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Risk assessment of "other substances" – Piperine

Opinion of the Panel Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics of the Norwegian Scientific Committee for Food Safety

Risk assessment of "other substances" – Piperine

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Assessed and approved

The opinion has been assessed and approved by Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics. Members of the panel are: Inger-Lise Steffensen (Chair), Ellen Bruzell, Berit Granum, Ragna Bogen Hetland, Trine Husøy, Jens Rohloff, Trude Wicklund.

(Panel members in alphabetical order after chair of the panel)

Acknowledgment

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

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has, at the request of the Norwegian Food Safety Authority (Mattilsynet, NFSA), assessed the risk of "other substances" in food supplements and energy drinks sold in Norway. VKM has assessed the risk of doses given by NFSA. These risk assessments will provide NFSA with the scientific basis while regulating the addition of "other substances" to food supplements and other foods.

"Other substances" are described in the food supplement directive 2002/46/EC as *substances other than vitamins or minerals that have a nutritional or physiological effect*. It is added mainly to food supplements, but also to energy drinks and other foods. In this series of risk assessments of "other substances", VKM has not evaluated any claimed beneficial effects from these substances, only possible adverse effects.

The present report is a risk assessment of piperine, and it is based on previous risk assessments and articles retrieved from a literature search.

According to information from NFSA, piperine, derived from black pepper, is an ingredient in food supplements sold in Norway. NFSA has requested a risk assessment of the dose 1.5 mg/day of piperine in food supplements.

The total exposure to piperine from other sources than food supplements, such as foods or cosmetics, is not included in the risk assessment.

Piperine ((E,E)-piperine) is a naturally occurring alkaloid which is the major pungent compound found in spices like black pepper (*Piper nigrum* L.) and long pepper (*Piper longum* L.), but it also occurs in Grains of Paradise (*Aframomum melegueta* K. Schum.). (E,E)-piperine is the isomeric form which is used in food supplements. Several isomers structurally related to (E,E)-piperine are found in pepper with less hot taste, including isopiperine, chavicine and isochavicine. In the European/Western cuisine, black pepper is the major source of piperine in the human diet. Other sources in the diet are piperine (pepper)-flavoured finished food products, including beverages and spirits. Piperine is also used in cosmetics as a perfuming agent (CosIng, 2016).

The range of doses reported to cause interactions with drugs and phytochemicals when studied *in vivo*, 5 to 20 mg/kg bw per day in humans and 10 to 50 mg/kg bw per day in animals (Chinta et al., 2015; Srinivasan, 2007; Srinivasan, 2013), exceeded estimated daily intake levels of piperine. Provided that the ingestion of piperine via pepper (food flavouring) or intake of dietary supplements containing *P. nigrum* or *P. longum* does not exceed common dietary levels, the risk of adverse piperine-drug and piperine-phytochemical interactions is minimal.

Based on a 90-day toxicity study in rats, a no observed adverse effect level (NOAEL) of 5 mg/kg bw per day was set in 2015 by the European Food Safety Authority (EFSA). In the present risk assessment, VKM has used this NOAEL of 5 mg/kg bw per day for the risk characterisation.

The risk characterisation is based on the margin of exposure (MOE) approach; the ratio of the NOAEL to the exposure. An acceptable MOE value for a NOAEL-based assessment of piperine based on an animal study is ≥100, which includes a factor 10 for extrapolation from animals to humans and a factor 10 for interindividual human variation.

From a daily dose of 1.5 mg piperine, the calculated intake levels are 34.6, 24.5, and 21.4 µg/kg bw per day for children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years), respectively. Using the MOE approach, for a daily intake of 1.5 mg piperine from food supplements and a NOAEL of 5 mg/kg bw per day, the MOE values are 145, 204 and 234 for children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years), respectively. Thus, for a daily intake of 1.5 mg piperine, the MOE values are above 100 for all age groups.

VKM concludes that it is unlikely that a daily dose of 1.5 mg piperine from food supplements causes adverse health effects in children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years).

