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Risk assessment of melatonin

Opinion of the Norwegian Scientific Committee for Food and Environment

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Risk assessment of melatonin

Preparation of the opinion

The Norwegian Scientific Committee for Food and Environment (Vitenskapskomiteen for mat og miljø, VKM) appointed a project group to draft the opinion. An interdisciplinary VKM approval group, appointed specifically for the assignment, assessed and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work. The authors have contributed as members of the project group and/or as members of the interdisciplinary VKM approval group.

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Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

In 2019, the Norwegian Medicines Agency reclassified melatonin as a drug at a dose up to 1 mg per day to be classified as a food supplement; doses above 1 mg melatonin per day are still classified as medicine. In 2020, addition of 1 mg melatonin per day in food supplements to adults (persons over 18 years of age) were permitted by the NFSA.

NFSA requested VKM to examine whether daily intake of 0.2 mg melatonin for 3-6-years-olds, 0.3 mg melatonin for 7-10-years-olds, and 0.5 mg melatonin for 11-18-year-olds might constitute a health risk. VKM was further requested to identify the highest safe dose up to and including 1 mg of melatonin per day for children and adolescents, if the food supplement doses given above were considered to be safe, and to evaluate for how long melatonin food supplement doses considered not to constitute a health risk can be used continuously. In addition, VKM was requested to examine whether there is a health risk associated with a continuous daily intake of food supplements added 1 mg melatonin for three months for healthy adults (>18 years), and to evaluate for how long 1 mg melatonin from food supplements can be used continuously without causing negative health effects in adults (>18 years), if no health risk is considered to be associated with continuous daily intake of food supplements added 1 mg melatonin for three months.

Melatonin is produced in the human vertebrate pineal gland, the retina and possibly in some other organs. Melatonin is a hormone with relatively low solubility in water, and the physiological effects of melatonin are mediated by melatonin receptors located in the brain and periphery (TGA, 2009). Serum melatonin concentrations vary considerably according to age. Melatonin displays a marked circadian rhythm, with high levels at night and low levels during the day (SCCS, 2010). Melatonin secretion increases soon after the onset of darkness, peaks in the middle of the night (between 2 and 4 am), and gradually falls during the second half of the night (Brzezinski, 1997). Endogenous plasma levels of melatonin are reported to range from about 10 pg/mL (during the day) up to 100 pg/mL (during the night) (SCCS, 2010; TGA, 2009).

Orally ingested melatonin is efficiently and almost completely absorbed from the gastrointestinal tract, however, the bioavailability in humans is low primarily due to extensive first-pass metabolism (EMA, 2018; MEB, 2020; TGA, 2009). The liver is the primary site of melatonin metabolism, and the main catabolite is 6-hydroxymelatonin (EMA, 2018; MEB, 2020). Excretion of melatonin catabolites, and a small quantity of melatonin (<1%), occurs predominantly via the kidneys (MEB, 2020).

Several potential adverse effects following melatonin exposure, mainly endocrine effects, were pinpointed (EMA, 2018; MEB, 2020; SCCS, 2010; TGA, 2009). Delay of sexual maturation and development were identified as important potential risks (EMA, 2018). Clinical human studies reported side effects such as headache, nausea and drowsiness. Case reports provides concern for increased risk for seizure frequency and autoimmune reaction,

and there was a call for caution in using melatonin in immune compromised patients and in patients with renal or hepatic impairment (MEB, 2020). Data on use during pregnancy and lactation, data on long-term safety in children (>2 years) and data on fertility effects, are lacking for human studies.

Results from experimental animal studies identified endocrine effects, organ toxicity, reproductive and developmental toxicity and carcinogenicity following repeated doses of melatonin. The total body of evidence on toxicity of melatonin in animal experimental studies showed a large variation in the melatonin doses causing an adverse effect (EMA, 2018; MEB, 2020; SCCS, 2010; TGA, 2009). Moreover, animal findings indicate developmental effects following melatonin exposure, and there is a need for more data on safety of melatonin exposure during pregnancy and lactation.

Based on the lack of evidence from long-term studies in children and adolescents on pubertal development and high-quality animal experiments, VKM conclude that a PoD (characterising the hazard) for children and adolescents cannot be identified.

For adults, VKM identified a no observed effect level (NOEL) of 0.005 mg/kg bw/day from a 90-day repeated dose toxicity study in rat. This dose equals a dose of 0.26 mg/day for a person weighing 52 kg which is the default 5th percentile body weight reported by EFSA (2012). An identified no observed adverse effect level (NOAEL) of 0.4 mg/kg bw/day (6-month study in dogs) equals a dose of about 21 mg/day. VKM consider that due to data insufficiency and lack of quality assessment of the studies that the default factor of 100 do not sufficiently cover the uncertainties related to the identified PoDs. VKM also considers that there is insufficient information to quantify the exact uncertainty related to the identified PoDs. For illustrative purpose only, applying an uncertainty factor of 100, results in doses of 0.0026 and 0.21 mg/day. VKM cannot conclude on the safety of 1 mg/day of melatonin for three months.

Conclusions:

VKM cannot conclude on the safety of daily intake of 0.2 mg melatonin for 3-6-years-olds, 0.3 mg melatonin for 7-10-years-olds, and 0.5 mg melatonin for 11-18-year-olds.

VKM cannot conclude on the safety of continuous use of 1 mg/day of melatonin for three months for adults.

Key words: Adverse health effect, melatonin, Norwegian Food Safety Authority, Norwegian Scientific Committee for Food and Environment, other substances, risk assessment, VKM.

Sammendrag på norsk

Doser på opptil 1 mg melatonin per døgn er ikke lenger klassifisert som legemiddel i Norge. Våren 2020 ga Mattilsynet tillatelse til å tilsette doser på 1 mg melatonin per døgn i kosttilskudd til voksne (personer over 18 år). Doser over 1 mg melatonin per døgn er klassifisert som legemiddel.

På oppdrag fra Mattilsynet har Vitenskapskomiteen for mat og miljø (VKM) vurdert om daglig inntak av følgende doser melatonin i kosttilskudd kan utgjøre en risiko for negative helseeffekter: 0,2 mg melatonin for 3-6-åringer, 0,3 mg melatonin for 7-10-åringer, og 0,5 mg melatonin for 11-18-åringer. Hvis disse dosene ble vurdert å ikke utgjøre en risiko for negative helseeffekter, skulle VKM identifisere den høyeste trygge dosen til og med 1 mg melatonin per dag for barn og ungdom, og vurdere hvor lenge sammenhengende daglig inntak av dosene er trygt. I tillegg ble VKM bedt om å undersøke om det er en helserisiko forbundet med et daglig inntak av kosttilskudd tilsatt 1 mg melatonin i tre måneder for friske voksne over 18 år. Hvis dette ble vurdert å ikke utgjøre en risiko for negative helseeffekter, skulle VKM vurdere hvor lenge 1 mg melatonin fra kosttilskudd kan brukes sammenhengende uten å forårsake negative helseeffekter hos voksne (>18 år).

Melatonin er et hormon som er viktig for døgnrytmen og som produseres i kroppen. Nivåene som produseres varierer fra rundt 10 pg/ml om dagen og opp til rundt 100 pg/ml om natten (SCCS, 2010; TGA, 2009). Melatonin tas raskt opp i kroppen etter oralt inntak, men fordi mye metaboliseres i lever ved førstepassasjemetabolisme er biotilgjengeligheten lav (EMA, 2018; MEB, 2020; TGA, 2009).

Det er påpekt flere potensielle negative effekter av melatonin, hovedsakelig endokrine effekter (EMA, 2018; MEB, 2020; SCCS, 2010; TGA, 2009). Forsinkelse av pubertetsutvikling er trukket frem som en viktig potensiell risiko (EMA, 2018). I kliniske studier er det rapportert bivirkninger som hodepine, kvalme og døsighet. Basert på rapporter om effekter på enkeltindivider er det uttrykt bekymring for økt risiko for epileptiske anfall og for autoimmune reaksjoner, og det er uttrykt behov for forsiktighet ved bruk av melatonin hos pasienter med nedsatt immunforsvar og hos pasienter med nedsatt nyre- eller leverfunksjon (MEB, 2020). Det mangler humane studier på bruk av melatonin under graviditet og amming, data på langtidsbruk hos barn (>2 år), og data om effekter på fertilitet.

I eksperimentelle dyreforsøk hvor det er gitt gjentatte doser av melatonin over tid, er det rapportert om negative effekter på det endokrine systemet, på organer, på reproduksjon og utvikling, og om kreftfremkallende effekter. I disse studiene var det stor variasjon i hvilke doser som førte til negative effekter (EMA, 2018; MEB, 2020; SCCS, 2010; TGA, 2009).

Basert på mangel på studier av effekt på pubertetsutvikling, både langtidsstudier på barn og ungdom og dyreforsøk av høy kvalitet, konkluderer VKM med at det ikke kan identifiseres noe utgangspunkt for å utlede en trygg dose melatonin for barn og ungdom.

I en 90-dagers rottestudie hvor det ble gitt gjentatte doser av melatonin over 90 dager, med unntak av ferier og helger, var 0,005 mg melatonin/kg kroppsvekt den høyeste dosen som ikke førte til helseeffekter (no observed effect level; NOEL). Dette tilsvarer en dose på 0,26 mg/dag for en person som veier 52 kg. I en annen dyrestudie var den høyeste dosen som ikke førte til negative helseeffekter (no observed adverse effect level; NOAEL) på 0,4 mg/kg kroppsvekt/dag, som tilsvarer en dose på ca. 21 mg/dag for en person som veier 52 kg. Ved å bruke en faktor på 100 for å justere for usikkerheten ved at disse dosene er fra dyrestudier og usikkerheten som er knyttet til individuelle forskjeller hos mennesker, blir dosene på henholdsvis 0,0026 og 0,21 mg/dag for en person som veier 52 kg. I og med at disse dosene er under 1 mg melatonin per dag, kan VKM ikke konkludere på om et daglig inntak av 1 mg melatonin per dag sammenhengende i en periode på tre måneder er trygt for voksne.

Konklusjoner

VKM kan ikke konkludere på om daglig inntak av 0,2 mg, 0,3 mg eller 0,5 mg melatonin fra kosttilskudd kan utgjøre en risiko for negative helseeffekter for henholdsvis 3-6-åringer, 7-10-åringer og 11-18-åringer.

VKM kan ikke konkludere på om det er en helserisiko forbundet med et daglig inntak av kosttilskudd tilsatt 1 mg melatonin i tre måneder for friske voksne over 18 år.

