362.5) pg/ml. Mean $T_{1/2}$ elimination was 39.4 ± 3.6 min, mean V_d 1.2 ± 0.6 l kg^{-1} and mean CL 0.0218 ± 0.0102 l min^{-1} kg^{-1}. It was concluded that the bioavailability of oral melatonin was only 3 % [81]. Exogenous administration of melatonin with a loading dose of 3 mg (as solution through subjects' feeding tube), followed by an hourly dose of 0.5 mg, results in supraphysiological and sustained concentrations of serum melatonin during 12 hours overnight in subjects (in critically ill patients). These findings support 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 [84].

Oral solution of melatonin (10 mg) was also administered in patients, who had undergone a tracheostomy in a randomized double-blind placebo-controlled trial [50]. Melatonin appeared to be rapidly absorbed from the oral solution and peak concentrations were higher than those reported for comparable doses in healthy individuals [41, 72]. 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 % [40], although there is high variability due to factors such as cytochrome P450 1A2 (CYP1A2) activity and co administration of interacting drugs [55].

Melatonin soft capsules showed similar pharmacokinetic parameters compared with the highest dosed of melatonin in powder form but its bioavailability was improved [52]. 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 PK and was not well absorbed.

The bioavailability of a new oral spray of melatonin emulsion was compared with a standard oral formulation in healthy subjects [redacted] 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 hour overnight fast, subjects received a single capsule of long acting of melatonin 5 mg 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-t} 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 [71].

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 [70]. The saliva melatonin 60 minutes after the ingestion showed the highest level (1177 ± 403 pg/ml) which was one-third of the plasma level. The highly 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 [75].

2.5.3.1.2 Distribution

It has been estimated that the mean steady state volume of distribution (Vd_{ss}) in healthy adult volunteers, following an intravenous infusion of D7 melatonin, to be 0.98 L/kg distribution. No gender

difference in the Vd_{ss} normalized to body weight was observed: 0.99 ± 0.063 L/h/kg and 0.97 ± 0.13 L/h/kg in males and females, respectively [83].

The in vitro plasma protein binding of melatonin is about 60.0 %. Melatonin is mainly bound to albumin, alpha1- acid glycoprotein and high density lipoprotein [85]. The level of melatonin binding appears to be constant over range of different serum concentrations. Data from the literature indicates that melatonin is distributed in all body fluids and is accessible at all tissues. The mean binding of melatonin to erythrocytes is 49.0 %.

Melatonin is not strongly or extensively bound to plasma proteins, therefore protein binding effects on pharmacokinetics should not be expected to be significant.

Distribution from serum to saliva, and passing through the blood-brain barrier is rapid. Concentration in the cerebrospinal fluid is 2.5 times lower than it is in the plasma. Plasma elimination T½ is usually approximately 45 minutes [86].

Melatonin reaches all tissues of the body within a very short period. Melatonin half-life is bi-exponential, with a first distribution half-life of 1.4 min and a second of 28.4 minutes [77]. 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 [87]. 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 [88]. Additionally, another human PET study was performed with carbon-11 labelled melatonin in a healthy volunteer (case report). Plasma pharmacokinetics of melatonin and 6-sulfatoxymelatonin were simultaneously determined using radioimmunoassay. Analysis of tracer kinetics showed maximum activity in the brain 8.5 minutes following injection, which was different from the curve observed for the plasma radioactivity (maximum at 3.5 minutes). This result confirmed that melatonin readily crosses the blood brain barrier and that 6-sulfatoxymelatonin is the main plasma metabolite. In this study, the distribution of tracer as a function of time, failed to reveal any specific binding [76].

2.5.3.1.3 Metabolism

The literature provides information regarding the metabolic fate of melatonin. The metabolic pathway of melatonin is presented in Figure 7.

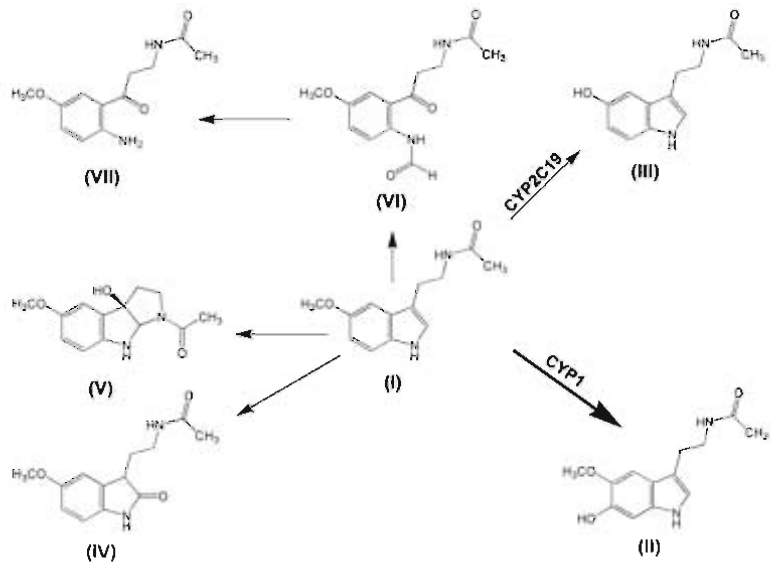

Figure 7 Metabolites derived from melatonin. In humans, melatonin (I) is converted by CYP2C19 to NAS (III) and by CYP1A1, CYP1A2 and CYP1B1 to 6-HMEL (II). Melatonin also undergoes conversion to the ring opened metabolites AFMK (VI), AMK (VII), and to the oxidation products 3-HMEL (V) and 2-.OMEL (IV) [89]

Circulating melatonin is metabolized primarily in the liver where it is first hydroxylated in the C6 position by cytochrome P450 mono-oxygenases (isoenzymes CYP1A2, CYP1A1 and, to a lesser extent, CYP1B1) and thereafter conjugated with sulphate to be excreted as 6-sulfatoxymelatonin (aMT6S); glucuronide conjugation is extremely limited. CYP2C19 and, at lower rates, CYP1A2 also demethylate melatonin to N- acetylserotonin, being otherwise its precursor [87] [73, 90]. [redacted] addressed this issue by measuring the production of the main metabolite 6-hydroxymelatonin sulphate following oral and intravenous administration.

The metabolism in extrahepatic tissues exhibits substantial differences. Tissues of neural origin, including the pineal gland and retina, contain melatonin-deacetylating enzymes, which are either specific melatonin deacetylases or less specific aryl acylamidases; as eserine-sensitive acetylcholinesterase has an aryl acylamidase side activity, melatonin can be deacetylated to 5-methoxytryptamine in any tissue carrying this enzyme. Melatonin can be metabolized non-enzymatically in all cells, and also extracellularly, by free radicals and a few other oxidants. It is converted into cyclic 3-hydroxymelatonin when it directly scavenges two hydroxyl radicals [87]. From one of the studies reviewed, it appears that repeated dose administration does not alter the metabolic profile of melatonin.

In the study [redacted] a markedly increased area under the curve (AUC) for the ratio of 6-sulfatoxymelatonin to melatonin in plasma after oral as compared with intravenous administration (13 ± 13 vs. 1 ± 1) was found, which can be explained only if one assumes that there was considerable first-pass hepatic extraction after oral administration, giving rise to the conversion of melatonin to 6-sulfatoxymelatonin and thereby decreasing the bioavailability of melatonin [46]. The study confirmed that there was a clear inverse relation between the AUC ratio and bioavailability after oral melatonin administration, confirming that the low bioavailability was a consequence of hepatic first-pass extraction, which converts melatonin to its metabolite before it enters the systemic circulation.

2.5.3.1.3.1 *Active Metabolites*

A substantial fraction of melatonin is metabolized 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 [81].

2.5.3.1.4 Excretion and Elimination

The primary metabolite of melatonin, 6-sulphatoxymelatonin, accounts for around 90 % of the dose excreted in the urine [91-94]. The other main metabolite results from melatonin O-demethylation, yielding N-acetylserotonin [95]. 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 min 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 [redacted] approximately 1 % of blood melatonin is excreted in the urine without being metabolized. The authors concluded in a positive correlation between AUC_{MLT} and 6-OH-MLT-S in the urine [96]. In another study, it is reported that 6-Sulphatoxymelatonin (aMT6s) is the major urinary metabolite of melatonin [93, 94] 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 h urine sample and the remainder in the next 24 h [93].

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 [48]. The half-life of melatonin following single intravenous and oral doses in healthy volunteers has been reported to be approximately 1 hour [40]. Elsewhere, the elimination half-life has been reported as 43.6 minutes following intravenous administration in human subjects [67]. 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 [48].

[redacted] 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 hours 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 [97].

Pharmacokinetic parameters of melatonin after i.v. infusion, i.v. bolus or oral administration are presented in Table IX.

2.5.3.1.5 Pharmacokinetic studies in special population

2.5.3.1.5.1 *Paediatrics*

To determine whether melatonin pharmacokinetics change during puberty, [redacted] infused melatonin i.v. in 9 pre-pubertal (8.4 ± 1.5 years), 8 pubertal (12.9 ± 1.7 years), and 16 adult subjects and measured melatonin in serum and saliva, and 6-hydroxymelatonin sulfate in urine [65]. A pilot study of 3 adult males showed dose linearity, absence of saturation kinetics, and unaltered metabolism and urinary excretion for doses of 0.1 mcg/kg, 0.5 mcg/kg, and 5.0 mcg/kg. All other subjects received 0.5 mcg/kg melatonin. The results of pharmacokinetic parameters calculated from serum melatonin showed no significant gender differences in adults. However, developmental differences were significant between pre-pubertal children and adults for terminal elimination rate constant (1.08 ± 0.25 h^{-1} versus. 0.89 ± 0.11 h^{-1}), elimination half-life (0.67 ± 0.12 h vs. 0.79 ± 0.10 h), and area under the concentration-time curve [250.9 ± 91.8 vs. 376.9 ± 154.3 (pg/mL) h, respectively]. At all time-points melatonin levels were higher in serum than in saliva, and the ratio between serum and salivary melatonin varied up to 55-fold within and between individuals. Results based on salivary melatonin showed significant differences between pre-pubertal children and adults for the terminal elimination rate constant (1.90 ± 0.95 h^{-1} versus. 1.06 ± 0.28 h^{-1}). The described group differences in pharmacokinetic parameters suggest that pre-pubertal children metabolize melatonin faster than adults.

2.5.3.1.5.2 *Young Adults and Elderly*

As previously described, melatonin is rapidly metabolized by the liver and eliminated in the urine as 6-sulphatoxy-melatonin (6-S-MT) and therefore, the urinary excretion of 6-S-MT can serve as a reliable measure of serum melatonin profile. Serum melatonin concentrations decrease in old age and it has been reported that in healthy elderly people suffering from insomnia, urinary 6-S-MT was significantly lower and its onset and peak time delayed, in comparison to age-matched controls with no sleep disorders. Similarly, in elderly females, 6-sulfatoxy melatonin levels were found to be significantly lower in poor compared to good sleepers [98].

In a study by [redacted] the magnitude and duration of melatonin secretion were measured over a period of 25 hours with pharmacokinetic studies employing administration of D7 melatonin at midday and at midnight in two separate studies and two groups of subjects, 12 young (26.7 ± 4.4 years) and 11 older men and women (70.0 ± 3.3 years). For all subjects, endogenous melatonin concentrations were lower than 0.5 pg/ml between 10 am and 6 pm. A rapid rise was observed for all subjects and steady state was reached in, 3 - 4 h (3 or 4 half-lives). Secretion started at 20:10 ± 50 min (19:15 - 21:25) and 20:40 ± 40 min (19:50 - 21:45) in young men and women, respectively. Offset of secretion was at 04:05 ± 50 min (range, 03:05 - 05:25) and at 04:20 ± 35 min (range, 03:35 - 05:10) for men and women, respectively. No significant gender difference in duration of secretion (7.9 ± 0.8 h and 7.6 ± 0.8 h), C_{max} (54.7 ± 23.1 and 54.2 ± 26.4 pg/ml) and AUC (375.5 ± 178.6 and 349.7 ± 174.8 pg.h/ml) was observed [83]. The values of terminal half-life, $T_{1/2}$ = 1.2 ± 0.3 h and 1.1 ± 0.5 h, were determined by regression of the terminal portion of the log-plasma concentration-time profile and showed no significant gender difference. There was also no significant gender difference in the amount of nocturnal secretion normalized to body weight of subjects: 0.48 ± 0.23 and 0.40 ± 0.19 µg/kg in men and women, respectively. Steady state melatonin concentrations were equal to 47.2 ± 20.4 pg/ml (range, 17.0 - 85.7) and 46.3 ± 25.5 pg/ml (range, 18.7 - 91.5) in men and women, respectively, with no significant gender difference.

In a randomized, double-blind, placebo-controlled study of low (0.4 mg) and high (4 mg) dose melatonin (25 % immediate release + 75 % controlled release) in 27 older adults with insomnia complaints and low endogenous melatonin levels, the pharmacokinetic properties of melatonin were determined. The time to maximum level (1.3 hours versus 1.5 hours), elimination half-life (1.8 hours versus 2.1 hours) and apparent total clearance (379 l/hr versus 478 l/hr) did not differ significantly between the low and high dose arms, respectively. The maximum concentration was 405 ± 93 pg/ml for the low dose arm and 3999 ± 700 pg/ml for the high dose arm, both of which are substantially higher than physiologic melatonin levels for this age group. Additionally, subjects in the high dose arm maintained melatonin levels > 50 pg/ml for an average of 10 hours, which could result in elevated melatonin levels beyond the typical sleep period. Renal and liver function parameters remained stable after 6 weeks of treatment. The authors concluded in a linear pharmacokinetic behavior of melatonin in this older group of patients [45].

2.5.3.1.5.3 *Impaired Renal Function*

The melatonin status of patients in end-stage Chronic Renal Failure (CRF) was evaluated by the determination of daytime plasma melatonin levels and by the investigation of the circadian rhythmicity of melatonin secretion [99]. A significant increase in plasma melatonin concentration was found in all CRF patient groups investigated, i.e. CRF patients on conservative treatment (CT; n = 48), CRF patients on maintenance haemodialysis treatment (HD; n = 39) and CRF patients on peritoneal dialysis (PD; n = 32). Successful transplantation led to a marked reduction in plasma melatonin levels. The circadian rhythm of melatonin secretion also appeared to be suppressed in CRF as the nocturnal secretory surge was absent in all HD patients and in 80 % of the post-transplantation patients studied. Another study on patients with end stage renal disease receiving haemodialysis, showed that melatonin plasma concentrations were not affected by the process suggesting that haemodialysis is unable to eliminate melatonin [100]. Thus, it appears that renal insufficiency affects melatonin elimination and is not compensated by haemodialysis.

2.5.3.1.5.4 *Impaired Hepatic Function*

Plasma melatonin levels have been studied in seven patients with cirrhosis and seven age-, sex-, and education-matched controls. It was found that patients with cirrhosis and subclinical hepatic encephalopathy had abnormal plasma melatonin pattern compared with healthy controls [101]. Three variables of the melatonin profile were analysed. Time of onset of melatonin secretion was significantly displaced from 7:50 p.m. ± 2 6 min to 9:30 p.m. ± 13 min (p = 0.013). Time of peak plasma melatonin levels was significantly displaced from 12:36 a.m. ± 33 min to 5:36 a.m. ± 29 min. In addition, significant increases in absolute melatonin levels were seen during daytime and night time hours (P < 0.05 at every measurement between 2:30 a.m. and 10:00 a.m.).