Short summary

The Norwegian Scientific Committee for Food Safety (VKM) has, at the request of the Norwegian Food Safety Authority, assessed the risk of intake of 1.5 mg/day of piperine ((*E,E*)-piperine) in food supplements. Piperine is a naturally occurring alkaloid which is the major pungent compound found in spices like black pepper (*Piper nigrum* L.) and long peppers (*Piper longum* L.).

Using the MOE approach, for a daily intake of 1.5 mg piperine from food supplements and a NOAEL of 5 mg/kg bw per day based on a 90-day toxicity study in rats, the MOE values are 145, 204 and 234 for children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years), respectively. Thus, the MOE values are above 100 for all age groups.

VKM concludes that it is unlikely that a daily dose of 1.5 mg piperine from food supplements causes adverse health effects in children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years).

Key words: Adverse health effect, food supplements, piperine, negative health effect, Norwegian Food Safety Authority, Norwegian Scientific Committee for Food Safety, other substances, risk assessment, VKM

Sammendrag på norsk

På oppdrag for Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved tilsetting av «andre stoffer» i kosttilskudd og energidrikk som selges i Norge. VKM har risikovurdert ulike doser brukt av kosttilskudd og konsentrasjoner i energidrikker oppgitt fra Mattilsynet. Disse risikovurderingene vil gi Mattilsynet vitenskapelig grunnlag for å regulere andre stoffer.

«Andre stoffer» er beskrevet i kosttilskuddsdirektivet 2002/46/EC som *stoffer som har en ernæringsmessig og/eller fysiologisk effekt, og som ikke er vitaminer og mineraler*. De tilsettes i hovedsak til kosttilskudd, men også til energidrikker og andre næringsmidler. I disse risikovurderingene har VKM ikke sett på påståtte gunstige helseeffekter, men kun vurdert mulige negative helseeffekter.

I denne rapporten har VKM vurdert risiko ved inntak av piperin. Risikovurderingen er basert på tidligere risikovurderinger av piperin og artikler som er funnet ved litteratursøk.

I følge informasjon fra Mattilsynet er piperin en ingrediens i kosttilskudd som selges i Norge. Oppdraget fra Mattilsynet var å risikovurdere inntak av 1,5 mg/dag av piperin i kosttilskudd for aldersgruppene barn (10 til <14 år), ungdom (14 til <18 år) og voksne (≥18 år).

Andre kilder til piperin, som for eksempel mat og kosmetikk, er ikke inkludert i denne risikovurderingen.

Piperin ((*E,E*)-piperin) er et alkaloid som finnes naturlig i krydder som svart pepper (*Piper nigrum* L.), langpepper (*Piper longum* L.) og Guinea pepper [Grains of Paradise] (*Aframomum melegueta* K. Schum.). Piperin er hovedsakelig ansvarlig for den skarpe smaken i pepper. (*E,E*)-piperin er den isomere formen som er brukt i kosttilskudd. Det finnes flere isomere former med mindre skarp smak som er strukturelt beslektet med (*E,E*)-piperin, inkludert isopiperin, chavicin og isochavicin, i pepper. I det europeiske/vestlige kostholdet er svart pepper den viktigste kilden til piperin. Andre kilder i kosten er ferdigmat smaksatt med piperin (pepper), også inkludert drikkevarer og brennevin. Piperin er også brukt i kosmetikk som duftstoff (CosIng, 2016).

Det er rapportert fra *in vivo* studier med piperin at 5-20 mg/kg kroppsvekt per dag hos mennesker og 10-50 mg/kg kroppsvekt per dag hos dyr kan gi interaksjoner med legemidler og plantestoffer (Chinta et al., 2015; Srinivasan, 2007; Srinivasan, 2013). Dette er høyere enn estimert daglig inntak av piperin. Forutsatt at inntak av piperin fra pepper (som krydder) eller inntak av kosttilskudd som inneholder *P. nigrum* eller *P. longum* ikke er høyere enn det som er estimert daglig inntak, er risikoen for interaksjon med legemidler og plantestoffer minimal.