Abbreviations and glossary

Abbreviations

ADME	absorption, distribution, metabolism and excretion
AE	adverse event
bw	body weight
EFSA	European Food Safety Authority
EMA	European Medicines Agency
HBGV	Health based guidance value
LOEL	lowest observed effect level
MEB	Medicines Evaluation Board (Netherland)
NFSA	Norwegian Food Safety Authority
NOAEL	no observed adverse effect level
NOEL	no observed effect level
PoD	point of departure
PPCS	persistent postconcussive symptom
RCT	randomised controlled trial
RoB	risk of bias
SCCS	Scientific Committee on Consumer Safety (European Commission)
TGA	Therapeutic Goods Administration (Australia)
VKM	Norwegian Scientific Committee for Food and Environment
WHO	World Health Organization

Glossary

Absorption, distribution, metabolism and elimination
The four key processes which describe how drugs and chemicals get into the body, what happens to them while they are there, and how they are eliminated.

Adverse health effect
A change in morphology, physiology, growth, development, reproduction or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences (WHO, 1994).

Health-based guidance value
Guidance on safe consumption of substances that takes into account current safety data, uncertainties in these data, and the likely duration of consumption (EFSA glossary).

Lowest observed adverse effect level
The lowest level of a substance that has been observed to cause harm in an exposed population.

No Observed Adverse Effect Level
The greatest concentration or amount of a substance at which no detectable adverse effects occur in an exposed population (EFSA glossary).

"Other substances"
A substance other than a vitamin or mineral that have a nutritional or physiological effect (Regulation (EC) No 1925/2006 of the European Parliament and of the Council).

Point of departure
The point on a dose-response curve established from experimental data used to derive a safe level (EFSA Glossary).

"Positive list"
Annex to Regulation (EC) No 1925/2006 including "other substances" and levels thereof allowed for addition to foods.

Background as provided by the Norwegian Food Safety Authority

"Other substances" are substances that have a nutritional or physiological effect but are not vitamins or minerals. Examples of "other substances" include fatty acids, amino acids, coenzyme Q10 and caffeine. Excessive intake of certain "other substances" may be associated with health risks.

In the European Economic Area (EEA), the provisions on the addition of "other substances" to foods are currently only partially harmonised in Regulation (EC) No 1925/2006. This means that Member States may lay down national supplementary provisions on the aspects that are not harmonised. Any national supplementary provisions must comply, inter alia, with the general principles of EEA law on the free movement of goods, "mutual recognition" and the legal exceptions to these EEA principles.

In Norway new supplementary national provisions regarding the addition of certain "other substances" to foods including food supplements entered into force on 1 January 2020. The new national supplementary provisions are included in the Norwegian regulation "Forskrift 26. februar 2010 nr. 247 om tilsetning av vitaminer, mineraler og visse andre stoffer til næringsmidler", which also implements Regulation (EC) No 1925/2006 in Norwegian internal law.

A so-called "positive list" for the addition of certain "other substances", was introduced as an Annex to the regulation. The intention is to reduce health risks that can occur when consuming certain "other substances" in foods, including food supplements.

The new national supplementary provisions only apply to the addition of "other substances" that a) have a purity of at least 50% or are concentrated 40 times or more, and b) are not normally consumed as a food in themselves and not normally used as an ingredient in foods.

Furthermore, the supplementary national provisions do not apply to the addition of the following "other substances": a) plants or parts of plants in fresh, dried, chopped, cut or powdered form, b) extracts of plants or parts of plants exclusively made through basic aqueous extraction, possibly followed by dehydration, c) enzymes and microorganisms and d) "other substances" listed in Parts A and B of Annex III to Regulation (EC) No 1925/2006.

It is only permitted to add "other substances" that are listed in the "positive list" in Annex 3 to foods, including food supplements. Such addition to foods must be in accordance with the terms and conditions set in the "positive list", including the limits that are set for the different substances. Substances regulated by other legislations like those for novel foods, food additives, flavourings, Foods for Specific Groups, etc. is outside the scope of the national supplementary provisions.

If a food business operator wants to add different quantities or use different conditions of a substance that is included in the "positive list", the food business operator must notify the NFSA. If a food business operator wants to add new substances, not currently included in the "positive list", the food business operator must apply for authorisation to the NFSA.

When needed for the NFSA to process an application or notification, the Norwegian Scientific Committee for Food and Environment (VKM) is requested to perform a risk assessment so that new substances or higher amounts of substances listed in the "positive list" are risk assessed.

Terms of reference as provided by the Norwegian Food Safety Authority

NFSA hereby ask the Norwegian Scientific Committee for Food and Environment (VKM) to examine whether the exposure to melatonin (CAS No. 73-31-40) in food supplements that is regulated by the national supplementary provisions might constitute a health risk for Norwegian children (both sexes) aged from 3 years up to 18 years. In November 2019 The Norwegian Medicines Agency reclassified melatonin as a drug at a dose up to 1 mg/day to be classified as a food supplement. Doses above 1 mg melatonin/day are still classified as a drug in Norway. In the spring of 2020, the NFSA has given permission to addition of 1 mg of melatonin per day in food supplements to adults (persons over 18 years of age).

Food supplemented with melatonin for children and adolescents

The NFSA requests a risk assessment of different doses of melatonin added to food supplements for children and adolescents. In the risk assessment the NFSA requests an assessment to be made of various doses of melatonin in food supplements for children and adolescents as shown in the table.

Age	Daily dose
3 years to 6 years	0.2 mg melatonin
7 years to 10 years	0.3 mg melatonin
11 years to 18 years	0.5 mg melatonin

If the daily doses of melatonin given in the table in the various age groups are safe in food supplements, the NFSA would like to know the highest safe dose up to and including 1 mg of melatonin per day that is safe for children and adolescents?

The NFSA also would also like to know the daily doses of melatonin in food supplements that are safe for children and adolescents (in the various age groups) to be considered for how long such food supplement doses can be used continuously without causing negative health effects?

This includes:

- Identify and characterise adverse health effects.
    - Identify and describe toxicological reference point(s).
    - Describe uncertainty related to the toxicological reference point(s).
- Estimate the exposure
    - Estimate exposure for the doses and age groups given above.
    - Describe uncertainty related to the exposure estimates.

- Characterise health risks associated with exposure to the substance (melatonin), and describe uncertainty that may have an impact on the conclusions.
- Identify and describe main knowledge gaps that may have an impact on the conclusions.

Food supplement added 1 mg melatonin per daily dose for adults

NFSA also wants to know whether there is a health risk associated with a continuous use for three months of food supplements added melatonin (1 mg/day) in healthy people over the age of 18 in the Norwegian population.

- If there is no health risk associated with using such a supplement for three consecutive months, how long can food supplements added 1 mg melatonin/day be used continuously without causing negative health effects in adults?

Assessment

1 Introduction

"Other substances" are substances that have a nutritional or physiological effect but are not vitamins or minerals (Regulation (EC) No 1925/2006 of the European Parliament and of the Council). Excessive intake of certain "other substances" may be associated with health risks.

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food and Environment (VKM) to:

- Examine whether daily intake of 0.2 mg melatonin for 3-6-years-olds, 0.3 mg melatonin for 7-10-years-olds, and 0.5 mg melatonin for 11-18-year-olds might constitute a health risk.
- Identify the highest safe dose up to and including 1 mg of melatonin per day for children and adolescents, if the food supplement doses given in bullet point 1 are considered to be safe.
- Evaluate for how long melatonin food supplement doses considered not to constitute a health risk (bullet point 1 or 2) can be used continuously.
- Examine whether there is a health risk associated with a continuous daily intake of food supplements added 1 mg melatonin for three months for healthy adults (>18 years).
- Evaluate for how long 1 mg melatonin from food supplements can be used continuously without causing negative health effects in adults (>18 years), if no health risk is considered to be associated with continuous daily intake of food supplements added 1 mg melatonin for three months (bullet point 4).

A protocol for the risk assessments of "other substances" was published (VKM et al., 2020).

Melatonin is produced in the human vertebrate pineal gland, the retina, and possibly in some other organs. Melatonin is a hormone with relatively low solubility in water, and the physiological effects of melatonin are mediated by melatonin receptors located in the brain and periphery (TGA, 2009). Plasma melatonin concentrations vary considerably according to age. Melatonin displays a marked circadian rhythm, with high levels at night and low levels during the day (SCCS, 2010). Melatonin secretion increases soon after the onset of darkness, peaks in the middle of the night (between 2 and 4 am), and gradually falls during the second half of the night (Brzezinski, 1997). Endogenous plasma levels of melatonin is about 10 pg/mL during the day (SCCS, 2010; TGA, 2009). According to TGA (2009), endogenous plasma levels of melatonin is about 30 pg/mL at night, whereas SCCS (2010) reported that endogenous plasma levels of melatonin is about 100 pg/mL at night.

Food, including vegetables, cereals, fruits, nuts, seeds, grapes, red wine and beer, contain considerable amounts of melatonin (Tan et al., 2010), and food consumption may in some cases alter the plasma melatonin level (Hattori et al., 1995). Melatonin is indicated as an antioxidant in cosmetic products in the CosIng database (2021).

1.1 Limitations

- The assessment is performed for melatonin, and only for the doses in the mandate given by NFSA.
- The assessment covers the general healthy population, not groups in the population that may have a high exposure due to e.g. certain dietary habits, or population groups that may be especially vulnerable due to e.g. certain genetic variants, diseases, drug use or age/life stages.
- The age groups to be included are given in the mandate from the NFSA.
- Exposure from other sources of melatonin is not estimated.
- Documentation of any claimed beneficial effects is not evaluated.
- Stability of melatonin in a product is not addressed.
- Interaction with other components in a product is not addressed.
- Potential impurities are not addressed.

2 Substance specifications

Name and other identifiers of melatonin are presented in Table 2-1 and physical and chemical properties are presented in Table 2-2. (https://pubchem.ncbi.nlm.nih.gov/compound/Melatonin)

Table 2-1. Name and other identifiers.

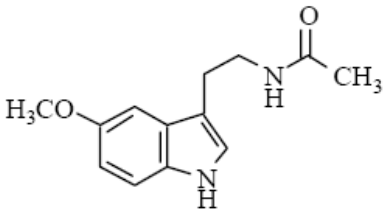
Substance name	Melatonin
Synonym	Melatonine, N-acetyl-5-methoxytryptamine
CAS number	73-31-4
EINECS number	200-797-7
Molecular formula	$C_{13}H_{16}N_2O_2$
Molecular weight	232.28 g/mol
Structural formula	
SMILES	CC(=O)NCCC1=CNC2=C1C=C(C=C2)OC

Table 2-2. Physical and chemical properties.

Physical state	Solid
Boiling point (liquids), melting point (solids)	Melting point 116-118 °C
Density	Not found
Vapor pressure	1.4X10-7 mm Hg at 25 °C (est)
Water solubility	In water, 2 g/L at 20 °C; 5 g/L at 50 °C
Partition coefficient (LogP)	1.6

3 Exposure

Exposure to melatonin was estimated for daily intake from food supplements for the doses and age groups given by the NFSA (Table 3-1). Exposure from other sources of melatonin is not included in the estimation.

Table 3-1. Doses and age groups given by the NFSA.