In a subsequent study, urinary 6-sulfatoxymelatonin levels in 21 hospitalized cirrhotic patients with normal renal function was measured (14 men and 7 women; median age, 50 years [range, 29 to 80 years]; Child class A, 7 patients; class B, 11 patients; class C, 3 patients) and in 9 healthy persons (3 men and 7 women; median age, 49 years [range, 32 to 69 years]). Sixteen had alcoholic liver disease, 2 had hepatitis C, and 1 each had primary biliary cirrhosis and Wilson disease. Two patients had signs of clinically overt hepatic encephalopathy [102]. Eight-hour urine excretion (10:00 p.m. to 6:00 a.m.) was assayed for 6-sulfatoxymelatonin and finally cirrhotic patients had a significantly decreased concentration (mean ± SE, 19.01 ± 2.76 ng/mL compared with 39.2 ± 5.41; p = 0.001) and total excretion (median, 8.28 mg [range, 0.85 to 28.1 mg] compared with 12.21 mg [range, 9.12 to 29.04 mg]; P < 0.05) of 6-sulfatoxymelatonin, compared with controls. Urine volumes were similar in

the two groups. No correlation to child class or liver function measures was seen. These findings indicate that the elevated plasma melatonin levels seen in cirrhotic patients are at least partly due to impaired hepatic catabolism.

2.5.3.1.5.5 *Critically ill patients*

Critically ill patients exhibit reduced melatonin secretion, both in nocturnal peaks and basal daytime levels. Its early enteral absorption and daily pharmacokinetics were determined in two cohort of six high-risk patients in the prospective trial of [redacted] Following enteral administration, pharmacological levels were already reached in 5 minutes with a serum peak after 16 minutes half-absorption time: 3 min and 17 sec). The maximum serum level observed was 11040 pg/mL and the disappearance rate indicated a half-elimination time of 1 hour and 34 minutes. Serum melatonin levels decreased significantly after midnight; pharmacological levels were maintained up to 10 hours following administration. Critically ill patients exhibited reduced melatonin secretion and despite the critical illness, the oral bioavailability was satisfactory (serum levels after oral administration showed basically unchanged intestinal absorption, while disappearance rate was slower than reported in other studies in healthy volunteers [53].

2.5.3.1.5.6 *Pregnancy*

Melatonin has protective actions on both the foetus and the mother during pregnancy. It can easily cross the placenta to enter the foetal circulation leading the photoperiodic information to the foetus [103]. During pregnancy there is a high metabolic demand for oxygen, which leads to a higher ROS production and, consequently, oxidative stress. The placenta is a major source of oxidative stress because it is rich in poly-unsaturated fatty acids. Spontaneous abortion and recurrent pregnancy loss have been associated with systemic oxidative stress [104]. Due to melatonin level increase, during gestation in normal pregnant humans, reducing the oxidative stress and abortion rate, melatonin has been suggested as a potential molecule to be administered throughout compromised pregnancy such as in pre-eclampsia and foetal undernutrition, two entities associated with oxidative stress. In preeclampsia, lipid peroxide levels in maternal blood and placental tissue are increased and total antioxidant activities are lowered. Melatonin levels were found to be decreased in severe pre-eclampsia [105]. Some recent evidence has suggested supplements of melatonin to prevent pre-eclampsia in humans [106].

2.5.3.2 PHARMACODYNAMICS

A melatonin receptor nomenclature was recently proposed by the International Union of Pharmacology (IUPHAR) [107]. Two subtypes, MT1 and MT2, of mammalian melatonin receptors have been cloned. Both subtypes are members of the seven-transmembrane G protein-coupled receptor family.

Physiologically, melatonin secretion increases soon after the onset of darkness, peaks at 2 – 4 am and diminishes during the second half life of the night [64].

Melatonin binds to two receptors co-localized in the SCN (Supra-Chiasmatic Nuclei) (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 [108]. The MT1 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 [109]. A binding protein originally thought to represent a third membrane receptor (MT3) turned out to be the primarily cytosolic enzyme quinone reductase 2 (QR2).

Some effects of melatonin cannot be explained by membrane receptors or radical scavenging. Melatonin appears to be the natural ligand for the orphan nuclear hormone receptor superfamily RZR/ROR. Melatonin nuclear receptors are involved in the immunomodulator effect of melatonin [110].

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 [111, 112]. This last effect may be the prominent mechanism of action of exogenous melatonin [33, 113].

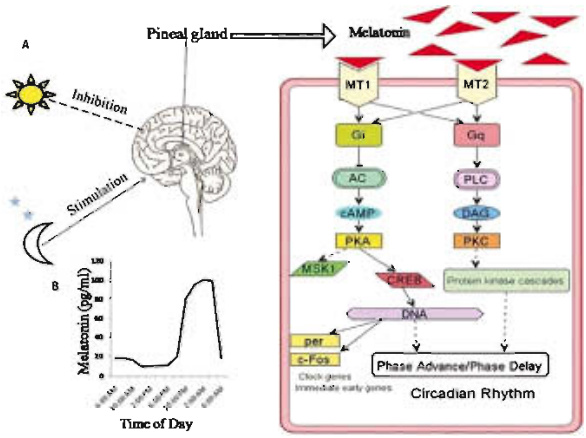

Figure 8 Regulation of melatonin production and MT1/MT2 receptor function.

The difference between the physiological and pharmacological effects of melatonin is not always clear, but is based upon the dose, and not the duration, of the hormone message. It is accepted that a "physiological" dose provides plasma melatonin levels of the same order of magnitude as a nocturnal peak (equal to, or lower than, 100 pg/ml or 400 pM) [107].

2.5.3.2.1 Circadian Regulation of Sleep and Mechanism of action

The neurons of the major circadian clock, the Suprachiasmatic Nuclei (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 synchronization) and with the periodic changes in the environment (external synchronization).

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 [114, 115]. Melatonin is also the endogenous agonist of two G-protein-coupled membrane receptors, named MT1 and MT2 [107] as presented in Figure 8, 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 [116]. Moreover, melatonin has been found to interact with other cellular targets, such as calmodulin [117]. MT1 and MT2 receptors are negatively coupled to adenylate cyclase, and they can also interact with other intracellular second messengers [118, 119]. In humans, they are expressed in different areas of the CNS, such as the SCN, cerebellum, hippocampus, substantia nigra and nucleus accumbens [120, 121]. They have also been found in peripheral tissues, such as retina, coronary arteries, immune cells, intestine and epithelial cells [122-124]. The differential role of the two receptor subtypes has been elucidated only partially. Activation of MT1 receptors inhibits neuronal firing within the SCN [125] and inhibits the release of hormones, such as prolactin [126], as seen in Figure 8, while activation of MT2 receptors induces splenocyte proliferation, vasodilatation of coronary arteries and inhibits dopamine release in the retina [122, 127, 128]. 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 [129, 130].

	mt_1	mt_2	MT_3
Nomenclature			
Other Names	Mel_{1a} ML_{1A}	Mel_{1b} ML_{1B}	ML_2
Affinity (K_D)	45 pM	140 pM	0.3-2 nM
Pharmacology	MLT >> NAS	MLT>>NAS	MLT=NAS
Selective Agonists	---	---	N-Acetyl 5HT 5-MCA-NAT
Selective Antagonists	--- ---	4P-ADOT 4P-PDOT	PRAZOSIN

Figure 9 Function of Melatonin Receptor subtypes

The human melatonin receptor subtypes show 60 % homology at the amino acid level and distinct pharmacological profiles of partial agonists and antagonists. The use of cell lines expressing the human MT1 and MT2 melatonin receptors has led to the discovery of subtype selective analogues.

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 [131]. 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 [132, 133]. 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 synchronization with the environment and entrain the physiological rhythms to a 24-hour cycle TUL [134].

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 [135]. This effect of melatonin is pertussis toxin sensitive, indicating coupling of the receptor to a Gi protein [136]. Thus, the classical effect of MT1 and MT2 receptors are primarily coupled, in an inhibitory manner, to the AC → cAMP → PKA signaling pathway, via a pertussis toxin sensitive Gi protein [137-141], as described in Figure 10. 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 [142], and Giαs, Gαz, and Gα16 [143, 144]. 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 Ca^{2+} and 1,2-diacylglycerol [145-147]. In addition. Activated MT1 receptors inhibit cAMP responsive element binding protein (CREB) phosphorylation, a nuclear transcriptional activator of cAMP-sensitive gene factor [148-150], and also inhibit the formation of immediate early gene products, c-Fos and Jun B [151].

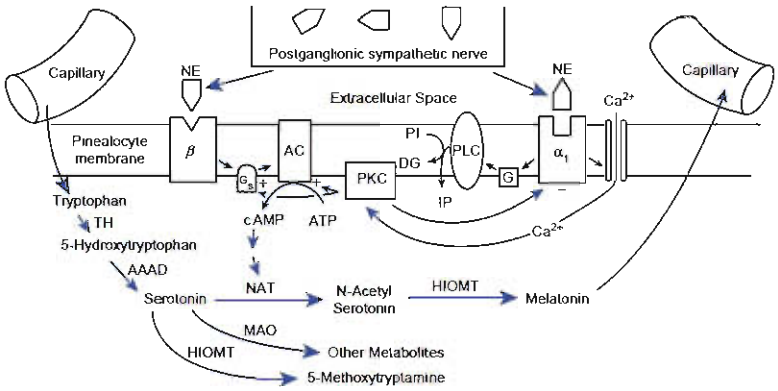

Figure 10 Interactions of NE (noradrenalin) released from postganglionic sympathetic fibres with beta-adrenergic receptors in the pinealocyte membrane. This interaction initiates a series of intracellular events, which culminate in a large rise in the acetylation of serotonin to N-acetylserotonin by the enzyme N-acetyltransferase (NAT). Once produced, melatonin is quickly discharged into the capillary bed in the pineal gland and possibly directly into the CSF of the third ventricle [152]

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 [142, 147]. 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 [144]. Stimulation of recombinant human MT1 receptors potentiates also the

prostaglandin F_{2a}-induced release of arachidonate and hydrolysis of phosphoinositide [153]. MT1 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 [147, 153].

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 [144].

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 MT1 receptor expression found in the SCN.[154]

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.

2.5.3.2.2 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 2000 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 [155].

2.5.3.2.3 Polysomnography for sleep disorders diagnosis and monitoring of melatonin efficacy

Polysomnography (PSG) is a recording of multiple physiologic parameters relevant to sleep. Clinical studies have used a typical recording montage that includes electroencephalography (EEG), electrooculography (EOG), chin electromyography (EMG), respiratory effort, airflow, electrocardiography (ECG), oximetry, and anterior tibialis EMG and PSG. That type of studies are useful for diagnosing sleep-related disorders and there is a large number of studies supporting the PSG as a successful tool of diagnosis of sleep disorders.

However, the studies that have used PSG to evaluate circadian rhythm sleep disorders found PSG useful in identifying sleep-structure changes in subjects with circadian rhythm sleep disorders. None of these studies presented in Section 2.5.4 (Clinical Efficacy) demonstrate a value for PSG in recognizing specific circadian rhythm sleep disorders or in directing treatment. Thus, PSG is not useful in the clinical diagnosis or treatment of circadian rhythm sleep disorders.

There are only few studies available in the public domain that use polysomnography for assessment of melatonin effects on subjects with jet lag or shift work disorders. Other methodologies that have been used and included in the provided literature of the clinical overview are wrist actigraphy, sleep diaries, VAS, questionnaires. According to literature data, compared to polysomnography, both actigraphy and sleep diary instruments underestimate total sleep time and sleep efficiency [156]. The results of Vallieres study suggest that actigraphy is a useful device for measuring treatment response and it should be used as a complement to sleep-diary evaluation.

Further details are presented in Section 2.5.4.

2.5.4 OVERVIEW OF EFFICACY

There are two general approaches to address the symptoms of jet lag and shift work disorder. The first is to accelerate realignment of the circadian system with the external environment and the second is to treat the symptoms of insomnia and excessive sleepiness. Multimodal approaches are typically needed and should be tailored to the individual, because the severity of symptoms and timing of treatments depends on the direction of travel and number of times zones crossed or the type of work schedule [27]. Phase shifting, or resetting the circadian clock, is achieved by timed light – dark exposure and or melatonin administration.

The inclusion criteria of the studies were assessed for methodological quality as well as for melatonin effectiveness. Most of the clinical studies included in this clinical overview, compare melatonin's effect on circadian rhythm disorders versus placebo. Participants completed sleep logs, symptoms questionnaires and POMS (Profile of Mood States) before melatonin administration. These studies examine also the evidence for the effectiveness of different dosage regimen. Additionally, in most of the studies immediate release oral dosages forms of melatonin were used.

The "gold standard" methodology for studying sleep physiology has long been considered to be sleep-stage scoring of the PSG. This methodology has limitations and there are also other potential methodologies that could be considered for sleep quality characterization. Among these are sleep EEG spectral analysis, scoring methods for characterizing the cycling alternating pattern and use of actigraphy [157]. In the studies that are presented in the following sections several methods have been used to evaluate the efficacy of melatonin such as self-rated VAS (Visual Analog Scale), polysomnographic recordings, sleep-log diaries, individual actograms and wrist Actigraphy, SSS (Stanford Sleepiness Scale), POMS (Profile of Mood States), MEMS (Medication Event Monitoring System), sleep EEG, Electrooculography (EOG), Electromyography (EMG) and several other questionnaires, as presented also in tabulated format in Module 2.7.

Since the most common used methodologies to evaluate the effects of melatonin on prevention and treatment of jet lag and treatment of shift work disorder include self-rated VAS, POMS, actigraphy, sleep diaries and questionnaires, the correlation of concentration – time curves of melatonin and desired clinical effects is difficult. Most of the clinical studies evaluate the fatigue, the daytime tiredness, the onset of sleep at destination the onset and quality of sleep, the psychological functioning and the duration of return to normal. On the other hand, melatonin displays a high inter-individual variability in the parameter of serum levels and the correlation of different doses of melatonin with the clinical effect is therefore difficult. The study [redacted] demonstrated a dose-effect relationship of melatonin with induced acrophase shift, by administering 0.05 mg, 0.5 mg and 5 mg of melatonin and recording advances of the melatonin acrophase from less than 1 hour to 1.5 hour.

Additionally, acute dose-dependent temperature suppression and decrements in alertness and performance efficiency were induced after melatonin administration [158].

According to the Guideline on medicinal products for the treatment of insomnia [EMA/CHMP/16274/2009 previously (EMEA/16274/2009) Rev. 1] [159], two complementary types of trials are required to demonstrate efficacy in the clinical development programme:

(1) Trials documenting effects on objective (usually self-rating) endpoints in the "natural" setting and
(2) Trials documenting effects on objective endpoints (polysomnography).

The following clinical efficacy criteria should be evaluated as a minimum acceptable standard:

- Sleep onset latency
- Sleep continuity
- Sleep duration
- Feeling of restorative sleep and quality of sleep
- Subsequent daytime functioning in the natural setting

In the following studies, at least one of these outcomes is assessed.

In all studies included in the clinical overview and Module 2.7.3, the effect of melatonin on jet lag patients or patients suffering from work shift disorder, is described following the abovementioned criteria.

Additionally two mores studies concluded in dose depended and time-depended effects of melatonin [160, 161].

2.5.4.1 PREVENTION AND TREATMENT OF JET LAG

As described in Section 2.5.1.2.1, jet lag commonly affects air travellers who cross several time zones. It results from the body's internal rhythms being out of step with the day-night cycle at the destination. Melatonin is a pineal hormone that plays a central part in regulating bodily rhythms and has been used as a drug to re-align them with the outside world.