I 2015 ble en NOAEL (no observed adverse effect level; «null-effektsnivå»)-verdi på 5 mg/kg kroppsvekt per dag fastsatt av den europeiske myndighet for næringsmiddeltrygghet (EFSA) ut i fra en 90-dagers toksisitetsstudie i rotter.

Risikokarakteriseringen er basert på beregning av eksponeringsmargin («margin of exposure» (MOE)), som er ratio mellom NOAEL-verdien og eksponeringen. En akseptabel MOE-verdi for piperin i en risikovurdering basert på NOAEL fra et dyreforsøk er 100, som inkluderer en faktor 10 for ekstrapolering fra dyr til mennesker og en faktor 10 for interindividuell variasjon mellom mennesker.

Ved en daglig dose på 1,5 mg piperin blir inntaket 34,6, 24,5 og 21,4 mg/kg kroppsvekt per dag for henholdsvis barn (10 til <14 år), ungdom (14 til <18 år) og voksne (≥18 år). Ved denne eksponeringen blir MOE-verdiene 145, 204 og 234 for henholdsvis barn (10 til <14 år), ungdom (14 til <18 år) og voksne (≥18 år). For alle aldersgrupper ligger de beregnede MOE-verdiene over 100.

VKM konkluderer at det er usannsynlig at en daglig dose på 1,5 mg piperin fra kosttilskudd forårsaker negative helseeffekter hos barn (10 til <14 år), ungdom (14 til <18 år) og voksne (≥18 år).

Kort sammendrag

På oppdrag fra Mattilsynet har Vitenskapskomiteen for mattrygghet (VKM) vurdert risiko ved inntak av 1,5 mg piperin ((*E,E*)-piperin) i kosttilskudd. Piperin er et alkaloid som finnes naturlig i krydder som svart pepper (*Piper nigrum* L.), langpepper (*Piper longum* L.) og Guinea pepper [Grains of Paradise] (*Aframomum melegueta* K. Schum.). Piperin er hovedsakelig ansvarlig for den skarpe smaken i pepper.

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Abbreviations and glossary

Abbreviations

ADI	- acceptable daily intake
ADME	- absorption, distribution, metabolism, excretion
ALP	- alkaline phosphatase
ALT	- alanine aminotransferase
AST	- aspartate aminotransferase
bw	- body weight
CYP	- cytochrome P450
EFSA	- European Food Safety Authority
GRAS	- Generally Recognized as Safe
MNT	- micronucleus test
MOE	- margin of exposure
MTD	- maximum tolerated dose
MSDI	- maximised survey-derived daily intake
NBT	- nitroblue tetrazolium
NFSA	- Norwegian Food Safety Authority [*norw*.: Mattilsynet]
NOAEL	- no observed adverse effect level
NOEL	- no observed effect level
P-gp	- P-glycoprotein
PHA	- phytohemagglutinin
TTC	- threshold of toxicological concern
VKM	- Norwegian Scientific Committee for Food Safety [*norw*.: Vitenskapskomiteen for Mattrygghet]

Glossary

"Other substances": a substance other than a vitamin or mineral that has a nutritional or physiological effect (EU, 2006).

"Negative health effect" and "adverse health effect" are broad terms. VKM uses the definition endorsed by EFSA for "adverse 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 (EFSA, 2006; WHO, 1994).

Background as provided by the Norwegian Food Safety Authority

«Other substances» are substances other than vitamins and minerals, with a nutritional and/or physiological effect on the body. "Other substances" are mainly added to food supplements, but these may also be added to other foods and beverages, such as sports products and energy drinks. Ingestion of these substances in high amounts presents a potential risk for consumers.

In Norway, a former practice of classification of medicines had constituted an effective barrier against the sale of potentially harmful "other substances". Ever since this practice was changed in 2009, it has become challenging to regulate and supervise foods with added "other substances". Meanwhile, in the recent years, the Norwegian market has witnessed a marked growth in the sales of products containing "other substances". In 2011, food supplements containing "other substances" constituted more than 50% of the market share.