Age	Daily dose
3 years to 6 years	0.2 mg melatonin
7 years to 10 years	0.3 mg melatonin
11 years to 18 years	0.5 mg melatonin
Adults ≥18 years	1.0 mg melatonin

The default body weights (bw) determined by EFSA (2012), the 5th percentile and the median, was used for the exposure estimation (Table 3-2).

Daily exposure, individuals with the 5th percentile body weight

From a daily dose of 0.2 mg melatonin, the exposure is 0.014 mg/kg bw/day for 3-6-year-olds, 0.021 mg/kg bw/day for 7-<10-year-olds, 0.017 mg/kg bw/day for 10-<14-year-olds, and 0.011 mg/kg bw/day for 15-<18-year-olds (Table 3-2). For adults, the exposure from a daily dose of 1 mg melatonin is 0.019 mg/kg bw/day.

Daily exposure, individuals with the median body weight

From a daily dose of 0.2 mg melatonin, the exposure is 0.009 mg/kg bw/day for 3-6-year-olds, 0.014 mg/kg bw/day for 7-<10-year-olds, 0.012 mg/kg bw/day for 10-<14-year-olds, and 0.008 mg/kg bw/day for 15-<18-year-olds (Table 3-2). For adults, the exposure from a daily dose of 1 mg melatonin is 0.014 mg/kg bw/day.

Table 3-2. Daily melatonin exposure from the doses given by the NFSA.

Population group	Daily melatonin dose	5th percentile bw	Median bw	Daily exposure (individuals with the 5th percentile bw)	Daily exposure (individuals with the median bw)
3-6-year-olds	0.2 mg	14.0 kg	21.7 kg	0.014 mg/kg bw	0.009 mg/kg bw
7-<10-year-olds	0.2 mg	14.0 kg	21.7 kg	0.021 mg/kg bw	0.014 mg/kg bw
10-<14-year-olds	0.3 mg	29.4 kg	42.0 kg	0.017 mg/kg bw	0.012 mg/kg bw
14-<18-year-olds	0.5 mg	45.0 kg	60.0 kg	0.011 mg/kg bw	0.008 mg/kg bw

Population group	Daily melatonin dose	5th percentile bw	Median bw	Daily exposure (individuals with the 5th percentile bw)	Daily exposure (individuals with the median bw)
Adults ≥18 years	1.0 mg	52 kg	72 kg	0.019 mg/kg bw	0.014 mg/kg bw

3.1 Other sources

Endogenous plasma levels of melatonin are about 10 pg/mL during the day (SCCS, 2010; TGA, 2009). According to TGA (2009), endogenous plasma levels of melatonin is about 30 pg/mL at night, whereas SCCS (2010) reported that endogenous plasma levels of melatonin is about 100 pg/mL at night.

A variety of food stuffs, including vegetables, cereals, fruits, nuts, seeds, grapes, red wine and beer contain considerable amounts of melatonin (Tan et al., 2010).

Melatonin is indicated as an antioxidant in cosmetic products in the CosIng database (CosIng database, 2021).

4 Hazard identification and characterisation

The questions for the hazard identification and characterisation for oral intake of melatonin are presented in Table 4-1. The negative effects were divided into genotoxicity and other adverse effects (referred to as adverse effects). An overview of the hazard identification and characterisation process is given in Figure 4-1.

Table 4-1. Hazard questions.

Absorption, distribution, metabolism and elimination	1	What is the ADME of melatonin in humans? Is human and animal (rodent) ADME similar?
	2	Is melatonin metabolised to innocuous metabolites?
	3	Is melatonin endogenous to humans? If yes, is the dose given in the mandate from NFSA resulting in body levels within the range normally metabolised and eliminated?
Hazard identification	4	Is there a concern for genotoxicity?
	5	Is exposure to melatonin associated with adverse health effects?
Hazard characterisation	6	What is the dose-response relationships between exposure to melatonin and the adverse effects?
	7	Can a health-based guidance value be established or a point of departure be identified?

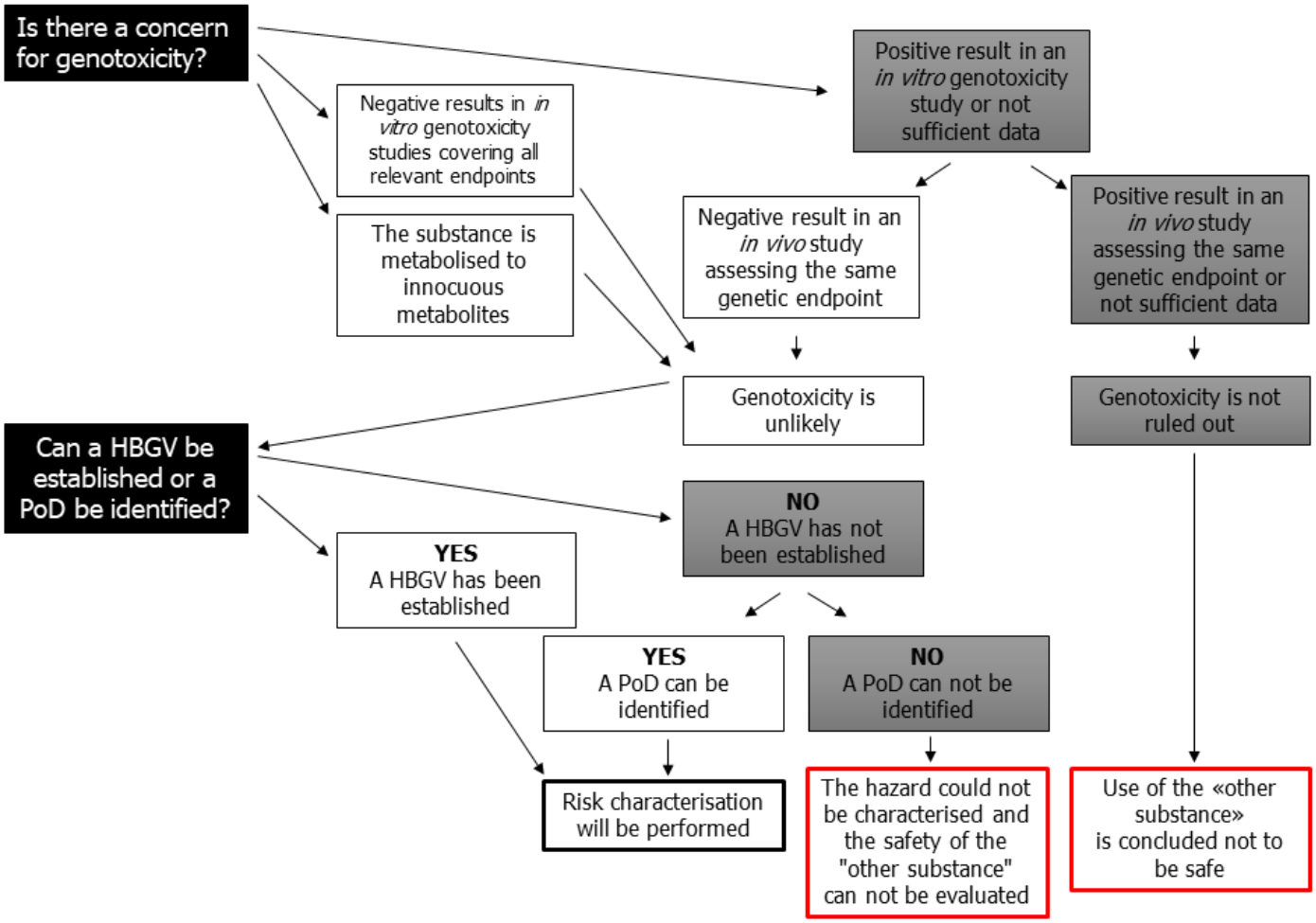


Figure 4-1. Flow chart for the hazard identification and characterisation. HBGV = health-based guidance value; PoD = point of departure.

To identify relevant data to answer the research questions in Table 4-1, websites of international risk assessment and medicine evaluating organisations and agencies were searched to identify opinions, risk or safety assessments of melatonin, and the following four reports (EMA, 2018; MEB, 2020; SCCS, 2010; TGA, 2009) were considered relevant:

- **Assessment report Slenyto. European Medicines Agency (EMA, 2018)**
  An application for a paediatric use marketing authorisation for Slenyto for treatment of insomnia characterised by maintenance problems and/or sleep onset difficulties in children aged 2-18 with autism spectrum disorders was evaluated. The product was Slenyto prolonged-release tablets, the paediatric formulation of Circadin, containing 1 or 5 mg melatonin as the active substance. The active ingredient in Circadin is melatonin.
- **Public Assessment Report, Melatonin Orifarm. Medicines Evaluation Board, Netherlands (MEB, 2020)**

Melatonine Orifarm 3 mg, indicated for the short-term treatment of jet-lag in adults, was evaluated.

- **Opinion on Melatonin. Scientific Committee on Consumer Safety, European Commission (SCCS, 2010)**
  The safety of melatonin in cosmetic products was evaluated. The specific request addressed was "Does the SCCS consider the use of melatonin in a concentration of 0.0033% w/w (33 µg/g) in hair care products safe for the consumer?"
- **Australian Public Assessment Report for Melatonin. Therapeutic Good Administration (TGA, 2009)**
  The safety of Circadin, 2 mg modified release tablets for short term treatment (up to 3 weeks) of primary insomnia characterised by poor quality of sleep in patients who are aged 55 or over, was evaluated.

In addition, literature searches for randomised controlled trials (RCTs) on children and adolescents (age 0-18 years) published after the publication year of the most recently published report, were performed in MEDLINE (Ovid) and Embase (Ovid) (section 4.3.1).

4.1 Absorption, distribution, metabolism and elimination (ADME)

An overview and a comparison of the human and animal ADME data available for melatonin in humans and animals, are given in Table 4.1-1.

Table 4.1-1. An overview and a comparison of the human and animal ADME data.

ADME	Human findings	Animal findings	Common findings for ADME in humans and animals
Absorption	Melatonin is efficiently and almost completely absorbed from the gastrointestinal tract. Bioavailability in humans is low, in the range 5-20%, and this is primarily due to extensive first-pass metabolism (EMA, 2018; MEB, 2020; SCCS, 2010; TGA, 2009). The pharmacokinetics in in humans appear to be non-linear, likely as a result of saturable first-pass metabolism (EMA, 2018; TGA, 2009). According to MEB (2020), a 3 to 6 mg dose results in a peak in the plasma melatonin concentration about 50 minutes after ingestion, and a single dose of 3 mg is expected to result in a maximum plasma concentration of about 3400 pg/mL. After oral administration to human volunteers of up to 3 times 80 mg of melatonin/person in one hour, the highest melatonin values were measured in serum 60 to 150 minutes after administration (SCCS, 2010). When 3 mg of melatonin/person was administered orally, an increase in serum melatonin within 20 minutes after oral administration was reported, followed by a rapid decrease at 240 minutes (SCCS, 2010).	Melatonin is rapidly and extensively absorbed in rats (MEB, 2020; TGA, 2009). The mean oral bioavailability of a single dose of 10 mg/kg was 54% in rats and about 100% in dogs and monkeys (EMA, 2018; MEB, 2020; TGA, 2009). The pharmacokinetics in rats, dogs and monkeys appear to be non-linear, likely as a result of saturable first-pass metabolism (EMA, 2018; TGA, 2009).	In both humans and experimental animals, melatonin is rapidly absorbed. In humans, the bioavailability is low (5-20%) due to extensive first-pass metabolism, whereas the bioavailability in rats is about 50%.
Distribution	About 50-60% binds to plasma proteins (EMA, 2018; MEB, 2020). Literature data indicates that melatonin is distributed in all body fluids and is accessible at all tissues (SCCS, 2010; TGA, 2009).	Studies in rats and hamsters indicate that melatonin is widely distributed to tissues and readily crosses the blood-brain barrier (TGA, 2009). Intravenous administration of melatonin to the	Melatonin is widely distributed in both humans and experimental animals. More than 50% is bound to plasma proteins in humans.