Melatonin's utility in the management of jet lag has been the subject of many studies. When making travel plans, jet particularly over a distance of five or more time zones, travellers should take melatonin on the day of travel at the projected night time hour in the new time zone and on subsequent days in the new time zone. In the case of flights that cross seven to eight time zones, it may be beneficial to initiate melatonin one to three days before the intended day of travel in order to better acclimate the traveller to the new time zone [33-36, 162-165]. Melatonin should be administered in the mid-afternoon of the departure city (at approximately 3 p.m.) to mimic an approximate bedtime in the destination city (at approximately 9 p.m.). On the day of arrival, travellers should avoid evening light and should take melatonin at the new bedtime in the destination city. Circadian rhythms should advance by one to two hours each day with time zone changes, and melatonin can be taken one to two hours earlier each day until the traveller has adjusted.

2.5.4.1.1 Comparative studies

The impact of various dosage forms of melatonin and placebo on jet lag symptoms was evaluated by [redacted] in a double-blind, randomized trial [39]. The efficacy of melatonin was evaluated by electronic medication event monitoring system and questionnaires. The study showed that 5 mg melatonin significantly alleviated the jet lag syndrome, improved self-rated sleep quality, shortened sleep latency and reduced fatigue. Additionally, melatonin proved more effective than a slow-release formulation (2 mg controlled release formulation). Lower (0.5 mg) physiological doses were almost as effective as pharmacological doses (5.0 mg). Only the hypnotic properties, such as sleep latency, were significantly greater with melatonin 5.0 mg. A dose-effect relationship with melatonin has also been demonstrated in artificially induced acrophase shift, in a double-blind placebo-controlled crossover study. While single 5 mg doses of exogenous melatonin administered in the late afternoon induced an advance of the melatonin acrophase of approximately 1.5 hour, melatonin doses of 0.5 mg and 0.05 mg resulted in advances of less than 1 hour [158]. Melatonin treatment induced acute, dose-dependent temperature suppression and decrements in alertness and performance efficiency. These observations suggest that pharmacological doses of melatonin (5 mg) induce larger advances of the endogenous melatonin rhythm and therefore faster resynchronization of the sleep-wake cycle than physiological doses (10.5 mg). A significant dose-response relationship existed between the dose of oral melatonin, the magnitude of temperature suppression and the degree of advance phase shift in the endogenous melatonin and temperature rhythms, suggesting that acute changes in body temperature by melatonin may be a primary event in phase-shifting mechanisms. Furthermore, the bioavailability of melatonin, which fluctuates between 10 % and 56 %, is very poor, and at lower doses, sufficiently high blood levels of melatonin may not be achieved, [redacted]

[redacted] studied the effects of slow-release caffeine (SRC) and melatonin (Mlt) on sleep and daytime sleepiness after a seven-time zone eastbound flight. In a double-blind, randomized, 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 Mlt on pre-flight D-1 (1700), flight day D0 (1600), and from D1 to D3 (2300), or placebo (Pbo) 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 Pbo and Mlt and a significant decrease in rapid eye movement sleep on N1 (Pbo) and N1–N3 (Mlt). Sleepiness was objectively increased under Pbo (D1 – D6) and Mlt (D1 – D3). SRC reduced sleepiness but also tended to affect sleep quality until the last drug day [33].

[redacted] examined the effects of low doses of melatonin (0.1 – 10 mg orally) or placebo on sleep latency and duration, mood, performance, oral temperature and changes in serum melatonin levels in healthy volunteers. Administration of a small melatonin dose (0.1 - 0.3 mg, p.o.) during the daytime, which raises serum melatonin concentrations to within the normal nocturnal range, or of slightly higher doses (1.0 - 10 mg, p.o.) was shown to cause hypnotic effects relative to placebo. These effects include a decrease in objective and self-estimated sleep-onset latency, an increase in sleep duration, and sleepiness upon waking. Self-reported feelings of sleepiness and fatigue were increased and feelings of vigour diminished. Oral temperature and the number of correct responses on the Wilkinson auditory vigilance task were found to decrease significantly after ingestion of 1.0 and 10 mg of melatonin. These results are similar to those reported after ingestion of benzodiazepines and suggest that melatonin may find use as a hypnotic drug [41]. These data are consistent with other observations, with reported decrease in daytime latency of sleep onset in subjects given 1.7 mg of melatonin and increased feelings of sleepiness after 9 hours of melatonin administration.

2.5 Clinical overview

The efficacy of oral melatonin in alleviating jet lag in flight crew after a series of international flight has been investigated [redacted]. A double-blind placebo-controlled trial resulted in reduced feelings of let lag and a more rapid recovery of sleep and energy levels. The timing of melatonin dose seems also crucial. In aircrew returning from a duty that includes a large number of time-zone changes over 1 week or more, melatonin taken a few days prior to returning home results in a worse adjustment. One explanation for this finding is that it may be caused by the natural circadian rhythm being so disrupted at this end of the duty that melatonin started before arrival does not re-entrain unless it is taken in the context of a stable day-night cycle. Another possible explanation comes from recent work that suggests melatonin shifts circadian rhythms according to a phase-response curve. In another study [redacted] subjects taking melatonin reported less jet lag and took less time to recover from their shift across 12 time zones [36]. Subjects reported also that they were less tired during the day and required less time to establish a normal sleeping pattern and reach their normal level of energy. The lack of adverse side effects in subjects taking melatonin suggests that it is well tolerated at the dose used.

In another double-blind cross-over study, melatonin (2 mg) or placebo, was administered daily for 4 weeks to 12 volunteers [166]. The most consistent effect observed was an increase in tiredness during the evening when taking melatonin. The first recorded episode of excessive sleepiness occurred 4 days after beginning treatment. In the group as a whole, evening tiredness (19.00 h) was first significantly different from placebo ratings on day 5 after beginning treatment. A circadian covariation of fatigue and urinary melatonin from which they infer that their results are compatible with melatonin being involved in the regulation of the human sleep-wake cycle. Melatonin treatment was generally well tolerated, albeit with a few reports of poor mood in the early evening. The dose given, while small, must nevertheless be considered as pharmacological. The same group determined the plasma concentrations of melatonin in healthy subjects after ingestion of three different oral preparations [49] (a dose of 2 mg was given as either a gelatine capsule, a solution or as a slow-release tablet).

[redacted] examined melatonin's ability to transduce light-dark information, its hypnotic effects in man and its low toxicity in a double blind study [167]. Subjects took a daily dose of melatonin (5 mg in gelatine lactose) or placebo. Subjects were asked to rate their jet lag on a 10 cm visual analogue scale from 0 (insignificant) to 100 (very bad). Jet lag was deliberately not defined as its nature and severity vary from person to person but it was considered to be present at scores of 50 or above. Fisher's exact test for small sample sizes indicated that jet lag was significantly less severe among subjects treated with melatonin. In another study of the same group, it has been reported that in sensitive individuals melatonin can induce rapid drowsiness after late afternoon ingestion and hence detection of treatment [31]. Most subjects reported no significant jet lag. The rate of resynchronization of aMT6s rhythms was consistent with that previously reported for melatonin [168].

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. Melatonin (5 mg/ day^{-1}) or placebo capsules were administered to 14 experimental and 17 control subjects, respectively, in a double-blind study; the time of administration was in accord with the current consensus for maximizing its hypnotic effect [35]. The greatest amount of adjustment occurred in the first 3 days. There was also a significant time-of-day effect, jet-lag being higher in the afternoon and evening than in the morning and at noon. The authors hypothesized that melatonin works only in those individuals in whom fatigue is high and motivation is low; in the current study, all subjects were motivated to be active in the new environment, and many were determined to `throw off' any negative effects due to sleep loss, for example.

On the other hand, a new rating scale for measuring severity of jet lag was validated [redacted] in a randomized, 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 [38]. 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.

The effects of 5 mg melatonin in comparison with placebo when administered at 12:00, 17:00, 19:00 and 21:00 hours were investigated by [redacted] in double-blind study. Eighteen young adults were studied with the 7/13 ultrashort sleep-wake paradigm after an overnight sleep deprivation. After each administration, melatonin significantly increased sleep propensity, the spectral power in the theta, delta and spindles bands, and subjective sleepiness. It significantly decreased the power in the alpha and beta bands and oral temperature. The latency to maximum effect varied linearly from 3 hours 40 minutes at 12:00 hours to 1 hour at 21:00 hours. These findings indicate that melatonin possesses a time-dependent hypnotic effect. It is possible that the nocturnal rise in melatonin production initiates a cascade of events leading to activation of somnogenic structures, or perhaps that one of the melatonin metabolites possesses hypnotic effects. This hypothesis is further supported by additional findings, showing diminished levels of endogenous melatonin in elderly insomniacs compared to normal elderly who did not suffer from sleep disorders [170]. These findings also raise the possibility that precisely timed exogenous melatonin may be beneficial in some forms of insomnia. In the study of Haimov et al, there was a correlation between disturbances of rhythm of 6-sulphatoxymelatonin excretion and poor sleep quality in elderly subjects and they concluded that melatonin deficiency seems to be a key variable in the incidence of sleep disorders in elderly people and melatonin replacement therapy may prove beneficial [170].

In another study, the combined use of slow-release caffeine and melatonin improved several jet lag symptoms during an eastbound flight. For travel of 11 - 13 hours, whether eastbound or westbound, available data from limited field studies indicate that a combination of melatonin, exposure to outdoor light, and exercise have a potent ameliorative effect on jet lag symptoms [171].

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 [redacted]. 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.

In another study, 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 [173]. 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 [18]. The mean resynchronization rate was 2.27 ± 1.1 days during the eastbound flight and 2.54 ± 1.3 days for the westbound flight. These findings compared favourably to the expected minimal resynchronization rate after 13 hours of flight without any treatment, thus supporting the conclusion that jet lag symptoms can be significantly reduced by the carefully timed application of melatonin, light exposure, and physical activity.

In two studies included in the meta-analysis [redacted], 10 mg of melatonin maintained sleep duration of 7 – 8 hours at the destination (during a training mission involving rapid deployment to the Middle East and night operations) compared to 5 – 7 hours in placebo group [redacted] melatonin 5 mg at bedtime for 7 consecutive days after westbound flight produced significant effect on sleepiness [-14.00 (-29.08, 1.08) Mean difference (95 % CI)] in 36 volunteers.

[redacted] focused on the effects of air travel sleep deprivation and jet lag disorder. To analyse the results of the study, a phase response curve was generated for all patients. Results indicated that if 3 mg of exogenous melatonin is taken just prior to usual bedtime as a sleep aid, minimal phase advances occur (< 0.5 hours in 3 days) [174]. They demonstrated that exogenous melatonin can phase delay as well as phase advance the human circadian clock and shows the optimal time to administer the dosage form to achieve a desired phase shift. It also demonstrates that using exogenous melatonin as a sleep aid at night, has minimal phase shifting effects, but that taking it near the end of the sleep episode may inadvertently phase delay the circadian clock [175].

2.5.4.1.2 Open studies

The process of re-entrainment of circadian melatonin rhythm was investigated in six subjects by [redacted] Except during 24- h blood sampling, the subjects were exposed to natural zeitgeber (time giver) outdoors and given 3 mg melatonin at 23:00 h. The subjects were exposed to bright sunlight from 3000 to 120001x. 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-h eastward flight was assessed. Subjects were exposed to natural zeitgeber outdoors and took 3 mg of malatonin at 20:00 h 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 min per day and alleviated the jet lag symptoms [162].

2.5.4.1.3 Reviews and Meta-analyses

The Cochrane meta-analysis [redacted] found 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, by including ten trials that met the inclusion criteria.

2.5 Clinical overview

The results of meta-analysis are presented below in Figure 11, Figure 12 and Figure 13.

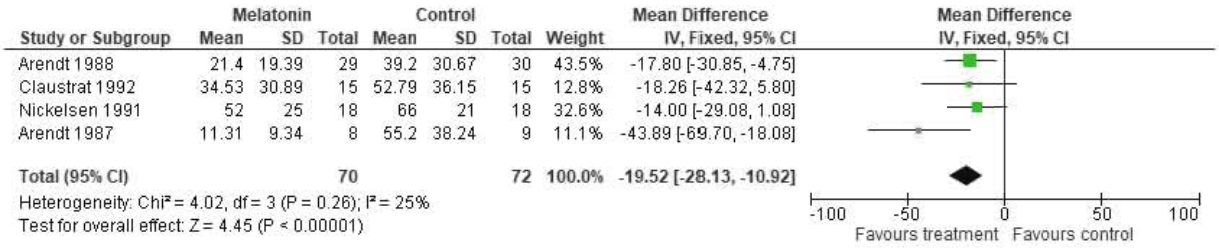

Figure 11 Comparison of Melatonin versus placebo – Outcome I for Global Jet Lag ratings: Eastward flights [10]

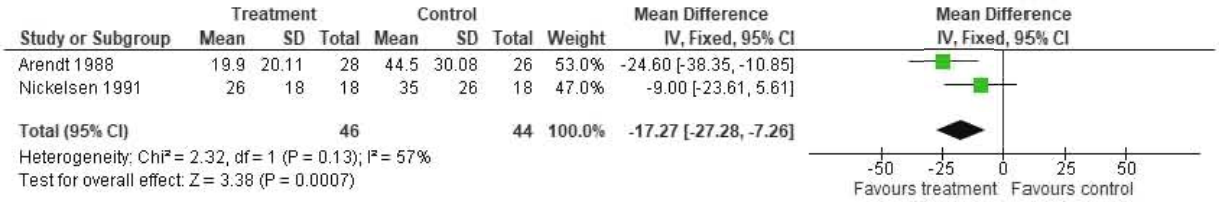

Figure 12 Comparison of Melatonin versus placebo – Outcome II for Jet Lag ratings: Westward flights [10]

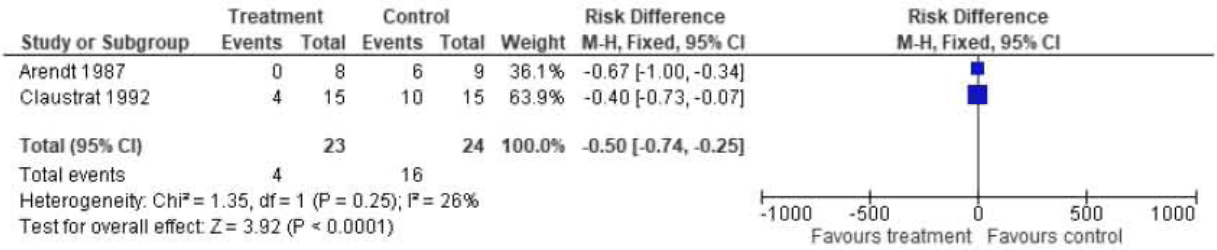

Figure 13 Comparison of Melatonin versus placebo: Eastward flights, proportion of people with jet lag score > 60 [10]

Table XI Outcomes from [redacted] Meta-analysis [177]. Moderate certainty: good indication of the likely effect

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: Meon Difference
Morgin of error: 95 % Confidence Intervol (CI)
Grode: Evidence grodes of the GRADE Working Group

**The risk Without melotonin is bosed on the risk in the control group of the triols. The risk With melotonin (ond its morgin of error) is colculoted from relotive effect (ond its morgin of error).*
[1]The certainty of the evidence wos lowered one level due to the risk of bias becouse most studies did not odequotely describe methods.