While within the European Economic Area, these substances fall under the scope of the European Regulation (EC) No. 1925/2006 on the addition of vitamins, minerals and certain other substances to foods and the European Regulation (EC) No 258/97 concerning novel foods and novel food ingredients, "other substances" remain largely unregulated. In order to ensure safe use of "other substances" many countries have regulated their use at a national level. For example, Denmark regulates these substances in a positive list, i.e. a list of substances with maximal daily doses, permitted for use in food supplements and other foods (FVM, 2014).

The Norwegian Food Safety Authority (NFSA) is working on the establishment of a regulation on the addition of "other substances" to foods at a national level. The regulation will include a list of substances with permitted maximal doses, based on the substances and doses found in products on the Norwegian market. In preparation for a regulation, NFSA has therefore requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of "other substances" found on the Norwegian market. NFSA, in consultation with the industry, has compiled a list of "other substances" found in products marketed in Norway. Only substances with a purity of minimum 50% or concentrated 40 times or more have been included in the list. Substances regulated by other legislations like those for novel foods, food additives, flavourings, foods for special medical purposes etc., have been excluded from the list.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority (NFSA) requested the Norwegian Scientific Committee for Food Safety (VKM) to assess the safety of piperine in food supplements at the following dose: 1.5 mg/day.

NFSA requested VKM to assess the safety of "other substances" (in accordance to the guidance document developed in Phase 2) at the doses specified (Phase 3). Safety assessments of "other substances" present in food supplements shall be carried out for a general population, ages 10 years and above.

Assessment

1 Introduction

"Other substances" are described in the food supplement directive 2002/46/EC *as substances other than vitamins or minerals that have a nutritional or physiological effect,* and may be added to food supplements or e.g. energy drinks (The European Parliament and the Council of the European Union, 2006).

This risk assessment regards the substance piperine per se, and no specific products.

VKM has in this series of risk assessments of "other substances" not evaluated documentation of any potential beneficial effects from these substances, but merely possible adverse effects at specified doses used in Norway. Thus, potential high intake consumer groups of the substance may not be identified and therefore not included in the assessment.

According to information from the Norwegian Food Safety Authority (NFSA), piperine, derived from black pepper, is an ingredient in food supplements purchased in Norway. NFSA has requested a risk assessment of 1.5 mg of piperine per day in food supplements. The total exposure to piperine from other sources than food supplements, such as foods, is not included in the risk assessment.

Piperine ((*E,E*)-piperine) (CAS no. 94-62-2) is a naturally occurring alkaloid, which is the major pungent compound found in spices like black pepper (*Piper nigrum* L.) and long pepper (*Piper longum* L.), but it also occurs in Grains of Paradise (*Aframomum melegueta* K. Schum.). (*E,E*)-piperine is the isomeric form which is used in food supplements. Several isomers structurally related to (*E,E*)-piperine are found in pepper with less hot taste, including isopiperine, chavicine and isochavicine (Attokaran, 2011). In the European/Western cuisine, black pepper is the major source of piperine in the human diet. Other sources in the diet are piperine (pepper)-flavoured finished food products, including beverages and spirits. According to (EFSA, 2015) the estimated exposure to piperine from natural sources when consuming black pepper as flavouring ingredient is 6.2 µg/day and 0.07 µg/day in the EU and USA, respectively

Piperine is rapidly absorbed in the intestine of rats, metabolised and excreted as piperonylic acid, piperonyl alcohol, piperonal and vanillic acid (Bhat and Chandrasekhara, 1987). Physiological effects of piperine include stimulation of digestive enzymes, increased digestive capacity (Srinivasan, 2007) and alterations in membrane dynamics and permeation characteristics, and thus, facilitated permeation through the epithelial barrier (Khajuria et al., 2002). Piperine shows inhibitory interaction with drug metabolising cytochrome P450 (CYP) enzymes of the liver and small intestine and P-glycoprotein (P-gp) (Chinta et al., 2015; Srinivasan, 2007). Excess intake of >10 mg doses of piperine due to high consumption of

pepper or intake of dietary supplements containing *P. nigrum* or *P. longum* above common dietary levels, might lead to clinically significant interactions with several drugs (Gurley et al., 2012).