ADME	Human findings	Animal findings	Common findings for ADME in humans and animals
		rat resulted in a rapid distribution into plasma and all tissues of the animal, including cerebrospinal fluid and brain (SCCS, 2010). It is shown in rats that most of circulating melatonin is bound to albumin (EMA, 2018; TGA, 2009).	In rats, most circulating melatonin is bound to the plasma protein albumin.
Metabolism	Melatonin is synthesized from the amino acid tryptophan, via the hormone serotonin. Endogenous plasma levels of melatonin is reported to range from about 10 pg/mL (during the day) up to 100 pg/mL (during the night) (SCCS, 2010; TGA, 2009). The liver is the primary site of melatonin catabolism and the key enzymes are the cytochrome P450 system enzymes CYP1A1 and CYP1A2. The major metabolic pathway of melatonin has been identified as being the hydroxylation of position 6, followed by conjugation, primarily with sulfate (70%) and, to a smaller extent, with glucuronic acid (6%) (SCCS, 2010). The main metabolite is 6-hydroxymelatonin (EMA, 2018; MEB, 2020). Approximately 2% melatonin is excreted unchanged in the urine (EMA, 2018).	The major catabolic pathway in rodents is via the hepatic P450 system, and the main metabolite is 6-hydroxymelatonin (EMA, 2018; MEB, 2020; TGA, 2009).	Melatonin is synthesized from the essential amino acid tryptophan. In both humans and rodents, the liver is the primary site of melatonin catabolism, the major catabolic pathway in rodents is via the hepatic P450 system, and the main metabolite is 6-hydroxymelatonin.
Elimination	Excretion of melatonin metabolites, and a small quantity of melatonin (<1%), occurs predominantly via the kidneys. 6-	The main excretion route of the melatonin metabolites is renal	In both humans and rodents, excretion occurs

ADME	Human findings	Animal findings	Common findings for ADME in humans and animals
	hydroxymelatonin is excreted as its sulphate (~70%) and glucuronide (~30%) conjugates. Melatonin half-life in healthy adults (including the elderly) is ~45 minutes (normal range ~30 – 60 minutes) and is essentially constant at oral doses up to 100 mg (MEB, 2020). When 23 µg/person of melatonin was administered by intravenous injection to humans, an apparent half-life of 36 to 42 minutes in the systemic circulation was determined (SCCS, 2010). A half-life of 36 to 45 minutes where reported for melatonin after oral administration of a dose of 250 µg/person. Limited data indicate that hepatic impairment can reduce the clearance of exogenous melatonin. Data regarding the effect of renal impairment on the clearance of exogenous melatonin are not available, however, as melatonin metabolites are predominantly excreted in the urine, renal impairment can be expected to reduce their elimination (MEB, 2020).	(EMA, 2018). The apparent elimination half-lives in the rat, dog and monkey has been estimated to be around 20-30 min (TGA, 2009). When melatonin was administered intravenously to the dog and the monkey at 3 mg/kg, the apparent elimination half-lives were measured to be 18.6 and 33.9 min, respectively (MEB, 2020; SCCS, 2010).	predominantly via the kidneys. Melatonin half-life in healthy adults is ~45 minutes, the half-life in rodents is 20-30 minutes.

4.2 Genotoxic potential

Gene mutations and structural and numerical chromosomal alterations are the endpoints that should be addressed to evaluate genotoxic potential. It was reported that a full battery of genotoxicity tests of sufficient quality, covering these endpoints, have been performed and that all results were negative (EMA, 2018; MEB, 2020; SCCS, 2010; TGA, 2009).

4.3 Adverse health effects

4.3.1 Literature search

The search strategies for the literature searches in MEDLINE (Ovid) and Embase (Ovid) are available in Section 8.1 (Appendix).

4.3.1.1 Publication selection

Literature retrieved from the searches were screened based on the eligibility criteria presented in Table 4.3.1.1-1.

Table 4.3.1.1-1. Hazard: eligibility criteria for human randomised controlled trials.

Study design	Human randomised controlled trials
Population	0-18-year-olds
Exposure	The substance is tested alone (not part of a mixture) Exposure route in prioritised order: 1. Oral 2. Intraperitoneal, intravenous, subcutaneous
Outcome of interest	Any adverse health effect related to exposure to the substance
Language of the full-text	English, Norwegian, Swedish, Danish, German
Publication type	Scientific publications

The literature search identified 100 publications. First, pairs of reviewers screened titles and abstracts independently, and five publications were included. Next, pairs of reviewers screened the full-text articles independently, and two was included (Figure 4.3.1.1-1). An overview of the excluded RCTs is given in Section 8.2.

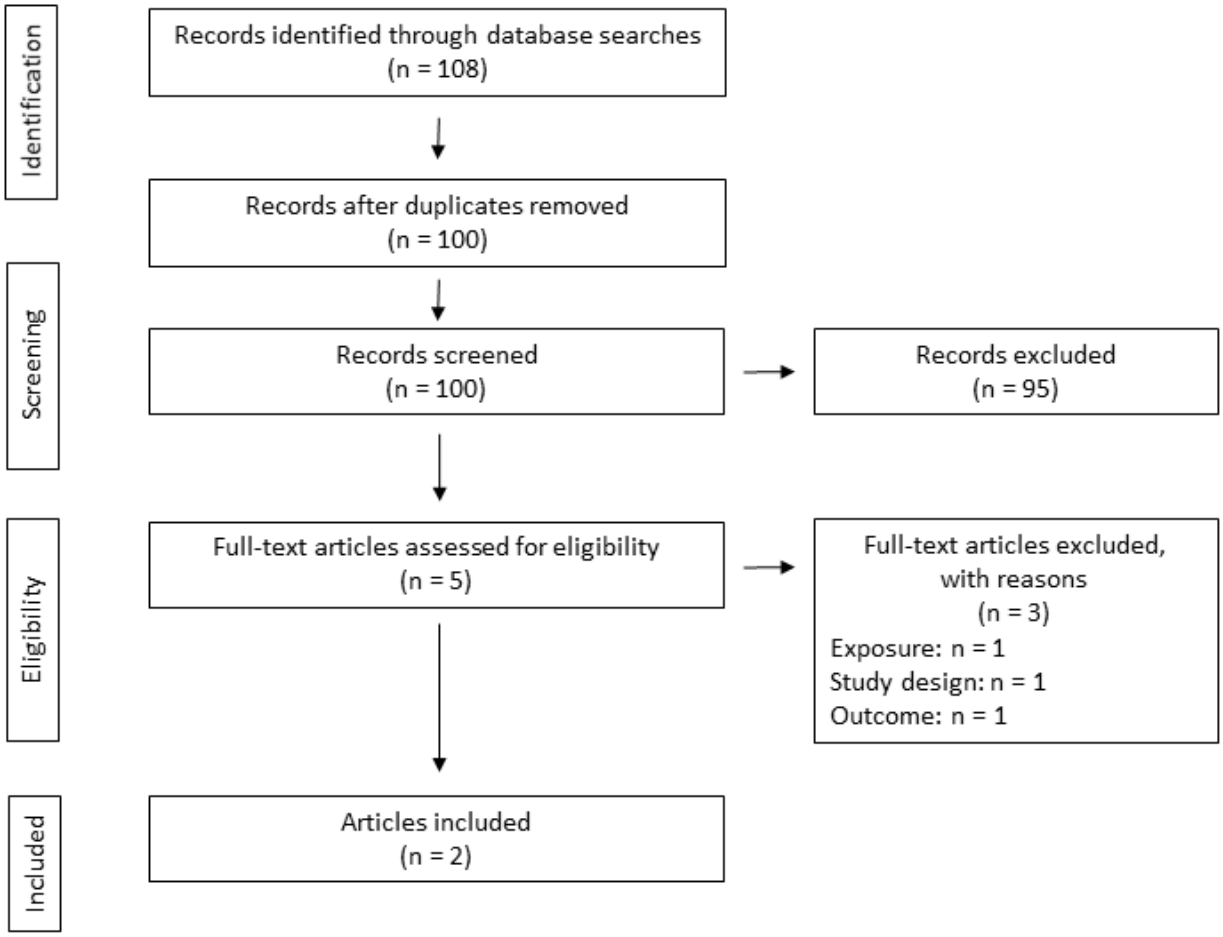


Figure 4.3.1.1-1. Flowchart for the selection of publications on adverse health effects (modified from Moher et al. (2009)).

4.3.1.2 Internal validity

Risk of bias (RoB) was evaluated using the OHAT (Office of Health Assessment and Translation) tool (OHAT, 2015; OHAT 2019). This tool includes questions considering aspects relevant for RoB evaluation of human and animal studies. The RoB questions (Table 4.3.1.2-2) addressing key elements such as exposure assessment and outcome assessment, were defined as key questions. The rating of all questions, key and non-key, was integrated to classify the studies into tiers to characterise the overall RoB as shown in Table 4.3.1.2-1. Tier 1 represents low RoB, tier 3 represents high RoB. Tier 2 studies, representing moderate RoB, did not meet the criteria for tier 1 or 3. For RCTs, we defined questions 1, 2, 3, 5 and 6 as key questions, whereas questions 4, 7 and 8 were defined as non-key questions. The key questions address the elements selection bias (randomisation and allocation to study groups), performance bias (identical experimental conditions across study groups and blinding of personnel and participants), and detection bias (confidence in the exposure characterisation and the outcome assessment). The non-key questions address the elements attrition/exclusion bias, selective reporting bias, and other sources of bias. The response options and symbols (in parentheses) used for the rating are i) definitely low risk of bias

(++); ii) probably low risk of bias (+); iii) probably high risk of bias/not reported (NR) (-); and iv) definitely high risk of bias (- -).

Table 4.3.1.2-1. Classification of studies into tiers according to overall RoB.