According to this meta-analysis, the daily doses of melatonin between 0.5 and 5 mg are similarly effective, except of people that fall asleep faster and sleep better after 5 mg than 0.5 mg. Doses above 5 mg appear to be no more effective. The relative ineffectiveness of 2 mg slow-release melatonin suggests that a short-lived higher peak concentration of melatonin works better. The benefit is likely to be greater the more time zones are crossed, and less for westward flights. The meta-analysis clearly demonstrates that Melatonin is remarkably effective in preventing or reducing jet lag, and occasional short-term use appears to be safe. It should be recommended to adult travellers flying across five or more time zones, particularly in an easterly direction, and especially if they have experienced jet lag on previous journeys. However, travellers crossing 2 - 4 time zones can also use it if need be. However, it should be noted that individuals differ greatly in the experience of jet-lag, with some travellers extremely affected while others who may have flown the same route may report no jet lag symptoms. This also suggests that individual differences may strongly influence the effectiveness of melatonin.

In the Cochrane review [redacted] it was concluded that melatonin is remarkably effective in preventing or reducing jet lag, and occasional short-term use appears to be safe. In most of the studies evaluated in this review, polysomnography records were evaluated, along with wrist actigraphy, visual analog scales and questionnaires [178]. The review demonstrated that melatonin decreases the symptoms of jet lag and accelerates the return of normal alertness and energy levels. However, it is recommended to adult travellers flying across five or more time zones, particularly in an easterly direction, and especially if they have experienced jet lag on previous journeys. Travellers crossing 2 - 4 time zones can also use it if need be [179].

[redacted] combined 11 randomized trials in order to evaluate the efficacy of melatonin in jet lag disorder. The authors followed the GRADE approach and concluded that the use of oral melatonin reduces the symptoms associated with jet lag syndrome [177]. This review did not conclude regarding the association of melatonin to adverse effects (nausea, tiredness, drowsiness and headaches), however no serious adverse effects were recorded in any of the clinical trials included in this review.

2.5 Clinical overview

The efficacy of exogenous Melatonin in managing secondary sleep disorders (jet lag and shift work) has been reviewed [redacted]. Sleep onset latency was the primary outcome in this meta-analysis. Pooled analysis of 7 studies (n = 154) demonstrate that exogenous administration of melatonin lowers Sleep Onset Latency (SOL) (Total mean difference: −2.48 min, 95 % CI: −4.56, −0.40) versus placebo (Figure 14).

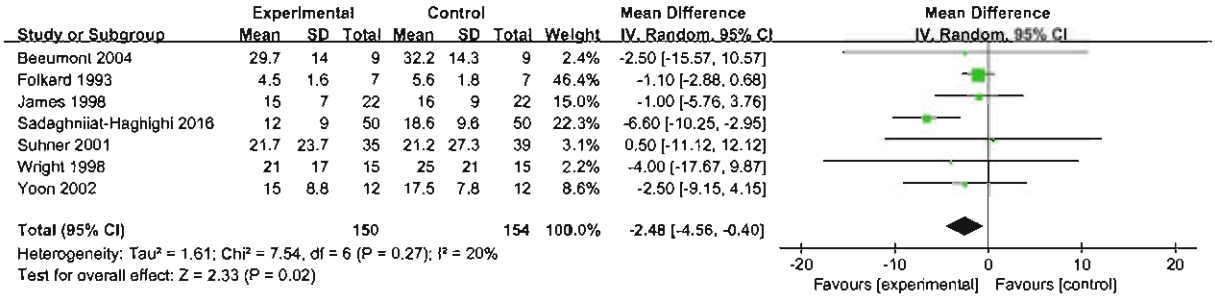

Figure 14 Effects of melatonin on SOL. This forest plot demonstrates that exogenous administration of melatonin lowers sleep onset latency [180].

Total sleep time was the secondary outcome in this meta-analysis and finally 3 studies reported the efficacy of melatonin in total sleep time. Exogenous administration of melatonin increased total sleep time (Total mean difference: 29.27 min, 95 % CI: 6.68, 51.86) (Figure 15).

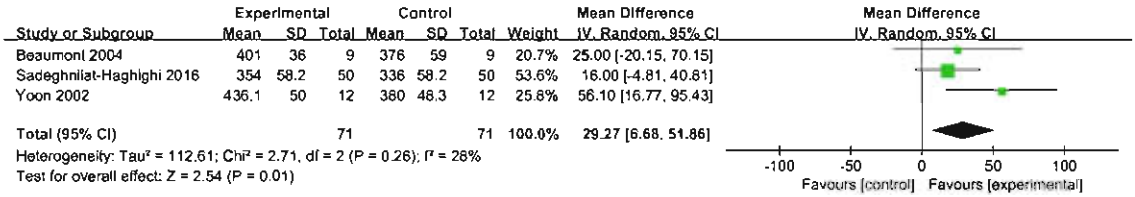

Figure 15 Effects of melatonin on TST. This forest plot suggests that exogenous administration of melatonin increases total sleep time [180].

Sleep efficiency was also the secondary outcome in this meta-analysis and 3 studies reported the efficacy of melatonin in sleep efficiency (SE). Analysis of the 3 studies (N=71) suggested that exogenous melatonin has no meaningful actions on sleep efficiency (Total mean difference: 1.46, 95 % CI: −0.43, 3.35) (Figure 16).

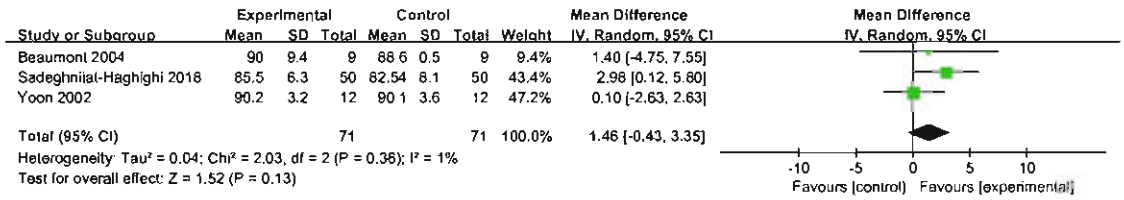

Figure 16 Effects of melatonin on SE [180].

The results of the meta-analysis demonstrated that exogenous melatonin lowers sleep onset latency and increases total sleep time, whereas it has little if any effect on sleep efficiency. The study of [redacted] clearly supports the use of melatonin as a management for patients with secondary sleep disorders [180].

2.5.4.2 OTHER USES

The actions of melatonin on the sleep-wake cycle in different disorders have been investigated and described below, in Sections 2.5.4.2.2, 2.5.4.2.3, 2.5.4.2.4, 2.5.4.2.5, 2.5.4.2.6 and 2.5.4.2.6.

2.5.4.2.1 Shift Work Disorder (SWD)

Shift work sleep disorder (SWD) is a common yet under recognized and undertreated sleep disorder caused by a sleep/wake pattern that is misaligned with the endogenous circadian rhythm [181], such as that which occurs when an individual works outside typical waking hours. Most commonly associated with night work, SWD can have potentially serious medical, social and economic consequences [182].

Shift work disorder is characterised by symptoms of insomnia and/or excessive sleepiness associated with the shift work schedule. It is diagnosed by history, but the use of sleep and work logs either alone or in combination with wrist actigraphy for either 2 weeks or for the duration of the shift rotation can be useful. Evening chronotype and sleep disturbance are both associated with depressive symptoms, and in shift workers sleep disturbance is prevalent [27].

The negative effects of shift work on alertness and sleeping can be classified as either work-directed, such as changes in the shift system, or worker-directed, such as pharmacological or non-pharmacological interventions [183]. Pharmacological interventions may help shift workers by either by reducing sleepiness and improving alertness during work-shifts, or by reducing sleep disturbances while off work. In this case, there are two categories of drugs, i.e. substances to help shift workers prevent drowsiness or improve alertness during shift work, or substances to improve sleep quality or sleep length after a shift work period.

During periods of night-shift work, the circadian pattern of melatonin secretion is advanced, resulting in maximum secretion during daylight and facilitation of daytime sleep. The altered pattern of secretion may result in a decrease in night-time sleep duration and an increase in number of daytime naps when the worker is not working nights. It has been suggested that melatonin use prior to retiring for night-time sleep may reset the pattern and facilitate sleep in individuals experiencing insomnia due to varying work schedules [184].

The following sections present the efficacy of Melatonin in shift work disorder by reducing the sleepiness and improving alertness in people who undertake shift works.

2.5.4.2.1.1 Comparative Studies

The efficacy of 3 mg melatonin taken 30 min before night time sleep on shift-workers with difficulty falling asleep was recently evaluated [redacted] Severity Index (ISI) were applied to find out shift workers with difficulty falling asleep. A randomized, double-blind, placebo-controlled crossover study with periods of 3 night and washouts of 2 weeks comparing melatonin with placebo was performed. Night time sleep parameters obtained from somnowatch including total sleep time (TST), sleep onset latency (SOL), sleep efficiency (SE) and wakening after sleep onset (WASO) were

analyzed. Among 295 workers, 103 had difficulty falling asleep. Finally, from 50 randomly selected workers with difficulty falling asleep, 39 workers completed the study. Melatonin treatment significantly increased SE and decreased SOL in comparison with baseline and taking placebo.The study showed that melatonin treatment significantly increased sleep efficiency and decreased sleep onset latency in comparison with baseline and taking placebo. Sleep efficiency was increased from 82.1 % at baseline to 85.5 % after melatonin therapy and sleep onset latency was decreased from 0.27 h at baseline to 0.20 h after melatonin therapy [185].

The effects of melatonin and bright light on adaption to night work were evaluated [redacted]. This was the first study (a randomized placebo-controlled crossover study) comparing the effects of melatonin (3 mg of melatonin, 1 hour before bedtime), with respect to subjective and objective measures of sleepiness and sleep in relation to night work among real shift workers. Melatonin gave a significant reduction in sleepiness, when compared with the placebo. This reduction in sleepiness was present during the day shift, but not during the night shift. Melatonin gave significant lower values for irresistible sleepiness and fighting sleep when compared with the placebo during the day shift. Additionally melatonin gave significantly longer total sleep time than the placebo and sleep efficiency was significantly higher after the intake of melatonin when compared with after exposure to bright light, whereas there were no significant differences between the placebo and the bright light. The participants also scored low on anxiety and depression, according to the rating scale. Melatonin increased the sleep onset latency when compared with the effect of bright light and the placebo during night shift. The authors concluded that the effects of melatonin are depended on the dose and time of administration [160, 161].

In the study [redacted], Melatonin treatment (3 mg) improved attention, evidenced by fewer omission errors, but had no significant effect on measures of sleep or mood, particularly fatigue. A beneficial effect of melatonin in attention may occur independently of improved sleep or decreased fatigue [187]. However, melatonin did not improve sleep duration, vigour or fatigue. The authors explained the variability in responses and the inconsistency of results among other studies, due to pharmacogenetic characteristics, individual tolerance to shift work and a relationship of response to melatonin with individual tolerance to shift work, however in all cases, administered melatonin for 2 weeks was well tolerated.

Compared to placebo, and to no treatment, melatonin (5 mg) taken at the desired bedtime improved problems related to sleep and increased alertness during working hours, especially during the early morning. Melatonin resulted in an increase in rated sleep quality and in its duration relative to both baseline and placebo conditions. The increased sleep duration with melatonin resulted in a somewhat delayed sleep offset, but there was no evidence that melatonin affected either sleep onset times or sleep latency. Melatonin improved also sleep quality compared to baseline and placebo during the seven night sleeps following the night shift period, but without significant effects on onset, offset or duration [188].

In another double blinded, randomized crossover study the authors examined whether melatonin is effective in helping emergency medical services personnel who work rotating night shifts, reset their biological clock and minimize circadian rhythm disruption [189]. Melatonin was associated with significantly fewer interim awakenings during day sleep when compared with placebo. However, this benefit was not associated with any significant effects on sleep latency, duration, or efficiency. Although these scores represent a 12 % improvement in sleep quality from melatonin, this difference was not statistically significant and did not produce concomitant improvements in night shift

performance. The rapid metabolism of melatonin is probably responsible for the inability to detect a significant improvement in overall sleep duration or sleep efficiency. It was concluded that high-dose (80 to 240 mg orally) or repeated low-dose administration are required, in order to maintain effective serum concentrations of melatonin throughout daytime sleep.

In another randomized, double-blind, placebo-controlled crossover trial, it was investigated whether melatonin taken prior to attempted daytime sleep sessions will improve daytime sleep quality, night-time sleepiness, and mood state in Emergency Medicine (EM) Residents, changing from daytime to night-time work schedules [190]. Among the 19 volunteers studied, there was no difference in sleep efficiency (91.16 % vs 90.98 %, NS), sleep duration (379.6 min vs 342.7 min, NS), or sleep latency (7.59 min vs 6.80 min, NS), between melatonin and placebo, respectively. In addition, neither the POMS (Profile of Mood States) total mood disturbance (5.769 baseline vs 12.212 melatonin vs 5.585 placebo, NS) nor the SSS (1.8846 baseline vs 2.2571 melatonin vs 2.1282 placebo, NS) demonstrated a statistical difference in night-time mood and sleepiness between melatonin and placebo, when 1 mg of melatonin was administered. The small sample size magnifies the detrimental effects of non-compliance with either medication dosing or mood and sleep testing.

The effect of oral intake of 5 mg melatonin taken 30 minutes before night time sleep, on insomnia parameters (associated with night work-shift-work nurses with insomnia) as well as subjective sleep onset latency, number of awakenings and durations of sleep was examined also in a double-blind, randomized, placebo-controlled crossover study with 118 participants. There was evidence of an effect of melatonin treatment on Sleep Onset Latency (SOL). Specifically, the mean SOL for subjects being treated with melatonin was significantly lower than the mean SOL for subjects given placebo. Furthermore, means for both the subjects given melatonin and those given placebo were significantly different from the baseline mean [191].

The effects of administration of exogenous melatonin (10 mg sublingual melatonin) in 18 subjects, in a double-blind, placebo-controlled crossover trial were determined [redacted] Melatonin was administered each morning during one string of nights and the other substance during another string of nights of equal duration. As measured on the basis of SSS (Stanford Sleepiness Scale), melatonin improved alertness at the end of a night shift over placebo (median difference, .5; 95 % CI, .04 to 1.0). They noted no difference between melatonin and placebo in alertness at the beginning of a night shift (median, 0; 95 % CI, –.4 to .9) or at the midpoint of a night shift (median, 0; 95 % CI, –.4 to .5). When the melatonin and placebo periods were combined, median SSS scores increased as the night shifts progressed from 2 just before a shift to 2.25 at the midpoint, to 4.75 just after a night shift.

The beneficial effects from exogenous melatonin in emergency physicians after intermittent night-shift duty were determined in the trial [redacted]. 5 mg of melatonin or placebo were administered to subjects and in the global assessment of recovery there was no difference between melatonin and placebo (60.4 ± 16.9 and 58.9 ± 14.5, respectively). There were no differences in sleep quality, duration or tiredness scores, sleep latency, hours of sleep obtained per night, and night or early awakening at any measurement point. Additionally, Profile of Mood States and neuropsychologic test performances were similar. On the other hand, Yoon et al administered melatonin or placebo on the second and third days of each treatment (6 mg melatonin) to twelve nightshift nurses. The SPTs (Sleep Period Time) and TSTs (Total Sleep Time) were significantly improved by melatonin administration. However, the improvement in nocturnal alertness was only marginal and attenuation of morning sunlight exposure had no effect on the findings [194]. This study indicates that the

beneficial effects of melatonin treatment are not increased by attenuation of morning sunlight exposure. This means that the masking sleep-promoting effect of melatonin is more influential than its phase-shifting effect in improving nightshift adaptation. This explanation is supported by the lack of melatonin benefit on the 4th day, when the phase-shifting effect of melatonin is expected to be distinguishable from its masking effect.

[redacted] tested whether melatonin can facilitate phase shifts in a simulated night-work protocol. Subjects (n = 32) slept in the afternoons/evenings before night work (a 7-hour advance of the sleep schedule). They took melatonin (0.5 mg or 3.0 mg) or placebo before the first four of eight afternoon/evening sleep episodes at a time when melatonin has been shown to phase advance the circadian clock. Melatonin produced larger phase advances than placebo in the circadian rhythms of melatonin and temperature. Average phase advances (± SD) of the dim light melatonin onset were 1.7 ± 1.2 h (placebo), 3.0 ± 1.1 h (0.5 mg), and 3.9 ± 0.5 h (3.0 mg). A measure of circadian adaptation, shifting the temperature minimum enough to occur within afternoon/evening sleep, showed that only subjects given melatonin achieved this goal (73 % with 3.0 mg, 56 % with 0.5 mg, and 0 % with placebo) [195]. There is probably an upper limit to the size of phase shift that can be induced in 1 day; dose-dependent curves for phase shifting with light exhibit a plateau (35), and in studies with large abrupt advances of sleep/dark, the maximum phase advances achieved were never more than 2 h/d [196, 197]. The second study [redacted] utilized a placebo-controlled, double-blind, cross-over design. Subjects participated in two 6 -day laboratory sessions. Each session included one adaptation night, two baseline nights, two consecutive 8-hour night shifts followed by 8-hour daytime sleep episodes and one recovery night. Subjects took 1.8 mg sustained-release melatonin 0.5 hour before the two daytime sleep episodes during one session, and placebo before the daytime sleep episodes during the other session. Sleep was recorded using polysomnography. Sleepiness, performance, and mood during the night shifts were evaluated using the Multiple Sleep Latency Test (MSLT) and a computerized neurobehavioral testing battery. Melatonin prevented the decrease in sleep time during daytime sleep relative to baseline, but only on the first day of melatonin administration. Melatonin increased also sleep time more in subjects who demonstrated difficulty in sleeping during the day. Melatonin had no effect on alertness on the MSLT, or performance and mood during the night shift. There were no hangover effects from melatonin administration. These findings suggest that although melatonin can help night workers obtain more sleep during the day, they are still likely to face difficulties working at night because of circadian rhythm misalignment.

In the latest randomized clinical trial [redacted] 3 mg of Melatonin were administered to emergency medicine residents who undertake shift work [199]. The study consisted of four phases within a month with intervention periods of two nights and washouts of six days. Shift workers had nine-hour shifts on 6 consecutive days. 3 mg of Melatonin or placebo were administered at the end of shift's cycle for 2 consecutive nights. Daytime sleepiness was calculated by KSS and mood status by POMS. The authors observed that in the melatonin group, night awakening on the first night was less than the placebo group (p=0.020), daytime drowsiness decreased by taking the second dose of melatonin (p = 0.021) (calculated by KSS score) and melatonin could significantly reduce this rate on the second night (p = 0.003) within its group [199].

2.5.4.2.1.2 *Reviews and Meta-analyses*

[redacted] performed recently a meta-analysis to evaluate the effects of pharmacological interventions to reduce sleepiness or to improve alertness at work and decrease sleep disturbances whilst off work, or both, in workers undertaking shift work in their present job and to assess their cost-

effectiveness. Sleep time (next day and next night [Outcomes 1 &2]), sleep onset latency (next day and next night [Outcomes 3 & 4]), sleep quality (assessed by Visual Analog Scale), alertness during the night shift work (assessed by Visual Analog Scale) and sleepiness during the night shift work (assessed by KSS-Karolinska Sleepiness Scale) were assessed (Figure 17, Figure 18, Figure 19).

Table XII Effect of Melatonin o Shift Work Disorder [200]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
Total sleep time, next day	7	263	Mean Difference (IV, Fixed, 95 % CI)	24.34 [9.82, 38.86]
Diary-based sleep time	6	225	Mean Difference (IV, Fixed, 95 % CI)	23.49 [8.49, 38.49]
Actigraphy based sleep time	1	38	Mean Difference (IV, Fixed, 95 % CI)	37.0 [-20.87, 94.87]
Total sleep time, next night	3	234	Mean Difference (IV, Fixed, 95 % CI)	16.97 [3.71, 30.22]
Diary-based sleep time	2	202	Mean Difference (IV, Fixed, 95 % CI)	19.05 [4.47, 33.63]
Actigraphy-based sleep time	1	32	Mean Difference (IV, Fixed, 95 % CI)	7.0 [-24.88, 38.88]
Sleep onset latency, next day	5	148	Mean Difference (IV, Random, 95 % CI)	0.15 [-2.18, 2.48]
Diary-based sleep onset latency	4	118	Mean Difference (IV, Random, 95 % CI)	0.80 [-1.15, 2.75]
Actigraphy-based sleep onset latency	1	30	Mean Difference (IV, Random, 95 % CI)	-9.0 [-18.60, 0.60]
Sleep onset latency, next night	3	-	Mean Difference (IV, Random, 95 % CI)	Totals not selected
Diary-based sleep onset latency, next night	2	-	Mean Difference (IV, Random, 95 % CI)	0.0 [0.0, 0.0]
Actigraphy-based sleep onset latency, next night	1	-	Mean Difference (IV, Random, 95 % CI)	0.0 [0.0, 0.0]
Sleep quality (visual analog scale)	4	291	Std. Mean Difference (IV, Fixed, 95 % CI)	0.08 [-0.15, 0.31]
Alertness during the night shift work (VAS)	1	-	Mean Difference (IV, Fixed, 95 % CI)	Totals not selected
Sleepiness during the night shift work (KSS)	1	-	Mean Difference (IV, Fixed, 95 % CI)	Totals not selected
Sleepiness during the day shift work (KSS)	1		Mean Difference (IV, Fixed, 95 % CI)	Totals not selected

2.5 Clinical overview

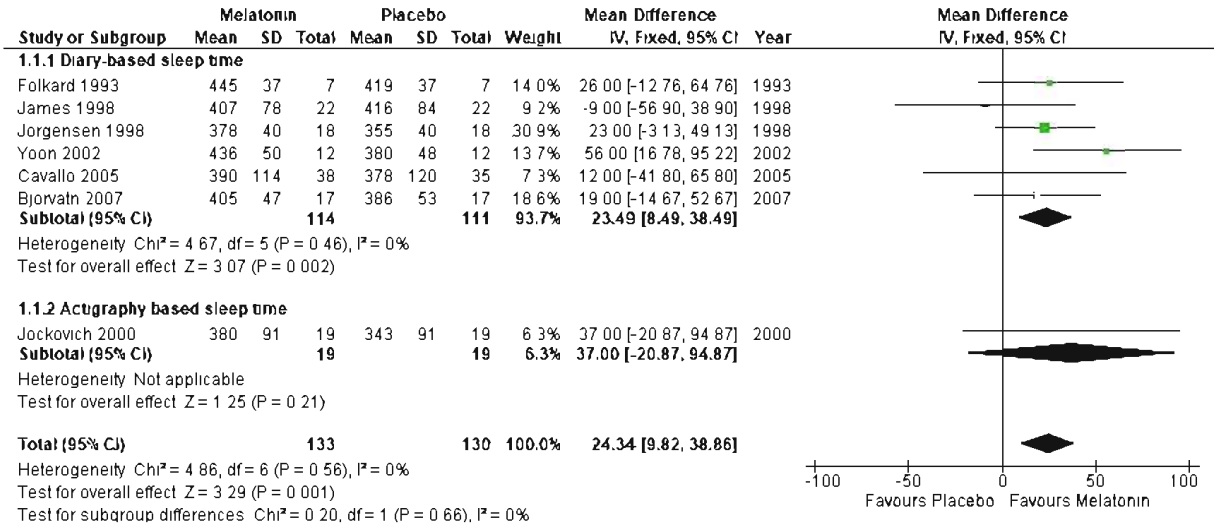

Figure 17 Melatonin versus placebo (Outcome I) [200] for sleepiness and sleep disturbances caused by shift work (next day)

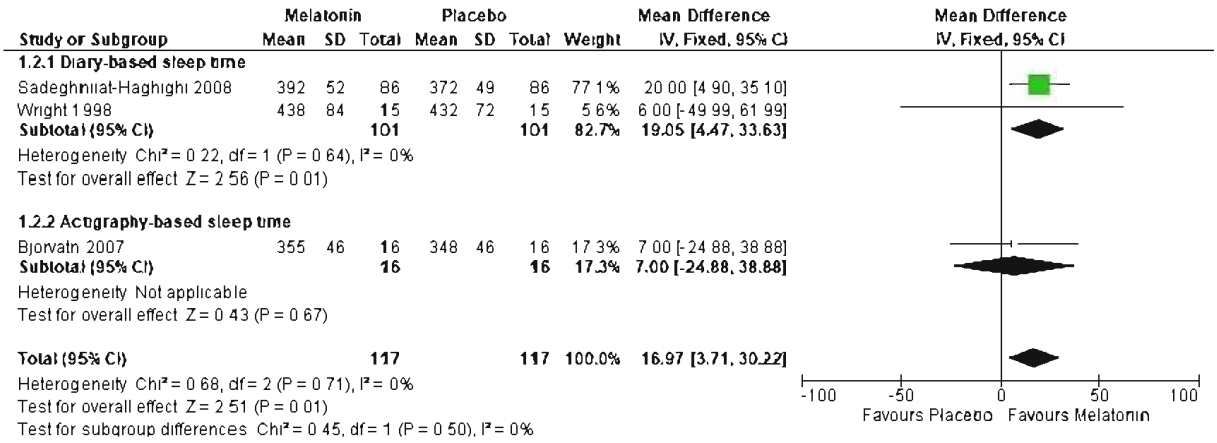

Figure 18 Melatonin versus placebo (Outcome II) [200] for sleepiness and sleep disturbances caused by shift work (next night)

2.5 Clinical overview

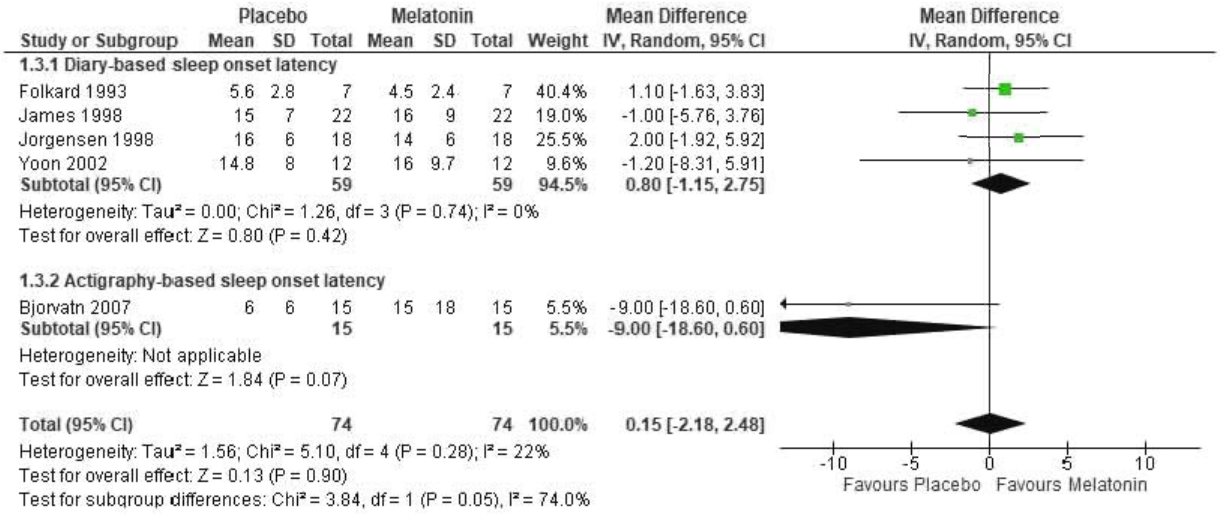

Figure 19 Melatonin versus placebo, Outcome III. Sleep onset latency, next day [200]

The results showed that melatonin (1 to 10 mg) after the night shift may increase sleep length during daytime sleep (mean difference (MD) 24 minutes, 95 % confidence interval (CI) 9.8 to 38.9; seven trials, 263 participants) and night-time sleep (MD 17 minutes, 95 % CI 3.71 to 30.22; three trials, 234 participants) compared to placebo. Outcome measures in the studies included in this meta-analysis were KSS (Karolinska Sleepiness Scale), MSLT (Multiple Sleep Latency Test) and psychomotor tasks. The authors did not find a dose-response effect.

As described also in Section 2.5.4.1.3 of the clinical overview, the recent meta-analysis of [redacted] demonstrated that exogenous melatonin lowers sleep onset latency and increases total sleep time, whereas it has little if any effect on sleep efficiency. This study clearly supports the use of melatonin as a management for patients with secondary sleep disorders [180].

2.5.4.2.1.3 *Open Studies*

There is only one open study available in the public domain [redacted] A total of 1533 nurses participated in a survey on shift work, sleep and health responded to questionnaires at baseline and about two years later at follow-up. The results of the study showed a significant reduction in the prevalence of SWD from baseline to follow-up, from 35.7 % to 28.6 %. Significant risks of having SWD at followed-up and the following variables measured at baseline; number of nights worked the last year (OR = 1.01, 95 % CI = 1.01 - 1.02), having SWD (OR = 5.19, 95 % CI = 3.74 - 7.20), composite score on the Epworth Sleepiness Scale (OR = 1.08, 95 % CI = 1.04 - 1.13), use of melatonin (OR = 4.20, 95 % CI = 1.33 - 13.33), use of bright light therapy (OR = 3.10, 95 % CI 1.14 - 8.39), and symptoms of depression measured by the Hospital Anxiety and Depression Scale (OR = 1.07, 95 % CI = 1.00 - 1.14) [202]. Additionally, leaving night work between baseline and follow-up was associated with a significant reduced risk of SWD at follow-up (OR = 0.12, 95 % CI = 0.07 – 0.22).

2.5.4.2.2 Advanced Sleep Wake Phase Disorder (ASWPD)

Advanced Sleep Phase Disorder (AWSPD) is characterized by a stable sleep schedule that is several hours earlier than the conventional or desired time [203]. The mechanisms leading to this condition are unknown, but hypotheses have usually been the opposite of those thought to underlie DSWPD. There are no systematic reports of melatonin administration for AWSPD, but consideration of the

melatonin PRC provides a rationale for low-dose administration after early morning awakenings and upon final arising in the morning [204].

2.5.4.2.3 Delayed Sleep-Wake Phase Disorder (DSWPD)

Delayed Sleep-Wake Phase Disorder (DSWPD) is a circadian rhythm sleep disorder characterized by abnormally late sleep and wake time [203]. It occurs mainly in young individuals and accounts for 10 % of chronic sleep disorders. The disorder is often misdiagnosed as sleep-onset insomnia [154].

The most common treatment approaches for DSWPD are based on the administration of bright light and/or exogenous melatonin with or without adjunct behavioural instructions. [redacted] investigated the short- and long-term effects on sleep of a DSWPD treatment protocol involving administration of timed bright light and melatonin alongside gradual advancement of rise time in adolescents and young adults with DSWPD in a randomized controlled trial and an open label follow-up study [57]. The participants were randomized to receive treatment for two weeks in one of four treatment conditions: dim light and placebo capsules, bright light and placebo capsules, dim light and melatonin capsules or bright light and melatonin capsules. In a follow-up study, participants were re-randomized to either receive treatment with the combination of bright light and melatonin or no treatment in an open label trial for approximately three months. Light and capsules were administered alongside gradual advancement of rise times. The main end points were sleep as assessed by sleep diaries and actigraphy recordings and circadian phase as assessed by salivary Dim Light Melatonin Onset (DLMO). During the two-week intervention, the timing of sleep and DLMO was advanced in all treatment conditions as seen by about 1 hour advance of bed time, 2 hours advance of rise time and 2 hours advance of DLMO in all four groups. Sleep duration was reduced with approximately 1 hour. At three-month follow-up, only the treatment group had maintained an advanced sleep phase. Sleep duration had returned to baseline levels in both groups. In conclusion, gradual advancement of rise time produced a phase advance during the two-week intervention, irrespective of treatment condition. Termination of treatment caused relapse into delayed sleep times, whereas long-term treatment with bright light and melatonin (three months) allowed maintenance of the advanced sleep phase.