Tier	**1**	**2**	**3**
Criteria for classification	All key questions are scored +/++ AND No more than one non-key question is scored – AND No non-key question is scored - -	All combinations not falling under tier 1 or 3	Any key or non-key question is scored - - OR More than one key question is scored -

Two reviewers independently assessed RoB. The overall RoB was classified as tier 1 for the study by Barlow et al. (2020), and tier 3 for the study by Malow (2021) (Table 4.3.1.2-2). The detailed evaluation for each RoB question is included in the appendix (Section 8.3).

Table 4.3.1.2-2. RoB rating and the classification into tiers for the eligible RCTs. *Key question.

	1. Was administered dose or exposure level adequately randomized?*	2. Was allocation to study groups adequately concealed?*	3. Were the research personnel and human subjects blinded to the study group during the study?*	4. Were outcome data complete without attrition or exclusion from analysis?	5. Can we be confident in the exposure characterisation?*	6. Can we be confident in the outcome assessment?*	7. Were all measured outcomes reported?	8. Were there no other potential threats to internal validity?	Tier
Barlow et al. (2020) Adverse events	++	++	++	++	+	+	++	++	1
Malow et al. (2021) Pubertal development	+	-	-	+	-	+	-	-	3
Malow et al. (2021) Child growth	+	-	-	+	-	++	-	-	3
Malow et al. (2021) Adverse events	+	-	-	+	-	+	-	-	3

4.3.1.3 Study characteristics

As publications classified as tier 3 (high RoB) is not included in the evidence synthesis due to the high concern for bias on key element(s), only study characteristics for the study classified as tier 1 (low RoB) (Barlow et al., 2020) are extracted. A brief overview is given in Table 4.3.1.3-1, a detailed data extraction form is available in Section 8.4 (Appendix).

Table 4.3.1.3-1. Human study on adverse health effects.

Reference	Aim	Participant characteristics	Dose and duration	Endpoints	Result (adverse health effects)
Barlow et al. (2020)	Evaluate if treatment with melatonin would result in a greater decrease in persistent postconcussive symptoms (PPCS) when compared with a placebo.	Youth (ages 8–18 years) with PPCS at 4 to 6 weeks after mild traumatic brain injury were included. In total, 99 children (mean age: 13.8 years; SD = 2.6 years; 58% girls) participated.	3 or 10 mg melatonin, 28 days treatment.	The primary outcome was change in the total youth self-reported PPCS-Inventory score measured after 28 days of treatment. Secondary outcomes included change in health-related quality of life, cognition, and sleep.	Side effects were mild and similar to the placebo.

4.3.2 Human studies on adverse health effects – an overview

MEB (2020) concluded that, altogether, the (limited) available evidence from clinical studies suggests that tolerability and safety of melatonin, especially when used for a short time period, is high, with headache, nausea and drowsiness as the most frequent side effects. Further, due to lack of evidence in children and the fact that it is unknown to what extent exogenous melatonin modulates gonadal axis pre-puberty in humans, it was agreed that the proposed summary of product characteristics advised not to use melatonin in children.

EMA (2018) concluded that the long-term safety up to 2 years of melatonin in children at the 2-5 (-10) mg daily dose is in line with the known safety profile of Circadin in adults and children, and that there is a need for more long-term safety data on melatonin in general and more specifically related to pubertal development.

TGA (2009) concluded that Circadin appeared to be safe and well tolerated in the target population.

The most common adverse events (AEs) reported included headache, nausea, drowsiness and sedation (EMA, 2018; MEB, 2020; TGA, 2009). The incidence of AEs was low, and no serious AEs or deaths were reported. TGA (2009) stated that most of the submitted studies, and all of the pivotal studies, were short-term interventions.

Overall, EMA (2018) and MEB (2020) reported that no important risks were identified. However, delay of sexual maturation and development (EMA, 2018) and off-label use in pediatric patients with sleep disorders (MEB, 2020), were identified as important potential risks. The following information was reported missing: data on use during pregnancy and lactation, data on long-term safety in children (>2 years), data on use in patients with renal or hepatic impairment, data on use in patients with autoimmune disorders, and data on fertility effects.

Endocrine effects

MEB (2020):

- Hormonal effects of melatonin have been observed. These observations included enhancement of luteinizing hormone levels in women during the follicular phase of the menstrual cycle, enhancement of cortisol levels in older women, enhancing prolactin secretion, and decreasing plasma progesterone and estradiol levels in healthy women, and reduced glucose tolerance and insulin sensitivity. In addition, melatonin may have an effect on fertility in women and men, on pregnancy, and on breast feeding.
- Typical therapeutic doses of melatonin seem to have, at most, a slight, transient effects on the level of other hormones.

EMA (2018):

- The long-term safety of melatonin in children has not been characterised in well-designed studies.
- The fact that melatonin plasma levels are high in prepubertal children and is dramatically reduced during puberty, has led to the suggestion that administration of exogenous melatonin leading to supraphysiological levels in pre-pubertal and pubertal children may lead to pubertal abnormalities. There is a lack of long-term safety studies in children investigating this issue. The best study, with regards to pubertal development, was conducted by van Geijlswijk et al. (2011). They studied 51 children (mean age 12.0 years, 8.6-15.7 years) who took melatonin during a mean time of 3.1 years (min 1.0 year, max 4.6 years) at a mean dose of 2.69 mg (0.3-10 mg). The parents reported Tanner stages of their children (n=46) using a questionnaire and there was no substantial deviation of the development of the children with respect to puberty. A drawback of this study was the low number of subjects and that Tanner staging was reported through questionnaires from the parents and not directly investigated by the physicians. Nevertheless, it is reassuring that there is no data so

far supporting the theoretical risk of hormonal disturbances or effects on the pubertal development from melatonin treatment. However, more long-term data is warranted.

Immunological effects

MEB (2020) reported that secondary pharmacology of melatonin suggests effects on the immune system, and that these effects may call for caution in using melatonin in immune compromised patients. Case reports concerning autoimmune reaction in response to treatment with melatonin have been published.

CNS effects

MEB (2020) reported that there are literature references suggesting that melatonin can increase seizure frequency, although clear causality between melatonin and epilepsy/seizure activity cannot be considered established.

4.3.3 Animal studies on adverse effects - an overview

An overview of animal experimental studies and the main results is given in Table 4.3.3-1 (single-dose toxicity), 4.3.3-2 (repeat-dose toxicity), and 4.3.3-3 (reproductive and developmental toxicity). For studies on pediatric use, TGA (2009) stated that "There are no adequately conducted nonclinical studies in young animals to support the use of melatonin in children, and treatment of paediatric patients is not proposed."

Table 4.3.3-1. Melatonin: single-dose toxicity.

Animal model	Dose/exposure	Outcomes assessed and results (adverse effects)
Low acute toxicology by the oral route. The main effects observed at high doses were sedation, lethargy, and vasodilatation. These effects are not expected to occur for a human dose of 5 mg melatonin, and not clinically relevant (EMA, 2018).		
Rats and mice (MEB, 2020).	Up to ≥200 mg/kg bw.	No special hazard was shown in single dose toxicity studies performed in rat and mice. Large doses (up to ≥200 mg/kg) of melatonin caused similar behavioural effects in mice and rats.
Rats and mice (SCCS, 2010; TGA (2009).	400 mg/kg bw and higher.	At doses of 400 mg/kg bw, vasodilatation of the extremities indicated by a reddening of the ears and feet, piloerection and ptosis were common. In addition, muscle relaxation, a marked lack of motor activity and ataxia were observed. At higher doses, an impairment of the righting, placing and hindlimb ipsilateral flexor reflexes, a marked reduction in body temperature and slow, laboured respiration preceded death.
VKM concludes: Acute toxicity of melatonin at doses of 400 mg/kg bw includes adverse effects such as ataxia and reduced motor activity. However, these doses are not relevant for human exposure. Melatonin appears to have low acute toxicity.		

Table 4.3.3-2. Melatonin: repeated-dose toxicity.

Animal model/ study design	Dose/ exposure	Endocrine effects	Alterations in hematology and clinical biochemistry	Effects on body weight	Organ effects	Carcinogenic effects
14-day oral study in the rat (Long-Evans and Fischer 344), 10 males and 10 females per dosage group (SCCS, 2010).	0, 0.005, 0.05, 5.0, 50 or 200 mg/kg bw/day.			No bw changes.	No organ weight changes.	
14-day rat juvenile toxicity study (EMA, 2018).	80 and 160 mg/kg bw/day.				Non-reversible minimal increase in extramedullary hematopoiesis in the spleen at 160 mg/kg bw/day.	
28-day subcutaneous study in the rat (Sprague-Dawley), 19 males and 19 females per dosage group, the observation period was 28 days (SCCS, 2010).	0.050, 0.50 and 4.8 mg/kg bw/day for the males and 0.074, 0.75 and 7.3 mg/kg bw/day for the females.		No effects.	No effects on bw.		
70-day study, juvenile male and female rats (EMA, 2018).	Oral administration of 80 and 160		Melatonin-related reversible increases in reticulocyte count and total		Reversible increases in liver weight were observed in males and females at 160 mg/kg bw/day, and	

Animal model/ study design	Dose/ exposure	Endocrine effects	Alterations in hematology and clinical biochemistry	Effects on body weight	Organ effects	Carcinogenic effects
	mg/kg bw/day.		bilirubin were observed in females dosed with 160 mg/kg bw/day.		reversible increased splenic weight was observed in females. Non-reversible extramedullary hematopoiesis in spleen was observed in females given 160 mg/kg bw/day.	
Subchronic 90-day oral toxicity study in rats (Long-Evans and Fischer 344 rats), 10 males and 10 females per dosage group, the observation period was 90 days (SCCS, 2010).	0, 0.005, 0.05, 5.0, 50 or 200 mg/kg bw/day by gavage, excluding weekends and holidays.	Increased T3 and T4 were observed at dosages starting from 0.05 mg/kg bw/day.		Mean weight gains over the entire study in all female Long-Evans melatonin treated groups were 7 to 10% less than their control. In the Fischer rats, a reduction in body weight gain was observed in dosages starting from 5 mg/kg bw/day.		
90-days rat study (EMA, 2018).	0.3, 1.2 and 6.0 mg/kg bw/day.			Decreased body weight gain at 1.2 mg/kg bw/day	Increased kidney weights at 6 mg/kg bw/day.	