The actions of melatonin on the sleep-wake cycle were investigated by means of a randomised, double-blind, placebo-controlled trial in 8 subjects with a delayed sleep phase syndrome attending a sleep disorders clinic [205]. Drug or placebo was given at 2200 hour, 5 hours before the mean time of sleep onset determined by pre-trial sleep logs. In all 8 subjects sleep onset time [mean advance 82 (range 19 – 124) min; $p < 0.01$] and wake time [117 (10 – 187) min; $p < 0.01$] were significantly earlier during melatonin treatment than during placebo. Mean total sleep time was slightly less on melatonin (8 h &12 min) than on placebo (8 h & 46 min). Alertness acrophase calculated from the subjects' ratings of alertness made every 2 h while awake was unaltered. Melatonin may act as a phase-setter for sleep-wake cycles in subjects with a delayed sleep phase syndrome.

The influence of 5 mg of melatonin on vigilance and cognitive processing speed in DSWPD patients was investigated [redacted] in a randomized controlled-placebo trial. Melatonin was administered 5 hours before the endogenous melatonin starts to rise in dim light (DLMO) and improved vigilance and cognitive processing speed in DSWPD patients. These observed effects might not completely within 10 days after stopping melatonin treatment.

[redacted] compared health-related quality of life of DSWPD patients with a random Dutch sample and four samples of patients with other chronic conditions [207]. The effectiveness of treatment with 5 mg of melatonin on the quality of life of DSWPD patients was also investigated. MOS SF-36 scales

scores (Medical Outcome Study Short Form -36) were significantly lower in DSWPD patients relative to age- and gender- adjusted norms for the Dutch sample. Melatonin treatment improved all scales, except the scale "role due to emotional problems".

In the randomized, double-blind, placebo-controlled crossover trial [redacted] the effects of exogenous melatonin (5 mg daily for 4 weeks) on sleep, daytime sleepiness, fatigue, and alertness were investigated in 22 patients with delayed sleep phase syndrome whose nocturnal sleep was restricted to the interval from 24:00 to 08:00 hours. In the 20 patients who completed the study, sleep onset latency was significantly reduced while subjects were taking melatonin as compared with both placebo and baseline. There was no evidence that melatonin altered total sleep time (as compared with baseline total sleep time), but there was a significant decrease in total sleep time while patients were taking placebo. Melatonin did not result in altered scores on subjective measures of sleepiness, fatigue, and alertness, which were administered at different times of the day. After an imposed conventional sleep period (from 24:00 to 08:00), subjects taking melatonin reported being less sleepy and fatigued than they did while taking placebo [208].

The effectiveness of melatonin to advance the timing of sleep and circadian phase in individuals with DSWPD was tested [redacted] Two doses of melatonin were tested: 0.3 mg and 3 mg and the administration of melatonin was double-blinded. Both doses of melatonin advanced the circadian phase of endogenous melatonin. The magnitude of phase advance in dim-light melatonin onset correlated strongly with the time of melatonin administration, with earlier times being more effective ($r2 = 0.94$, $P < 0.0001$). Administration of placebo had no effect on the timing of DLMO, whereas melatonin (0.3 and 3.0 mg) advanced DLMO and T_{min}. For the melatonin treatment group as a whole, 4 weeks of melatonin administration induced robust advances in DLMO and T_{min}. With this small sample size, no evidence of a difference in the ability of the 2 doses, 0.3 and 3.0 mg of melatonin, to phase advance DLMO when administered over the course of 4 weeks was obtained and this is consistent with the finding that low doses of melatonin can effectively entrain circadian rhythms in the blind [134, 210]. On average, subjects fell asleep 27 minutes earlier and woke up 47 minutes earlier than before treatment. The 47-minute advance in sleep offset, though not reaching statistical significance, could be clinically relevant for patients with difficulty in meeting work and school schedules Similar, though weaker, relationships were obtained between the timing of melatonin administration and changes in sleep time.

A meta-analysis of data of randomized controlled trials involving individuals with Delayed Sleep-Wake Phase Disorder that were published, compared melatonin with placebo, and reported one or more of the following: endogenous melatonin onset, clock hour of sleep onset, wake-up time, sleep-onset latency, and total sleep time. The 5 trials including 91 adults and 4 trials including 226 children showed that melatonin treatment advanced mean endogenous melatonin onset by 1.18 hours [95 % confidence interval (CI): 0.89 - 1.48 h] and clock hour of sleep onset by 0.67 hours (95 % CI: 0.45 - 0.89 h). Melatonin decreased sleep-onset latency by 23.27 minutes (95 % CI: 4.83 - 41.72 min). The wake-up time and total sleep time did not change significantly [211].

[redacted] administered 5 mg of melatonin, based on the favourable results of [redacted] who treated successfully more than 100 mentally handicapped children with 2.5 mg to 10 mg melatonin. This dose is much higher than the 0.3 mg that [redacted] used in the treatment of 13 children with Angelman's syndrome. [redacted] showed that melatonin maximally advances circadian rhythms when administered 5 hours before melatonin onset. Five hours before melatonin onset should have meant that several children should have to take the melatonin at 4 PM. In a pilot

study, we found that most children who received melatonin at that time became intolerably sleepy within 30 to 60 minutes after melatonin intake 2003 [214].

2.5.4.2.4 Non-24-h Sleep Wake Rhythm Disorder (N24SWD) of Free Running Disorder (FRD)

Normal (unaffected) subjects who are maintained in an inpatient research environment devoid of time cues eventually develop free-running rhythms [203]. The earliest studies of human subjects in time–free environments concluded that most people have an intrinsic circadian period much longer than 24 hours, averaging about 24.5 hours; however, more recent studies using the forced de-synchrony protocol have found the average to be significantly shorter; i.e., 24.15 hours [215]. In either case, the human circadian period is usually longer than 24 hours. Patients with free-running rhythms have circadian cycles that mimic those of subjects in time-free environments, and thus are thought to reflect a failure of entrainment. The condition is very rare in normally sighted people, but quite common in the totally blind who have no access to the entraining effects of the light/dark cycle [216].

Because the condition is rare in sighted people, the data consist almost entirely of level 4, single case reports, [217-226], or studies with few subjects, [227, 228] although [redacted] recently reported an accumulated series of 57 patients. A high proportion (about 25 %) of sighted people with FRD have associated psychiatric disorders [222]. A similar proportion of patients have a prodromal history of DSWPD [217].

Four level 4 case reports of successful treatment of sighted FRD with melatonin administered around the hour of the desired bedtime, when it would be predicted to cause a phase advance, are available [219, 224, 229, 230]. The most common dose was 3 mg and the duration of treatment ranged from one month to six years. In one study, [224] the treatment was interrupted for a double-blind, placebo-controlled dose escalation.

Following the demonstration of entrainment in animals with free-running rhythms [231], melatonin has been tested as a treatment in totally blind people. In addition to several positive case reports (level 4) [232-234], there have been two small single-blind, placebo-controlled melatonin treatment trials demonstrating successful entrainment of free-running rhythms in totally blind people (level 2). In one study [235], 3 of 7 subjects entrained to 5 mg of melatonin given for 35 - 71 days at 21:00. In the other study [134], 6 of 7 subjects entrained to 10 mg given at the usual bedtime for 3 to 9 weeks. In this study, three of the subjects were given a 10 mg dose that was gradually stepped-down every other week to 0.5 mg. Melatonin treatment on this step-down dosing schedule maintained entrainment, and free-running rhythms recurred after the cessation of treatment. Subsequently, these same subjects were successfully entrained with 0.5 mg de novo (level 4) [210]. The subject who failed to entrain in the initial trial to 10 mg was subsequently entrained with a 0.5 mg dose (level 4). The effectiveness of the lower dose was attributed to its selective activity on the advance zone of the melatonin phase response curve with no “spillover” to the delay zone. In another recent trial, the 0.5 mg dose entrained 6 of 10 subjects (level 2). In summary, the evidence is compelling that melatonin can entrain the majority of totally blind patients with FRD. Furthermore, a physiological dose (0.5 mg) appears to be as effective as a pharmacological dose (5 mg to 10 mg), and in some cases, more effective.

2.5.4.2.5 Irregular Sleep Wake Rhythm (ISWR)

ISWR is characterized by the relative absence of a circadian pattern to the sleep-wake cycle. Total sleep time may be comparatively normal, but instead of being consolidated into distinct bout or bouts, sleep times are shortened, and in extreme cases, almost randomly distributed throughout the day and night [203].

Melatonin has typically been used in studies seeking to improve sleep quality by increasing amplitude rather than phase shift sleep/wake rhythms.

[redacted] reported some success in treating sleep disturbances in children with presumed ISWR and severe psychomotor retardation. However, this study was a poorly controlled and employed a small sample size. [redacted] reported an incomplete, but nevertheless significant benefit in an open label trial of melatonin (2 to 20 mg) given at bedtime to neurologically multiply-disabled children with chronic sleep wake cycle disorders. A later report (level 4) [238] compared Controlled Release melatonin (CR) to Immediate Release (IR) (2 to 12 mg) in a similar population; the CR formulation was found to be superior to IR for sleep maintenance. A trial of melatonin which sought to improve sleep timing and quality in girls with Rett syndrome and associated mental retardation, was negative (level 2) [239].

[redacted] randomized forty-four participants with DSM-IV diagnosis of dementia (Diagnostic and Statistical Manual of Mental Disorders) and comorbid sleep disturbance to a seven-week double blind crossover trial of two weeks of slow release melatonin (6 mg) versus placebo. It should be noted that only 25 out of 44 patients completed the trial. Melatonin had no effect on actigraphically measured total time asleep, number of awakenings, or sleep efficiency. [redacted], in a large multi-centre trial, randomized [239] Alzheimer dementia patients with insomnia and daytime sleepiness to melatonin, 2.5 mg sustained-release; melatonin, 10 mg immediate-release, or placebo. The protocol consisted of 2 to 3 weeks of baseline measurement, 8 weeks of treatment, and 2 weeks placebo washout. Actigraphically monitored sleep was not significantly improved with either melatonin dose or placebo.

2.5.4.2.6 Sleep Onset Insomnia in children and adolescents with Attention Deficit Hyperactivity Disorder (ADHD)

In the open label study, the effect of a 3 mg dose of melatonin given in the evening, on insomnia, was investigated in 24 children with ADHD [242]. Immediately after the start of melatonin treatment the subjects fell asleep significantly earlier than before, varying between 15 min to 240 min, with a median value of 135 min in the first week of treatment. The long-term effect, after 3 months, was comparable with the immediate effect after 1 week. Also, a paired student t-test for the time of falling asleep before and after medication was significant ($t = 16.05$, $P < 0.01$). The time of falling asleep after melatonin had been given varied between 15 – 64 minutes. Immediate relapse of insomnia was reported twice when treatment with melatonin had been forgotten during the study period and twice after ending the study. Restarted use of melatonin restored the positive effect. Comparing the long-term effect to the immediate effect showed that melatonin remained effective for at least 3 months. No statistical difference could be shown in the short-term effect of melatonin between those patients who stopped recording data and those who did not ($P < 0.50$). The data indicate that melatonin has a significant effect on the time of falling asleep. Although the study design, a placebo effect and the change of sleeping ritual could have influenced the results, the highly significant difference strongly suggests a positive melatonin effect.

In the study of [redacted], the effect of melatonin treatment on sleep, behaviour, cognition and quality of life in children with ADHD and chronic onset insomnia was investigated [243]. Sleep onset advanced by 26.9 ± 47.8 minutes with melatonin and delayed by 10.5 ± 37.4 minutes with placebo ($p < 0.0001$). There was an advance in dim light melatonin onset of 44.4 ± 67.9 minutes in melatonin and a delay of 12.8 ± 60.0 minutes in placebo ($p < 0.0001$). Total time asleep increased with melatonin (19.8 ± 61.9 minutes) as compared to placebo (13.6 ± 50.6 minutes; $p = 0.01$). There was no significant effect on behaviour, cognition, and quality of life, and significant adverse events did not occur.

In a randomized double-blind, placebo-controlled study melatonin was evaluated for its efficacy on sleep hygiene (n = 27) in children with ADHD. Melatonin was found clinically and statistically significantly superior to placebo on actigraph measurement of Sleep Onset Latency in children [(SOL), $t (18) = -4.54$, $p < 0.01$)] in another trial [244]. Two-sample t tests of the period and crossover differences indicated a significant difference between the sleep latencies for the two treatments, $t (20) = -3.06$, $p < 0.01$ and a significant period effect, $t (20) = -2.20$, $p < 0.05$, respectively. The effect size of the difference between melatonin and placebo treatment was 0.6. Mean Somnolog SOL on placebo was 62.1 minutes (SD = 26.6) versus mean SOL on melatonin of 46.4 minutes (SD = 26.4). Two-sample t tests of the period and crossover differences indicated a significant difference between the sleep latencies for the two treatments, $t (20) = -3.06$, $p < 0.01$ and a significant period effect, $t (20) = - 2.20$, $p < 0.05$, respectively. SOL at the end of open label was 31 minutes (n = 17), which was not statistically significantly different from SOL during the randomized melatonin treatment. Sleep duration, however, continued to improve by 23 minutes [$t (12) = 3.90$, $p < 0.01$]. The effect size of the combined sleep hygiene and melatonin intervention from baseline to 90 days' post-trial was 1.7, with a mean decrease in initial insomnia of 60 minutes. Adverse events were generally mild and not different from those recorded with placebo treatment.

Melatonin was used in the study of [redacted] for the delayed sleep phase syndrome which is associated with ADHD in children. 5 mg of melatonin was administered to 27 children with sleep disorders. The total score of the RAND-GHRI (RAND General Health Rating Index) and FS-II (Functional Status) improved significantly more during melatonin treatment compared to placebo. The magnitude of change was much higher in the melatonin group than in the placebo group, with standardized response means for the RAND-GHRI of 0.69 versus 0.07 and for the FS-II of 1.61 versus 0.64. Melatonin treatment also significantly advanced sleep onset by 57 minutes, sleep offset by 9 minutes, and melatonin onset by 82 minutes, and decreased sleep latency by 17 minutes.

2.5.4.2.7 Effect of exogenous melatonin on sleep onset latency, sleep efficiency and total sleep duration

The effects of exogenous melatonin on sleep onset latency, sleep efficiency and sleep duration were evaluated [redacted]. The results of the meta-analysis are presented in Figure 20.

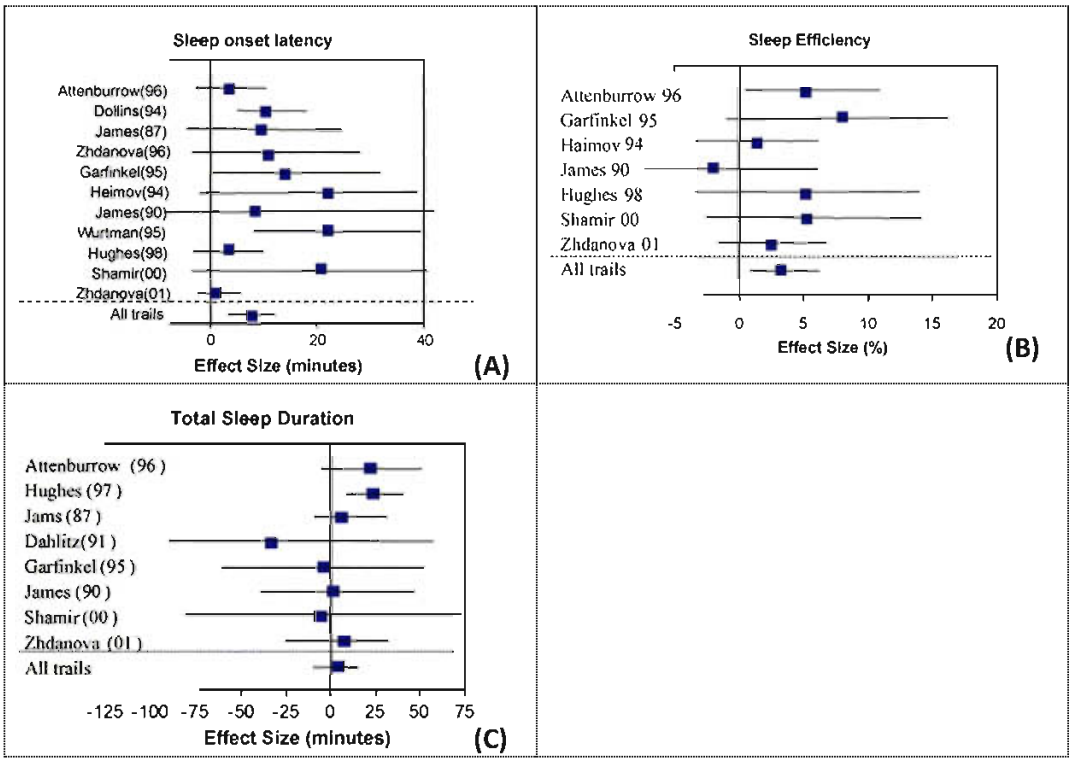

Figure 20 (A) Effects of exogenous melatonin on sleep latency. (B) Effects of exogenous melatonin on sleep efficiency. (C) Effects of exogenous melatonin on total sleep duration. Intervals are 95 % confidence intervals for the mean effect [246].

This meta-analysis supports the hypotheses that melatonin decreases sleep onset latency, increases sleep efficiency, and increases total sleep duration. In spite of the heterogeneity of the data, the present meta-analysis does lend statistical support to the notion that melatonin preparations can improve sleep quality with regard to sleep onset latency, sleep efficiency, and sleep duration.

2.5.4.3 OTHER STUDIES

The effects of exogenous melatonin on nocturnal sleep quantity in critically ill patients were evaluated [redacted] in a randomized double-blind placebo-controlled trial with 24 subjects who had undergone a tracheostomy to aid weaning from mechanical ventilation [50]. Melatonin 10 mg, formulated in an oral liquid and administered for four consecutive nights. Nocturnal sleep time was found 2.5 hours in the placebo group and was found 1 hour longer in the melatonin group, corresponding to an increase of 47 %, although the difference did not reach statistical significance. They obtained also a statistically significant reduction of 7 % in BIS (Bispectral Index) AUC with melatonin administration, suggesting sleep improvement.

In the double-blind placebo-controlled randomized pilot study [redacted], oral melatonin (3 mg) was administered to tracheostomised patients with nocturnal sleep. Although melatonin was well absorbed and this standard dose increased blood levels approximately 1000-fold, these high levels failed to increase observed nocturnal sleep or induce other observable benefits in tracheostomised ICU (Intensive Care Unit) patients [247]. In another study healthy volunteers were exposed to artificial insomnia participated in a double-blind, placebo controlled, parallel group design study. Melatonin

administered as a single oral pharmacological dose of 80 mg at bedtime has the properties of a real hypnotic substance. It affected sleep initiation and sleep maintenance by reducing the time awake before sleep onset, the sleep latency, and the number of awakenings during the total sleep period; in addition, it improved sleep efficiency. Moreover, sleep architecture was altered as sleep stage 1 decreased and sleep stage 2 increased, modifications repeatedly reported as typical for anxiolytic sedatives [248].

[redacted] examined how photic and non-photic time cues may be combined by the human circadian system by assessing the phase advancing effects of one evening dose of exogenous melatonin, alone and in combination with one session of morning bright light exposure, in a randomized placebo-controlled double-blind circadian protocol. The effects of four conditions, dim light (~1.9 lux, ~0.6 Watts/m^2)-placebo, dim light-melatonin (5 mg), bright light (~3000 lux, ~7 Watts/m^2)-placebo, and bright light-melatonin on circadian phase was assessed by the change in the salivary dim light melatonin onset (DLMO) prior to and following treatment under constant routine conditions. Melatonin or placebo was administered 5.75 hours prior to habitual bedtime and 3 hours of bright light exposure started 1 hour prior to habitual wake time. It was obtained that morning bright light combined with early evening exogenous melatonin induced a greater phase advance of the DLMO than either treatment alone. Bright light alone and melatonin alone induced similar phase advances [249].

Morning injections of melatonin did not affect sleep parameters in young adults; however, peripheral hand temperature was reported to increase. Administration of both melatonin and temazepam at 1400 hour resulted in decreased sleep-onset latency in young people and concomitant changes in peripheral skin temperature measured at the feet. In that study, sleep-onset latency was assessed using the multiple sleep latency test, a well-established method of assessing sleep propensity during wake-time. Compared with placebo, melatonin reduced sleep-onset latency by 4.8 ± 1.49 min and temazepam (a benzodiazepine used to treat insomnia) reduced sleep latency by 6.5 ± 1.62 min. In this crossover, randomized, placebo-controlled study, the degree of change of sleep-onset latency was similar between the benzodiazepine and melatonin [250]. In an older population, [redacted] no changes in polysomnographic sleep following short-term melatonin administration via a transbuccal patch [251]. Longer treatment periods in the elderly (oral) did result in actigraphically-recorded improvements in sleep parameters, such as sleep efficiency and wake after sleep onset [252]. Furthermore, no cognitive impairment was reported in association with treatment of healthy middle aged and elderly individuals with prolonged release melatonin on the day after treatment [253].

Another study in healthy young adults demonstrated that melatonin significantly improved sleep efficiency, particularly during the time of the so-called wake maintenance zone or forbidden zone for sleep [254]. Melatonin administered in the late afternoon just prior to an advanced and extended sleep opportunity (1600 – 0800 hours), significantly increased the total amount of sleep obtained in the period 1600 – 2400 h by 2 h (3.37 h in controls vs 5.37 h in the melatonin-treated group) [255]. Thus, melatonin facilitated sleep when the sleep opportunity occurred at a biologically inappropriate time. Supporting these findings, a study of the efficacy of melatonin in healthy volunteers maintained in a forced desynchrony protocol reported that melatonin improved sleep efficiency when it was administered when endogenous melatonin levels are low, but not when endogenous levels are high [256].

In the study [redacted] both light and melatonin were used in order to determine whether phase advances induced by morning intermittent bright light and a gradually advancing sleep schedule

could be increased with afternoon melatonin. There were significantly larger phase advances with 0.5 mg (2.5 hours, n = 16) and 3 mg melatonin (2.6 hours, n = 13), compared with placebo (1.7 hours, n = 15), but there was no difference between the two melatonin doses, although slightly larger phase shifts were obtained with the 3 mg dose.

Three different morning light patterns combined with a low dose of afternoon melatonin (0.5 mg) and a sleep/dark schedule that was advanced by 1 hour/day for 3 days have been also compared [257]. Despite different protocols, different melatonin doses, and different types of morning light among previous studies, the phase advancing effect of morning light alone and afternoon melatonin alone are roughly similar and when combined they are roughly additive. The authors tested these 3 different morning bright light patterns combined with a low dose of afternoon melatonin and a gradual advance of sleep/dark over three days to advance circadian rhythms. Such a strategy could be used to help night owls or people with DSPD, to help people adapt to an early work or school schedule, to help a shift worker who has to work early morning shifts, or help travellers to reduce or prevent jet lag. The 2-hour (30 minutes × 4) morning bright light exposure produced the largest phase advance; however, one 30-minute bright light exposure immediately upon waking each day is effective and takes a quarter of the time.

Melatonin capsules was administered to 21 students with sleep-onset insomnia, due to delay of circadian phase. Melatonin (1 mg) capsules or placebo was administered to subjects for three weeks. The study of Eckerberg et al showed that a small dose of melatonin, administered in the afternoon will result in a significant advanced sleep timing. The treatment was effective although the subjects continued with their often irregular sleep habits [258].

In a short-term pilot study, the sleep-promoting action of melatonin (3 mg p.o. for 6 months) was investigated in a small non-homogenous group of insomniac patients, under benzodiazepine treatment. Melatonin augmented significantly sleep quality, duration and decreased sleep latency and the number of awakening episodes. Overall, 20 out of 22 patients improved sleep at the end of treatment and estimates of next-day function. The observed effects lasted for the entire period examined [259].

The effectiveness of preoperative oral melatonin medication on sedation sleep quality was assessed [redacted]. patients received an oral placebo (n = 26) or 6 mg melatonin (n = 26) the night before and 1 hour before surgery and additionally all patients received a standard anaesthetic protocol. Extubation time and recovery time from anaesthesia were significantly longer in the melatonin group. Intraoperative fentanyl usage, pain scores and tramadol consumption were significantly better in the melatonin group than in the control group. The sedation scores were significantly higher in the melatonin group than in the control group at 1 hour and 2 hours after surgery. The authors concluded that patients had enhanced sleep quality during the postoperative period [260].

In a randomized, double-blind placebo-controlled study included 33 patients received either oral melatonin 5 mg (n = 17) or placebo (n = 16) the night before and 1 hour before surgery. The findings of the study suggested that preoperative melatonin produced clinically relevant anxiolytic and analgesic effects, especially in the first 24 postoperative hours. Melatonin also may accelerate the resynchronization of circadian rhythms in the postoperative period, suggesting better recovery quality which could be a consequence of melatonin's effects on pain and anxiety, which usually enhance rhythmicity disruption in stressful situations such as surgeries [261].

2.5.4.4 DOSAGE AND ADMINISTRATION

The recommended dose in adults is 3 mg (1 tablet) daily for a maximum of 5 days. The dose can be increased to 6 mg (2 tablets) if the standard dose does not adequately alleviate symptoms. The dose that adequately alleviates symptoms should be taken for the shortest period.

The first dose should be taken on arrival at destination at the habitual bed-time.

Due to the potential for incorrectly timed intake of melatonin to have no effect, or to cause an adverse effect, on re -synchronisation following jet-lag, Melatonin should not be taken before 20:00 hr or after 04:00 hr at destination.

The same posology is recommended by Melatonin Pharma Nord 3 mg film-coated tablets (Netherlands, 2018) and by the Finnish products Melatonin Vitabalans 3 mg & 5 mg tabletit and Melatonin Orion 3 mg kalvopäällysteiset tabletit, both approved in 2016.

The proposed dose has been used in a number of clinical trials, as presented in Sections 2.5.3 and 2.5.4. Although various doses of melatonin have been used in clinical practice, typically between 1 mg and 10 mg, a 3 mg dose is one of the most commonly used dose for the treatment of jet-lag and other circadian rhythm sleep disorders.

According to the literature, daily doses up to 300 mg of melatonin do not produce any clinically significant side effects.

The safety and efficacy of melatonin in children aged 0 to 18 years has not been established.

The metabolism of melatonin decreases with the progression of age. However, no dosing recommendation can be made for the elderly, due to the significant differences in the pharmacokinetics of melatonin between individuals, even between those of the same sex.

Although, the effect of any stage of renal impairment on melatonin pharmacokinetics has not been studied. Caution should be used when melatonin is administered to such patients. Melatonin is not recommended for those with severe renal impairment.

There is no experience of the use of melatonin in patients with liver impairment. Published data demonstrates markedly elevated endogenous melatonin levels during daytime hours, due to decreased clearance in patients with hepatic impairment. Therefore, melatonin is not recommended for use in patients with hepatic impairment.

2.5.5 OVERVIEW OF SAFETY

In this section, the potential adverse effects after Melatonin administration are based on literature data. Further data, regarding the safety outcomes of Melatonin and frequency of reported adverse reactions after oral administration of Melatonin are presented in Module 2.7.4. The most common adverse reactions after oral administration of Melatonin are: headache, hyperactivity, dizziness, drowsiness, abdominal pain, nausea and diarrhoea.

Single case reports of possible adverse effects of Melatonin outside clinical trials are also presented in Module 2.7.4.

2.5.5.1 TOXICITY

There is remarkably little published information regarding the potential adverse effects of melatonin administration. Melatonin appears to have a favourable adverse effect profile; headaches, dizziness, nausea, and drowsiness are the most common adverse events reported with short-term melatonin administration. Melatonin treatment appeared to be well tolerated in patients [30, 50]. This result did not change by dose, the presence or absence of a sleep disorder, type of sleep disorder, duration of treatment, gender, age, formulation of melatonin, use of concurrent medication, study design, quality score, and allocation concealment score [262]. Fatigue occurs when melatonin is administered in the morning at higher doses (> 50 mg) [263]. Indeed, most studies with melatonin point out that overall adverse effects of melatonin are insignificant and, in general, similar to those found with placebo [154]. No hangover effects have been observed with melatonin when administered at reasonable concentrations, partially as a consequence of its short half-life.

2.5.5.1.1 Acute Toxicity

Melatonin and analogues appear to have extremely low acute toxicity. In rats and mice, oral doses of melatonin in excess of 1000 mg/kg are needed to induce death; the estimated doses required to cause death in 50 % of the animals treated (LD_{50} values) are 1250 and 3200 mg/kg in mice and rats, respectively. 2-Iodomelatonin, which is at least 10-fold more potent than melatonin in affecting biological responses, caused death in a minority of animals even at the highest doses tested (800 mg/kg orally, 600 mg/kg by intraperitoneal injection) [264]. These doses are so far above the doses recommended for human consumption as to be nearly irrelevant (maximal intake in humans is approximately 5 mg/kg in women taking 300 mg/day). Acute administration of melatonin appears to have little consistent effect on hormone levels in adult humans. Several early studies indicated effects of melatonin on growth hormone secretion in men, whereas a more recent comprehensive study by [redacted] indicated that the only acute effect of melatonin was an elevation in prolactin levels, as determined in 24 young healthy males after oral administration of 240 mg melatonin. If melatonin administered in sufficiently high doses, may well also enhance GH (Growth Hormone) levels, whereas various other hormones are not influenced [266].

Melatonin has been also used for its hypnotic, anti-nociceptive and anticonvulsant properties as a hypnotic anaesthetic agent. [redacted] were the first to demonstrate clearly the orally administered 0.2 mg/kg melatonin produced loss of consciousness in human beings accompanied by a pattern of EEG activity similar to that seen during intravenous and volatile anaesthetic-induced loss of consciousness [267-269].

2.5.5.1.2 Long-term and Chronic Toxicity

The absence of detectable gross toxicity following several months of melatonin administration does not rule out significant adverse effects of the hormone. Some effects of melatonin administration may become apparent only after long latencies [262]. A relevant example is the development of osteoporosis, which occurs earlier, more frequently, and to a greater extent in women with premature removal of the ovaries (or premature menopause) than in controls.