Animal model/ study design	Dose/ exposure	Endocrine effects	Alterations in hematology and clinical biochemistry	Effects on body weight	Organ effects	Carcinogenic effects
				(males) and 6.0 mg/kg bw/day (males and females).		
26-weeks study on hemizygous Tg rasH2 mice (EMA, 2018).	Oral administration of up to 180 mg/kg bw/day.					No tumours induced.
6-month dog study (EMA, 2018).	1.5 and 8.0 mg/kg bw/day.		Increased serum glucose levels were observed at some time points.		Cytoplasmatic rarefaction of hepatocytes consistent with the presence of glycogen. Capsular fibrosiderosis of the spleen.	Pituitary gland and parathyroid cysts.
6-month repeated dose toxicity study in dogs (TGA (2009).	Includes 0.4, 1.5 and 8.0 mg/kg bw/day.	Thyroid weights were increased at melatonin doses of 8 mg/kg bw/day in males (8%) and at all doses (0.4-8 mg/kg bw/day) in females (5-19%) after 6 months dosing.			8 mg/kg bw/day increased liver weights in males (11%). 5 and 8 mg/kg bw/day for six months increased liver weights in females (7-16%). Chronic liver inflammation was observed in 4/8 dogs from all dose groups given 0.4-8 mg/kg bw/day	

Animal model/ study design	Dose/ exposure	Endocrine effects	Alterations in hematology and clinical biochemistry	Effects on body weight	Organ effects	Carcinogenic effects
					melatonin compared with 1/8 control dogs (for both genders combined).	
A combined 13-week rat study with a 4-week recovery period coupled to a 26-week toxicity phase (EMA, 2018; TGA, 2009).	Oral administration by gavage of 0, 15, 75 and 150 mg/kg bw/day.	Dark thyroid at 75 mg/kg bw/day (3/20 males, 1/20 females) and 150 mg/kg bw/day (9/19 males, 7/20 females), correlated with agonal congestion/haemorrhage microscopically. These thyroid findings were not observed in control animals. TSH levels were elevated in rat males after 150 mg/kg bw/day at weak 78 and 104, but this effect was not a clear dose effect.	Increased haemoglobin concentration and platelet counts at 75 and 150 mg/kg bw/day (13 and 26 weeks).		13 weeks: Increased liver weights at 75 and 150 mg/kg bw/day (10-13% in males; 8-10% in females). This was reversible. 26 weeks: Increased liver weight, males (7%) at 150 mg/kg bw/day No remarkable changes in liver enzymes or macroscopic liver changes were observed at 13, 26 or 104 weeks. Minor centrilobular hypertrophy in 9/10 (compared with 0/10 control) and 9/19 (compared with 0/20 control) males at 150 mg/kg bw/day after 13 and 26 weeks, respectively. Liver hypertrophy was not observed in any recovery animals, indicating	

Animal model/ study design	Dose/ exposure	Endocrine effects	Alterations in hematology and clinical biochemistry	Effects on body weight	Organ effects	Carcinogenic effects
					reversibility, and no microscopic liver changes were observed after 104 weeks dosing in either gender. Increased kidney weights at 75 and 150 mg/kg bw/day in males (5-7%) and 150 mg/kg bw/day in females (7%) after 13 weeks, a reversible effect following four treatment-free weeks. A kidney weight increase was observed in males (7%) only at 150 mg/kg bw/day after 26 weeks.	
A combined 13-week rat (males) study with a 4-week recovery period coupled to a 104-weeks carcinogenicity phase (EMA, 2018; TGA, 2009).	Oral administration by gavage of 0, 15, 75 and 150 mg/kg bw/day.	Increased dark and/or large thyroid, 104 weeks, 75 and 150 mg/kg bw/day. Microscopically, treatment-related increases in incidence and severity of thyroid pigment and thyroid follicular cell			A slight, significantly increased incidence of pituitary adenomas in males at 150 mg/kg bw/day (15/49 = 31%) compared with controls (8/50 = 16%, control1; 10/49 = 20%, control2) for 2 years, but not females. 4/15 pituitary adenomas in	Increased thyroid follicular cell tumours in males at 150 mg/kg bw/day (104 weeks; 7/50 compared with

Animal model/ study design	Dose/ exposure	Endocrine effects	Alterations in hematology and clinical biochemistry	Effects on body weight	Organ effects	Carcinogenic effects
		hypertrophy, at 75 and 150 mg/kg bw/day.			the 150 mg/kg bw/day group were fatal, compared with 0/8 and 1/10 for the control groups, respectively.	3/50 in control groups).

VKM concludes:

The body of evidence on adverse effects following repeated doses of melatonin in experimental animals indicate endocrine effects and organ effects (predominantly on kidney and liver). VKM notes that the occurrence of effects was not consistent across studies. Moreover, there are inconsistencies concerning at which dose levels the different effects occurred. From a subchronic (90 days) oral rat toxicity study, the following was reported in the description of the results (SCCS, 2010): "As far as clinical biochemistry is concerned, increases in T3 and T4 were observed at dosages starting from 0.05 mg/kg bw/day, but these measurements have been declared as not clinically significant, since no concurrent effects on thyroid histopathology were observed". SCCS (2010) concluded that "In light of the poor description of the test and given the fact that the raw data cannot be consulted, it is very difficult to establish a NOAEL value based on this study. An additional issue is that there is a large gap between the dosages of 0.05 and 5 mg/kg bw/day. Without full description of the test and without the raw data, the NOAEL value can be temporarily set on 5 mg/kg bw/day (based upon the significant treatment-related adverse effects, being the coloured faces and the dilated uterus)". VKM considers that the increase of T3 and T4 cannot be ignored; several studies report thyroid effects at different doses, possible mechanisms for thyroid effects of melatonin have been described, and increased levels of T3 and T4 can be caused by other mechanisms than thyroid toxicity, e.g. liver toxicity. From this study, the no observed effect level (NOEL) identified was 0.005 mg/kg bw/day, and the lowest observed effect level (LOEL) was 0.05 mg/kg bw/day. The NOELs and NOAELs identified in the different studies ranged from 0.005 mg/kg bw/day to 15 mg/kg bw/day.

In one 2- year rat study, increased incidence of adenomas in the pituitary gland and follicular cell tumours in the thyroid gland for male, but not female rats, were observed. VKM notes that both of these glands are part of the endocrine system which were also identified as a target organ for toxicity. Moreover, gross changes were observed for the thyroid in males which are consistent with changes observed in repeated-dose toxicity studies. There is no concern for genotoxicity for melatonin and the mechanism for adenoma and tumour formation is unknown. Inflammation due to high doses could be a

Animal model/ study design	Dose/ exposure	Endocrine effects	Alterations in hematology and clinical biochemistry	Effects on body weight	Organ effects	Carcinogenic effects
plausible cause, however, the body of evidence is too small to conclude on whether these adenomas and tumours are relevant for humans. Based on one study, VKM identifies a NOAEL for carcinogenicity of 75 mg/kg bw/day.						

Table 4.3.3-3. Melatonin: reproductive and developmental toxicity studies.

Animal model/ study design	Dose/ exposure	Fetal effects	Maternal toxicity	Reproductive toxicity
Fertility and early embryonic development study with 24 rats/sex/dose (EMA, 2018).	0, 15, 55 or 200 mg/kg bw/day by gavage.	No effects reported.		
Rat developmental toxicity study; 25 timed-mated CDR female rats (EMA, 2018).	50, 100 and 200 mg/kg bw/day, gestation day 6 to 19, by gavage.	The endpoints related to embryo/foetal growth, viability or morphological development were not modified.	No maternal deaths. Transient reduction of bw gain and relative decreased food intake at the high dose. Increased relative maternal liver weight at the mid and high dose.	
Rabbit developmental toxicity study; NZW rabbit (EMA, 2018).	Oral administration of 0, 15, 50 and 150 mg/kg bw/day from days 7 to 19 of gestation.	In all groups, including controls, visceral and skeletal malformations and/or variations were observed.	No dose-related maternal effects.	

Animal model/ study design	Dose/ exposure	Fetal effects	Maternal toxicity	Reproductive toxicity
Prenatal and postnatal development study, including maternal function. 24 pre-mated rats (EMA, 2018).	0, 15, 55 and 200 mg/kg bw/day, from day 6 of gestation to day 21 post-partum.	Growth and viability of the high dose offspring was slightly reduced during lactation. At weaning, a slight reduction of offspring maturity was observed in all dose groups, but the subsequent F1 development was not modified.		
70-day repeated-dose toxicity study, juvenile male and female rats (EMA, 2018).	Oral administration of 80 and 160 mg/kg bw/day.			
90-days rat study (EMA, 2018)	0.3, 1.2 and 6.0 mg/kg bw/day.			Decreased testis weight at 6 mg/kg bw/day.
A combined 13-week rat study with a 4-week recovery period coupled to a 26-week toxicity phase (EMA, 2018; TGA, 2009).	Oral administration by gavage of 0, 15, 75 and 150 mg/kg bw/day.			Increased testes, prostate and epididymides weights were seen in males administered 75 and 150 mg/kg bw/day.
Rats, postnatal development study (MEB, 2020).		Delay in sexual maturation.		
Squirrel, postnatal development study (MEB, 2020).		Delay in sexual maturation.		
Teratogenicity study, 25 female rat per group, the observation	Gavage, 50, 100 or 200 mg melatonin/kg bw/day or vehicle (0.5% aqueous	Melatonin had no effect on prenatal survival, foetal body weight, or incidence of foetal malformations/variations.	Mild sedation, reduced maternal food intake, and reduced body weight gain were found during initial	

Animal model/ study design	Dose/ exposure	Fetal effects	Maternal toxicity	Reproductive toxicity
period was 25 days (SCCS, 2010).	methylcellulose) on gestation day 6 through gestation day 19.		treatment with 200 mg/kg bw/day.	
28-day subcutaneous study in the rat (Sprague-Dawley), 19 males and 19 females per dosage group, the observation period was 28 days (SCCS, 2010).	0.050, 0.50 and 4.8 mg/kg bw/day for the males and 0.074, 0.75 and 7.3 mg/kg bw/day for the females.			Males; a trend toward decreasing serum prolactin concentration, all doses. Two of 10 males administered 4.8 mg/kg bw/day had decreased testes weights and testicular degenerative changes composed of reduced or absent spermatogenesis, spermatidic giant cells and oedema.
Subchronic (90 days) oral toxicity study in rats (Long-Evans and Fischer 344 rats), 10 males and 10 females per dosage group, the observation period was 90 days (SCCS, 2010).	0, 0.005, 0.05; 5.0, 50 or 200 mg/kg bw/day.		Cystic uterine endometrial hyperplasia was observed in a number of treated Long-Evans female rats, but also in their respective control group. One treatment-related finding in a 50 mg/kg bw/day treated Long-Evans female was a dilated uterus at necropsy.	
Rat study (TGA, 2009).	Oral doses ranging between 50-200 mg/kg bw/day during	Increased pre-implantation loss (15% compared with 7.5% in controls) was observed (not significant). Combined	Slight maternotoxicity (clinical signs and mild	

Animal model/ study design	Dose/ exposure	Fetal effects	Maternal toxicity	Reproductive toxicity
	all stages of the reproductive cycle.	embryofetal and peri- and postnatal treatment of rats with doses of 200 mg/kg bw/day oral melatonin was associated with reduced growth and viability of the offspring during lactation and some slight physical developmental delays in male offspring.	reductions in body weight and food consumption).	
6-month dog study (EMA, 2018).	1.5 and 8.0 mg/kg bw/day.		Adenomyosis of the uterus.	
Rabbit study, oral (TGA, 2009).	Oral doses of melatonin up to 150 mg/kg bw/day during the embryofetal development period.	The incidence of fetal malformations of the head, joint and spine appeared slightly increased with treatment at 150 mg/kg bw/day (1.9% compared with 0% control). A range of visceral (absence of a lung, abnormal eye texture, discoloured thymus) and skeletal (incomplete ossification, alignment shift, long or fused) variations were slightly, and in some cases, significantly increased with treatment at 150 mg/kg bw/day.		
VKM concludes: The body of evidence from experimental animals following melatonin exposure indicate low maternal toxicity for doses up to 150 mg/kg bw/day, where effects on food intake and body weight were observed. NOAEL for reproductive effects was identified to be 1.2 mg/kg bw/day. For fetal toxicity, adverse effects on the skeletal development, growth, sexual maturity and viability were reported. The lowest observed adverse dose level is 15 mg/kg bw/day, which was the lowest tested dose across studies. VKM notes that for both maternal, reproductive and fetal toxicity there are inconsistencies regarding the doses at which the effects occur that cannot be explained by exposure length, time of exposure, or species. In addition, there are inconsistencies for the direction of effect for testes weight. Moreover, VKM notes that the occurrence of effects was not consistent across studies.				

4.4 Summary of the hazard identification and characterisation

NFSA requested VKM to evaluate whether daily intake of 0.2, 0.3 and 0.5 mg melatonin in food supplements might constitute a health risk for 3-6-years-olds, 7-10-years-olds, and 11-18-year-olds, respectively. If the given doses are considered to be safe, NFSA requested VKM to identify the highest safe dose up to and including 1 mg of melatonin per day for children and adolescents. For the identified safe melatonin food supplement doses for these age groups, VKM was also requested to evaluate for how long the doses can be used continuously without causing negative health effects. For healthy adults (>18 years), NFSA requested VKM to evaluate whether there is a health risk associated with a continuous daily intake of food supplements added 1 mg melatonin for three months. Further, if no health risk is considered to be associated with continuous daily intake of food supplements added 1 mg melatonin for three months, VKM was requested to evaluate for how long 1 mg melatonin from food supplements can be used continuously without causing negative health effects in adults (>18 years).

The risk assessment of melatonin is part of the risk assessments of the so-called "other substances", and a protocol for these assessments was published (VKM et al., 2020). The time available for the risk assessment of "other substances" is limited, and as described in the protocol, websites of international risk assessment organisations are first searched for available opinions, risk or safety assessments. The need for literature searches in electronic databases is then evaluated. For melatonin, four relevant reports were identified in the website searches, and only a literature search for RCTs on children and adolescents were considered necessary. A literature search, covering the period from the most recently published safety evaluation until March 1, 2021, were performed. One RCT of sufficiently good quality was identified.

The hazard identification and characterisation are based on the four included reports (EMA, 2018; MEB, 2020; SCCS, 2010; TGA, 2009) and the included RCT (Barlow et al., 2020). EMA (2018) evaluated the safety of Slenyto prolonged-release tablets, the paediatric formulation of Circadin containing 1 or 5 mg melatonin as the active substance, for treatment of insomnia characterised by maintenance problems and/or sleep onset difficulties in children aged 2-18 with autism spectrum disorders. MEB (2020) evaluated the safety of Melatonine Orifarm, 3 mg, indicated for the short-term treatment of jet-lag in adults. SCCS (2010) evaluated the safety of melatonin in cosmetic products. TGA (2009) evaluated the safety of Circadin, 2 mg modified release tablet per day, for short term treatment (up to 3 weeks) of primary insomnia characterised by poor quality of sleep in patients who are aged 55 or over (TGA, 2009). Barlow et al. (2020) evaluated the effect of melatonin treatment on persistent postconcussive symptoms in youths (3 or 10 mg for 28 days, 8-18 years). Due to the choice of limiting the literature to existing assessments and a literature search for RCTs addressing avderse health effects on children and adolescents, there is a chance that relevant data might have been left out of the risk assessment. Moreover, for the majority of the studies described in the reports, details and results were poorly reported, and the quality was not assessed.

Bioavailability of melatonin in humans is stated to be low (5-20%) due to first-pass metabolism. In rats and dogs, the bioavailability of a single dose of 10 mg/kg was 54% and about 100%, respectively. In both humans and experimental animals, melatonin is widely distributed, but to a larger extent bound to plasma proteins in humans. In both humans and experimental animals, the major catabolic pathway in rodents is via the hepatic P450 system, and the main metabolite is 6-hydroxymelatonin. Melatonin half-life in healthy adults is ~45 minutes, the half-life in rodents and monkeys is 20-30 minutes. In both humans and rodents, excretion occurs predominantly via the kidneys. There is not enough information on metabolism and melatonin metabolites to assess if melatonin is only metabolised to innocuous metabolites. Data from toxicity studies pinpointed several potential adverse effects following melatonin exposure. There is no data on whether these effects are caused by melatonin itself or metabolites of melatonin.

Endogenous plasma levels of melatonin are reported to range from about 10 pg/mL (during the day) up to 100 pg/mL (during the night) (SCCS, 2010; TGA, 2009). According to MEB (2020) a single melatonin dose of 3 mg is expected to result in a maximum plasma concentration of about 3400 pg/mL, which is 34-340 times higher than the endogenous melatonin level.

The endpoints gene mutations and structural and numerical chromosomal alterations were addressed in the genotoxicity studies, and all tests were negative (EMA, 2018; MEB, 2020; SCCS, 2010; TGA, 2009). VKM therefore concludes that there is no concern for genotoxicity.

Several potential adverse effects following melatonin exposure, mainly endocrine effects, were pinpointed (EMA, 2018; MEB, 2020; SCCS, 2010; TGA, 2009). Delay of sexual maturation and development were identified as important potential risks (EMA, 2018). Clinical human studies reported side effects such as headache, nausea and drowsiness. Case reports provides concern for increased risk for seizure frequency and autoimmune reactions, and there was a call for caution in using melatonin in immune compromised patients and in patients with renal or hepatic impairment. Data on use during pregnancy and lactation, data on long-term safety in children (>2 years) and data on fertility effects are lacking for humans (EMA, 2018; MEB, 2020). The identified safety evaluations, with the exception of SCCS, did only to a small degree include a quality evaluation of the included studies. Without a full assessment of study quality and confidence in evidence, VKM have judged all studies as equally important.

Two studies assessed the effect of long-term melatonin exposure on pubertal development in children (van Geijlswijk et al. (2011) included in EMA (2018), and Malow et al. (2021) identified in the literature search). Malow et al. (2021) was not included in the body of evidence as the risk of bias was classified as high. For the study by van Geijlswijk et al. (2011), VKM considers that the participating children were of an appropriate age and Tanner staging is a well-known tool to assess physical development in children, including sexual maturity. However, from the description of the studies, VKM identified aspects that might indicate high risk of bias; the Tanner stages were reported by the parents and not a

physician, and it was not stated whether the parents were blinded to the allocation to intervention or placebo group. Moreover, the number of participants was too low to make any conclusions based on this study.

VKM notes that the clinical trials were not designed to study the safety following melatonin exposure, and it is therefore not possible to conclude on the safety of melatonin on these studies alone. Results from experimental animal studies identified endocrine effects, organ toxicity, reproductive and developmental toxicity and carcinogenicity following repeated doses of melatonin. In addition, animal findings indicate developmental effects following melatonin exposure, and there is a need for more data on safety of melatonin exposure during pregnancy and lactation. The lowest NOEL identified from animal experiments was 0.005 mg/kg bw/day based on increased T3 and T4 levels in a 90-day repeated dose toxicity study in rats. In the same study, the lowest dose reported to have an effect (lowest observed effect level; LOEL), was 0.05 mg/kg bw/day. With regard to this study, SCCS (2010) concluded that "In light of the poor description of the test and given the fact that the raw data cannot be consulted, it is very difficult to establish a NOAEL value based on this study". This increase in T3 and T4 was not accompanied with effects in the thyroid. However, several other studies have shown toxicity in these organs and VKM therefore considers this effect to be of relevance, although there is not sufficient information to assess whether this effect is adverse or not. The lowest NOAEL identified from animal experiments was 0.4 mg/kg bw/day based on increased thyroid weight of female dogs in a 6-months repeated dose toxicity study. For fetal toxicity, adverse effects on the skeletal development, growth, sexual maturity and viability were reported. However, a NOAEL could not be identified.

Based on the lack of evidence from long-term studies in children and adolescents on pubertal development and high-quality animal experiments, VKM conclude that a PoD for children and adolescents cannot be identified.

For adults, VKM identified a NOEL of 0.005 mg/kg bw/day from a 90-day repeated dose toxicity study in rats. This dose equals a dose of 0.26 mg/day for a person weighing 52 kg which is the default 5th percentile body weight reported by EFSA (2012). A NOAEL of 0.4 mg/kg bw/day was identified from a 6-month repeated dose toxicity study in dogs, and this dose equals a dose of about 21 mg/day for a person weighing 52 kg. To account for the uncertainty in the PoDs, uncertainty factors are applied. The default uncertainty factor to adjust for interspecies and interindividual differences is 100. However, the uncertainty factor should be derived on a case-by-case basis and justified (EFSA, 2012). VKM consider that due to data insufficiency and lack of quality assessment of the studies that the default factor of 100 do not sufficiently cover the uncertainties related to the identified PoDs. VKM also considers that there is insufficient information to quantify the exact uncertainty related to the identified PoDs. For illustrative purpose only, applying an uncertainty factor of 100, results in doses of 0.0026 and 0.21 mg/day. VKM cannot conclude on the safety of 1 mg/day of melatonin for three months.

Overall, as a PoD was not identified for children and adolescents and VKM cannot conclude on the safety of 1 mg/day of melatonin for three months, it is not possible to characterise the risk.

5 Conclusions

VKM cannot conclude on the safety of daily intake of 0.2 mg melatonin for 3-6-years-olds, 0.3 mg melatonin for 7-10-years-olds, and 0.5 mg melatonin for 11-18-year-olds.

VKM cannot conclude on the safety of continuous use of 1 mg/day of melatonin for three months for adults.

6 Data gaps

EMA (2018) and MEB (2020) identified the following missing information: data on use during pregnancy and lactation, data on long-term safety in children (>2 years), data on use in patients with renal or hepatic impairment, data on use in patients with autoimmune disorders, and data on fertility effects.