Correlation between a developmental decline in melatonin levels with the timing of puberty in humans led to speculation that melatonin regulates the timing of puberty [270]. Subsequent investigation indicated that this developmental decline in melatonin levels is due at least in part to developmental changes in body mass (and thus volume of distribution) and is without a strict relationship to pubertal

development [271-273], and references therein). Although endogenous melatonin does not appear to play a role in timing human puberty, no data are available to draw a conclusion with respect to the effects of exogenous melatonin on puberty in humans. These data indicate that the amplitude of nocturnal melatonin secretion does not have a role in the regulation of reproductive events in menstrual primates.

Melatonin is used mostly for short term administration in cases of jet lag or shift work disorders. 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.

Chronic administration of melatonin also appears to be well tolerated. Women taking melatonin as a contraceptive agent ingest up to 300 mg melatonin daily; the initial report of this regimen indicated that no toxic effects were noted in the 4-month treatment period [82]. Alterations in hormone concentrations noted in this study are viewed as evidence of melatonin's efficacy rather than as an indication of toxicity. Other examples of chronic melatonin treatment at lower doses (e.g., 5 mg/day for hypnotic effect) have been reported without obvious evidence of adverse effects [272], however abnormally high (or pharmacologic) concentrations of melatonin in women are associated with altered ovarian function and anovulation.

2.5.5.1.3 Effect of administered melatonin on suppression of endogenous melatonin secretion

It has been considered that endogenous melatonin was not modified by administration of D7 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 [83]. [redacted] 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 [274].

[redacted] 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 [66].

Exogenous melatonin did not affect the production of endogenous melatonin in terms of secretion rate, amplitude and duration. [redacted] 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 h), or sleepiness and mood levels on the subsequent day [275].

2.5.5.1.4 Effect of Melatonin on Cardiovascular System

The effect of 2 mg Melatonin or placebo on the Heart Rate Variability (HRV) of 26 healthy men was evaluated [redacted]. 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 [276].

2.5.5.1.5 Pre-Implantation Development

Limited evidence suggests that the contraceptive efficacy of melatonin in humans is due to effects on release of hypothalamic hormones rather than on uterine mechanisms, although effects on uterine "readiness for implantation" cannot be discounted. The uterus certainly is another tissue that should be examined when assessing the chronic effects of melatonin treatment in humans [262].

2.5.5.1.6 Mutagenicity

Assessment of melatonin and melatonin analogues or its major metabolites using the Ames test indicates that melatonin, 6-hydroxymelatonin and 2-iodomelatonin are devoid of mutagenic activity [264, 277].

2.5.5.1.7 Pregnancy and Lactation

2.5.5.1.7.1 Fertility

Infertility treatments are associated with significant levels of reactive oxygen species which have the potential to negatively affect the quality of oocytes and embryos. Melatonin shows promise as an adjunctive therapy in the treatment of infertility. Its unique anti-oxidative characteristics and safety profile make it an ideal potential adjuvant therapy [278].

A recent phase II double blind placebo controlled randomised trial has shown that melatonin can help reduce chronic pelvic pain in women with endometriosis potentially through its effects on brain-derived neurotrophic factor and beneficial effects on sleep quality [279]. Level II evidence has also determined the effectiveness of melatonin as an analgesic in temporomandibular disorders [280] and as a method of reducing oxidative stress and improving dyspnoea in patients with chronic obstructive pulmonary disease [281]. Despite this, melatonin use in infertility treatment still lacks adequate evidence to recommend routine use.

2.5.5.1.7.2 Pregnancy

The role of melatonin in embryo foetal development has been recently reviewed by [redacted]. Chronodisruption leads to reproductive dysfunction and appears to be a key contributor to offspring diseases that develop in adult life (the concept of foetal programming). Melatonin decreases in conditions associated with serious outcome for the foetus and seems to be involved in preeclampsia and intrauterine growth restriction [105]. Melatonin treatment during human normal or abnormal

pregnancy has been studied for a large range of conditions and at different times during the gestational period. Considering the ethical issues, it is more difficult to study a normally occurring pregnancy, than an in vitro fertilization (IVF) one. Melatonin administration started prior to IVF-cycles, continued during pregnancy and was associated with improved pregnancy outcomes [283]. Melatonin receptors are widespread in the human foetus from early foetal development. In addition, it appears that the foetuses' sleep patterns develop in the late pregnancy, melatonin being the regulating factor. A normal sleep pattern is involved in the neurodevelopment and there is solid evidence that melatonin is involved in foetal neuroprotection [284]. Thus, the influence of melatonin on the developing human foetus may not be limited to entertaining the circadian rhythmicity.

Melatonin crosses the placenta and has been found to show rhythmic variations in milk, in parallel with plasma, in both humans and goats. Thus, it is highly likely, if taken by pregnant women, that the foetus will be exposed to excess melatonin, and the possibility exists that it will modify subsequent development in terms of the circadian system and the timing of puberty [285].

It has been suggested that exogenous melatonin increases GSH-Px activity in the chorion and thereby may protect indirectly against free radical injury and thus it could be useful in treating preeclampsia and possibly other clinical states involving excessive free radical production, such as intrauterine foetal growth retardation and foetal hypoxia [286].

[redacted] suggest that alterations in maternal or placental melatonin might alter foetal melatonin levels and thus gene expression in the foetal nervous system [287, 288].

2.5.5.1.7.3 *Lactation*

Some authors suggest that mothers should nurse in the dark at night in order to avoid reductions in the melatonin content of breastmilk, which could disturb infant sleep patterns [289]. Differentiating milk pumped during the day from milk pumped during darkness has also been suggested for women expressing milk for their infants [290]. Some studies have attributed a longer sleep time in breastfed infants than in formula-fed infants due to melatonin in breastmilk [291]. Another study found higher colostrum melatonin levels at night which appeared to increase the phagocytic activity of colostral cells against bacteria [292]. 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 [293]. 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 [294].

In studies in which exogenous oral melatonin was given to women, the resulting serum melatonin was variable, but peak serum concentrations ranged from 1.1 to 2.6 µg/L for each 1 mg administered [40, 48, 56]. This would result in an average increase in breastmilk melatonin concentration from 0.4 to 1 µg/L for each 1 mg administered to the mother, based on an average milk concentration of 35 % of the maternal serum concentration. While the resulting concentrations would be higher than the typical physiologic peak milk concentrations of 0.02 µg/L [295, 296], it would present a considerably lower dose to the infant than the 10 mg/kg dosages of melatonin that have been safely administered to neonates in clinical studies [289]. It seems unlikely that prenatal melatonin exposure will strongly influence reproductive development in humans [262]. However, it seems unlikely that prenatal melatonin exposure will strongly influence reproductive development in humans [262].

2.5.5.2 DRUG INTERACTIONS

2.5.5.2.1 Pharmacokinetic Drug Interactions

Caffeine has been shown to increase the oral bioavailability of melatonin, probably due to inhibition of CYP1A2-catalyzed first-pass metabolism of melatonin [72]. In addition, this effect was found to be more pronounced in non-smoking subjects and in subjects with the CYP1A2*1F/*1F genotype. In a study of five healthy male volunteers, 50 mg of oral fluvoxamine, a potent CYP1A2 inhibitor, was shown to substantially increase the bioavailability of oral melatonin with AUC and C_{max} values increasing 17 fold and 12 fold, respectively. Whereas the combined oral contraceptive resulted in a 4- to 5-fold increase in AUC and C_{max} compared to non-OC users (non-Oral Contraceptive users) [73, 297].

Cimetidine increases plasma concentration of melatonin (via CYP1A2) by inhibiting metabolism of melatonin [298] which increases the levels of plasma melatonin.

The effect of melatonin on metabolic side effects of olanzapine was evaluated in a randomized double-blind placebo-controlled trial of 48 patients with first episode schizophrenia who were eligible for olanzapine treatment [299]. Patients were randomly assigned to olanzapine plus either melatonin 3 mg/day or matched placebo and were followed for 8 weeks. Metabolic parameters including weight, waist circumference, triglyceride, cholesterol, insulin, and blood sugar were assessed at baseline, week 4, and week 8. The study found that melatonin was associated with significantly less weight gain, increase in waist circumference and triglyceride than the placebo. Changes in cholesterol, insulin, and blood sugar did not differ significantly between the two groups.

CYP1A2 inhibitors (e.g. Quinolones) may increase the melatonin exposure and melatonin inhibits the antibacterial activity of ciprofloxacin [300]. The study [redacted] showed that the antibacterial activity of ciprofloxacin was inhibited by the pre-treatment of bacteria with antioxidant agents such as melatonin. This is likely to be related to the interference with induction of ROS by ciprofloxacin.

Results of a clinical study demonstrate that 8-methoxypsoralen (8-MOP or 5) intake is followed by correlated changes in melatonin levels and an independent decrease in serum aMT6s levels, suggesting a competitive inhibition of hepatic melatonin metabolism [301].

Melatonin down-regulates the circulating levels of gonadal oestrogens and acts as an antioestrogen with mechanisms of action different to those of the commercially available antioestrogens and inhibits aromatase expression in human breast cancer cells. The metabolism by CYP1A1 isoenzymes are inhibited and CYP1A2 increases melatonin levels [302]. Caution should be exercised in patients on oestrogens (e.g. contraceptive or hormone replacement therapy), which increase melatonin levels by inhibiting its metabolism by CYP1A1 CYP1A2 [303].

CYP1A2 inducers, such as carbamazepine and rifampicin may reduce melatonin plasma concentration [304].

Cigarette smoking may decrease melatonin levels due to induction of CYP1A2 [305]. [redacted] showed that exogenous, but not endogenous (at night), serum melatonin levels are influenced by cigarette smoking. When the melatonin levels are low, the influence of CYP1A2 levels appears to be less pronounced than when they are high and the enzyme capacity hugely utilized. These findings implicate that interactions between exogenous melatonin and substrates metabolized by CYP1A2 may differ in individuals before and after smoking abstinence [306].

There is a large amount of data in the literature regarding the activation/promotion of endogenous melatonin secretion by opiate agonists/antagonists [307], antidepressant medicinal products [308], prostaglandin inhibitors, benzodiazepines [309, 310], tryptophan [311] and alcohol [312]. Adrenergic agonists/antagonists decrease the 6-SMT excretion. Whether or not these active substances interfere with the dynamic or kinetic effects of melatonin or vice versa has not been studied.

2.5.5.2.2 Pharmacodynamic Drug Interactions

In humans, co-administration of melatonin and zolpidem showed pharmacodynamic interaction (increased sedation) [253]. 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 randomized, double-blind, placebo-controlled and four-way crossover study. A new pharmacodynamic 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 affect the CNS may result in pharmacodynamic drug interactions [313]. For example, relative to monotherapy with the CNS-active drug, patients receiving melatonin PR and imipramine had increased feelings of tranquillity and difficulty in performing tasks, and those receiving melatonin PR plus thioridazine had increased feelings of 'muzzy-headedness' [313]. 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 [314]. In the study of [redacted] AD 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 [58].

Alcohol should not be taken with melatonin, because it reduces the effectiveness of melatonin on sleep [315].

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 [redacted] Bleeding events, INR (International Normalized 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 [316]. The results of this study are consisted with previous cases reported in the meta-analysis of [redacted]

2.5.6 BENEFITS AND RISKS CONCLUSIONS

2.5.6.1 THERAPEUTIC CONTEXT

2.5.6.1.1 Disease or Condition

Melatonin, as a neurohormone that is primarily produced in the pineal gland, can acutely attenuate the activity of the 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. It is currently used exogenously in the treatment of primary and secondary sleep disorders, although the hormone contributes also to the protection of the organism from carcinogenesis and neurodegenerative disorders. Exogenous melatonin administration can be used to mimic the physiological functions of endogenous low level melatonin when administered in a specifically timed manner (i.e. to correct abnormalities in in circadian timing.

The planed medicinal product will be available as tablets of 3 mg Melatonin.

2.5.6.1.2 Current Therapies

Current therapies of jet lag include light therapy, melatonin, melatonin receptor analogues [Ramelteon (Rozerem©, Takeda, Japan; TAK-375), Agomelatine (Valdoxan©, Servier, France; S20098), TIK-301, Tasimelteon (Hetlioz, Vanda Pharmaceuticals, USA)], non-benzodiazepine hypnotics, caffeine, diphenhydramine and CNS stimulants such as armodafinil. The patient's flight schedule, physical condition and individual response to treatment play important roles. Benzodiazepines, antidepressants, antihistamines and anxiolytics have the potential for dependence and addiction. Moreover, some of these medications can gradually impair cognition.

Administering exogenous melatonin in the conventional afternoon to evening hours of a 24-hour day promotes a phase shift in circadian rhythm and thus promoting sleep.

[redacted]

2.5.6.2 BENEFITS

Melatonin is remarkably effective in preventing or reducing jet lag and has been the subject of many studies. When making travel plans, particularly over a distance of five or more time zones, travelers should take melatonin on the day of travel at the projected night time hour in the new time zone and on subsequent days in the new time zone. The impact of various dosage forms of melatonin on jet lag symptoms has been evaluated in several studies.

Most of the clinical trials included in the clinical overview and in Module 2.7.3 found 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 zone. The benefit is likely to be greater the more time zones are crossed and less for westward flights. The timing of the melatonin dose is important: if is taken at the wrong time, early in the day, it is liable to cause sleepiness and delay adaptation to local time.

2.5.6.3 RISKS

Melatonin is well tolerated with a good safety profile, with most adverse events being of mild severity. Headaches, dizziness nausea and drowsiness are the most common adverse events reported with short term melatonin administration. Reported adverse events from clinical trials are presented in Module 2.7.4. 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. Additionally, all the reported adverse events of melatonin since 2004 are presented in Module 2.7.4.

In all cases there was no significant differences found between melatonin and placebo despite tight confidence intervals.

The risk of overdose is low and does not change by dose, the presence or absence of a sleep disorder, type of sleep disorder, duration of treatment, gender, age, formulation of melatonin, use of concurrent medication, study design, quality score and allocation concealment score. Melatonin interacts only with few other active substances.

2.5.6.4 BENEFIT-RISK ASSESSMENT

Three physiologic effects:

1) promotion of sleep onset;
2) maintenance of sleep and
3) phase-shifting of circadian rhythms - an indirect action - and the diurnal rhythm in melatonin itself

have been associated with melatonin administration.

Many different melatonin preparations have been used in this clinical overview, including fast and long acting formulations, solid or liquid formulations and different doses of those formulations. Overall, the results in the different studies presented in this clinical overview suggest that the product is efficacious in patients suffering from jet lag, when the dose of melatonin is correctly timed. The most likely reason for some inconsistent results of the literature, particularly in field studies is that melatonin treatment was incorrectly timed. The large differences in individual response to, for example jet lag, mean that timing treatment is difficult unless internal clock timing is known.

The clinical overview along with Modules 2.7.3 and 2.7.4 describes also the safety of the proposed product. The most common adverse events reported in the clinical trials were nausea, headache, dizziness and drowsiness, which are common by MedDRA definition. No serious adverse events or health risks have been noted from melatonin use.

Melatonin offers an alternative treatment to the currently available pharmaceutical therapies for sleep disorders with significantly less side effects and limited potential for drug-drug interactions.

There is no doubt that the prescribing of melatonin is continuing to increase, since it has positive effects on sleep disturbances.

The suggested dose is in accordance with other European products and supported also by clinical data, as presented in the relevant sections of the clinical overview.

Additionally, the therapeutic equivalence of the product under assessment with the EU licensed product Bio-melatonin 3 mg filmtabletta has been established by two bioequivalence studies. The full details of the bioequivalence studies are included in the revised Modules 5 and 2.7.

2.5.7 LITERATURE REFERENCES

2.5 Clinical overview

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