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8 Appendix Adverse effects

8.1 Literature search

Database: Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions(R) <1946 to date of the search>
Date of search: March 1, 2021
Result: 29

1	("melatonin".mp. and (risk/ or risk assessment/ or risk factors/ or "Chemical and Drug Induced Liver Injury"/ or Immunosuppression/ or Endocrine Disruptors/ or Hypersensitivity/ or Food Hypersensitivity/ or Food Intolerance/ or Anaphylaxis/ or Inflammation/ or Poisoning/ or (adverse effects or toxicity or poisoning).fs. or (risk* or safety or adverse or "side effect?" or sideeffect? or hazard* or harm* or negative or toxicity or toxic or hepatotox* or "liver tox*" or nephrotox* or "nephro tox*" or "kidney tox*" or "renal tox*" or immunotox* or "immune system tox*" or "immune tox*" or "immuno tox*" or "immunosystem tox*" or "reproductive tox*" or "developmental tox*" or embryotox* or "embryo tox*" or "lung tox*" or pulmotox* or "pulmonary tox*" or "respiratory tox*" or respirotox* or neurotox* or "skin tox*" or "dermal tox*" or dermatox* or teratogenicity or teratogeneity or "endocrine tox*" or "immune effect" or "immune respons*" or "immuno respons*" or immunorespons* or immunogenesis or "immunologic respons*" or immunosuppress* or "immuno suppress*" or "immune suppress*" or "endocrine disrupt" or anaphylax* or anaphylactic or anaphylactoid or anaphylatoxin or "immune fever" or "food intoleranc*" or "Food Sensitivit*" or "nutritional intolerance*" or "nutrient intolerance*" or hypersensitiv* or hypersensitization or hypersensitisation or hyperergic or hyperergy or erethism or Allergy or Allergies or Allergic or allergen? or allergenic or sensitization or inflammation* or inflammatory or serositis or poisoning?).tw,kf.)) not (comment or editorial or letter).pt.	7868
2	limit 1 to (humans and yr="2020 -Current")	214
3	limit 1 to "all child (0 to 18 years)	568
4	limit 2 to "all child (0 to 18 years)"	29

Database: Embase 1974 to date of the search
Date of search: March 1, 2021
Result: 79

1	((("melatonin" or "melatonine*").mp. and (risk/ or risk assessment/ or risk factor/ or exp side effect/ or exp adverse drug reaction/ or adverse event/ or toxicity/ or acute toxicity/ or exp health hazard/ or hazard assessment/ or liver toxicity/ or nephrotoxicity/ or immunotoxicity/ or reproductive toxicity/ or chronic toxicity/ or embryotoxicity/ or lung toxicity/ or neurotoxicity/ or skin toxicity/ or teratogenicity/ or immune response/ or immunosuppressive treatment/ or endocrine disruptor/ or hypersensitivity/ or allergy/ or food allergy/ or food allergen/ or anaphylaxis/ or nutritional intolerance/ or inflammation/ or (risk* or safety or adverse or "side effect?" or sideeffect? or hazard* or harm* or negative or toxicity or toxic or hepatotox* or "liver tox*" or nephrotox* or "nephro tox*" or "kidney tox*" or "renal tox*" or immunotox* or "immune system tox*" or "immune tox*" or	7868

	"immuno tox*" or "immunosystem tox*" or "reproductive tox*" or "developmental tox*" or embryotox* or "embryo tox*" or "lung tox*" or pulmotox* or "pulmonary tox*" or "respiratory tox*" or respirotox* or neurotox* or "skin tox*" or "dermal tox*" or dermatox* or teratogenicity or teratogeneity or "endocrine tox*" or "immune effect" or "immune respons*" or "immuno respons*" or immunorespons* or immunogenesis or "immunologic respons*" or immunosuppress* or "immuno suppress*" or "immune suppress*" or "endocrine disrupt" or anaphylax* or anaphylactic or anaphylactoid or anaphylatoxin or "immune fever" or "food intoleranc*" or "Food Sensitivit*" or "nutritional intolerance*" or "nutrient intolerance*" or hypersensitiv* or hypersensitization or hypersensitisation or hyperergic or hyperergy or erethism or Allergy or Allergies or Allergic or allergen? or allergenic or sensitization or inflammation* or inflammatory or serositis or poisoning?).tw,kw.)) not (conference abstract* or letter* or editorial*).pt.) and Elsevier.cr.	
2	limit 1 to (humans and yr="2020 -Current")	214
3	limit 2 to (child <unspecified age> or preschool child <1 to 6 years> or school child <7 to 12 years> or adolescent <13 to 17 years>)	79

8.2 Studies excluded after full-text evaluation

An overview of the publications considered not to fulfil the eligibility criteria is given in Table 11.2-1.

Table 11.2-1. Publications considered not eligible.

Reference	Reason for exclusion
Gelfand et al. (2020)	Exposure
Iyer et al. (2020)	Outcome
Yuge et al. (2020)	Study design

8.3 Evaluation of internal validity

The detailed RoB evaluations for the eligible RCT (Barlow et al., 2020; Malow et al., 2021) is shown below. The response options and symbols used for the rating:

- Definitely low risk of bias ++
- Probably low risk of bias +
- Probably high risk of bias -
- Definitely high risk of bias - -

Barlow et al. (2020).

Type of bias	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	Was administered dose or exposure level adequately randomized?	Participants were randomly assigned by using a random-block-size design.	++
	Was allocation to study groups adequately concealed?	An independent pharmacist created sequentially numbered, identical treatment packages.	++
Performance bias	Were the research personnel and human subjects blinded to the study group during the study?	All investigators, assessors, parents and children were blinded. Melatonin and placebo pills were identical in appearance and flavour. The computer-generated randomization list was held by an external statistician.	++
Attrition/exclusion bias	Were outcome data complete without attrition or exclusion from analysis?	Loss to follow up is described.	++
Detection bias	Can we be confident in the exposure characterisation?	Purity of melatonin is not reported.	+
	Can we be confident in the outcome assessment?	Little information on the questionnaire.	+
Selective reporting bias	Were all measured outcomes reported?	All outcomes were reported.	++
Other sources of bias	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	A priori protocol was published. A sample size calculation was performed. Statistical methods were appropriate (both intention-to-treat and per-protocol analysis).	++
Overall RoB: Tier 1			

Malow et al. (2021)

Type of bias	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	Was administered dose or exposure level adequately randomized?	Participants were randomly assigned. Method not described.	+
	Was allocation to study groups adequately concealed?	No information	-
Performance bias	Were the research personnel and human subjects blinded to the study group during the study?	It is stated that the study is double-blinded, however, it is questionable whether the investigators were blinded optional. It is stated that: "decrease in dose was allowed at all times during the study, based on the investigator decision."	-

Attrition/exclusion bias	Were outcome data complete without attrition or exclusion from analysis?	Loss to follow up is described.	+
Detection bias	Can we be confident in the exposure characterisation?	Purity of melatonin is not reported. The participants were allowed to change dose during the study.	-
	Can we be confident in the outcome assessment?	Pubertal development: less participants in the intervention group	+
		Child growth	++
		Adverse events	+
Selective reporting bias	Were all measured outcomes reported?	Only aggregated data (all treatment groups combined) for the outcomes were reported.	-
Other sources of bias	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate, and researchers adhered to the study protocol)?	No protocol or sample size calculation. Statistical analysis is not adequately reported.	-
Overall RoB: Tier 3			

8.4 Data charting

Study characteristics	Title	Efficacy of Melatonin in Children With Postconcussive Symptoms: A Randomized Clinical Trial
	Author(s)	Barlow, K.M., Brooks, B.L., Esser, M.J., Kirton, A., Mikrogianakis, A., Zemek, R.L., MacMaster, F.P., Nettel-Aguirre, A., Yeates, O., Kirk, V., Hutchison, J.S., Crawford, S., Turley, B., Cameron, C., Hill, M.D., Samuel, T., Buchhalter, J., Richer, L., Platt, R., Boyd, R., Dewey, D.
	Year of publication	2020
	Country	Canada
	Funding	Canadian Institutes of Health Research (grant 293375), the Alberta Children's Hospital Research Institute, and the University of Calgary
	Reported conflict of interest	None
Methods/ intervention	Study design	Randomized, double-blind trial

	Blinding	An independent pharmacist created sequentially numbered, identical treatment packages. Melatonin and placebo tablets were identical in appearance and flavor. All investigators, outcome assessors, parents, and children were blinded to treatment groups.
	Randomisation	Participants were randomly assigned by using a random-block-size design (block sizes 3, 6, and 9) to 3 parallel treatment groups with a 1:1:1 allocation: placebo, melatonin 3 mg, and melatonin 10 mg. The computer-generated randomization list was created and held by an external statistician.
	Exposure (including duration of the study	The study drug was taken sublingually one hour before sleep time at night for 28 days and was continued even if symptom resolution occurred. No restrictions were placed on the use of other medications.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	Eligible: n=124 (n=14 refused, n=11 unable to commit to study requirements). Enrolled: n=99 children (mean age: 13.8 years; SD = 2.6 years; 58% girls). N=84 completed 6-month follow-up.
	Inclusion/exclusion criteria for participants	Children aged 8 to 18 years with PPCS and a ≥10-point increase in their total symptom score on the PCSI postinjury when compared with their preinjury score (assessed at enrollment). Children were ineligible if they had a significant medical or psychiatric history, a previous concussion within the last 3 months, persistent symptoms after a previous concussion, or a more severe TBI previously. Other exclusions included lactose intolerance, use of neuroactive drugs, and inability to complete questionnaires.
	Gender	Girls and boys
	Age	8-18
	Confounders and other variables as reported	Participants were advised to avoid analgesia overuse, abstain from contact sports, perform light exercise, and gradually return to school.
	Health and socioeconomic status of participants	

Results	Parameters measured, methods used, and measurement time points	The primary outcome was the change in Post-Concussion Symptom Inventory–Youth (PSCI-Y) total score after 28 days (filled out by the participants). Secondary outcomes were behavioral, cognitive, and sleep problems and functional impairment. The Post-Concussion Symptom Inventory–Parent (PCSI-P), a parent-proxy questionnaire, was also completed. Overnight urinary 6-sulphatoxymelatonin was analysed before treatment, mid-treatment, and at the end of treatment by using a solid-phase enzyme-linked immunosorbent assay (Cat#RE54031; IBL International, Hamburg, Germany).
	Reported outcome (for the outcome adverse effects)	Thirty-two adverse events were reported in 28 participants: placebo (n=8), melatonin 3 mg (n=13), and melatonin 10 mg (n= 11). One participant had a serious adverse event (appendicitis) unrelated to the study drug. Eight events involved a known melatonin side effect, and 5 were potentially related. Ten events were associated with a mild functional impact. The frequency and severity of adverse events did not differ between groups.
Statistical analysis	Power analysis	Data from a previous epidemiological study were used to calculate a Reliable Change Index. A 10-point change in PCSI score indicated a reliable change for subjects who have PPCS at one month and is similar to previous reports. On the basis of medical record review, the relevance of 10-point improvement in PCSI score is a difference of ~30% in overall clinical symptoms. Clinically meaningful examples of this would be a 50% reduction in posttraumatic headache burden.
	Statistical test	Statistical analyses were performed by using IBM SPSS version24 (IBM SPSS Statistics, IBM Corporation) and Stata release 14 (Stata Corp, College Station, TX).