Based on the MSDI (maximised survey-derived daily intake) approach, the estimated exposure to piperine from natural sources when consuming black pepper as flavour ingredient, is 6.2 µg/day and 0.07 µg/day in the EU and USA, respectively (EFSA, 2015).

In the present risk assessment, VKM has assessed the intake of 1.5 mg per day piperine from food supplements.

2 Hazard identification and characterisation

2.1 Literature

The present risk assessment is based on previous risk assessments of piperine and articles retrieved from a literature search.

2.1.1 Previous risk assessments

Evaluation of certain food additives - Joint FAO/WHO Expert Committee on Food Additives (JECFA). World Health Organization (JECFA, 2006a)

The JECFA committee evaluated the safety of various food additives in view of acceptable daily intakes (ADI), and specifications for their identity and purity. A no observed effect level (NOEL) of 20 mg/kg body weight (bw) per day for piperine was derived from a 56-day feeding study in rats. In this study, groups of 6 rats were given various doses of black pepper or oleoresin corresponding to up to approximately 20 mg/kg bw per day or 100 mg piperine/kg feed corresponding to up to approximately 10 mg/kg bw per day (Bhat and Chandrasekhara, 1986a). The NOEL was 50 000 times the estimated exposure to piperine from its reported use as a flavouring agent in Europe (0.4 µg/kg bw per day) and 20 000 000 times that in the USA (0.001 µg/kg bw per day). The Committee concluded that the margin between the estimated current exposures to piperine intended to be used as a flavouring agent, and the NOEL for this agent was adequate, and its use would not present a safety concern. Although the use of piperine would raise no safety concern at estimated exposure levels, JECFA pointed out that less uncertain exposure estimates were needed. JECFA considered this dose as the NOAEL.

SCIENTIFIC OPINION. Flavouring Group Evaluation 86, (FGE.86). Consideration of aliphatic and aromatic amines and amides evaluated by JECFA (65th meeting). The European Food Safety Authority (EFSA, 2008)

and

Scientific Opinion on Flavouring Group Evaluation 86, Revision 1 (FGE.86Rev1): Consideration of aliphatic and aromatic amines and amides evaluated by JECFA (65th meeting). The European Food Safety Authority (EFSA, 2011)

EFSA assessed and revised the safety of various aliphatic and aromatic amines and amides based on the previous JECFA evaluation (JECFA, 2006a). Based on the JECFA assessment from 2006 and studies cited therein, EFSA concluded that piperine is not genotoxic *in vitro* and *in vivo*. EFSA pointed out that no histopathology was performed. EFSA disagreed with

JECFA and concluded that the original 56-day feeding study in rats (Bhat and Chandrasekhara, 1986a) was not appropriate for deriving the NOEL (NOAEL) of 20 mg/kg bw per day for piperine. Accordingly, additional information including more recent toxicity data would be required.

Scientific Opinion on Flavouring Group Evaluation 86, Revision 2 (FGE.86Rev2): Consideration of aliphatic and arylalkyl amines and amides evaluated by JECFA (65th meeting). The European Food Safety Authority (EFSA, 2015)

In Revision 2 on the Flavouring Group Evaluation 86, the safety of piperine intended to be used as flavouring substance in food was re-evaluated. A stepwise approach was used to integrate information on structure-activity relationships, intake from current uses, threshold of concern and recent data on metabolism and toxicity. According to the concept of Threshold of Toxicological Concern (TTC) (EFSA, 2012c; EFSA/WHO, 2016), the TTC value for piperine was 90 µg/person per day.

The opinion included evaluation of additional toxicity data for piperine. A new 90-day rat study with piperine was performed according to OECD Guideline (TG 408) (Bauter, 2013) with an average daily intake of 0 (vehicle), 5, 15 or 50 mg/kg bw per day for males and females for at least 90 days. Prior to study initiation and again on day 86, the eyes of all rats were examined by focal illumination and indirect ophthalmoscopy. The animals were observed for viability, signs of gross toxicity and behavioral changes at least once daily during the study, and weekly for a battery of detailed clinical parameters. For further details from the study, please see 2.4.2 Animal studies.

Based on the dose-dependent increase in plasma cholesterol levels in males at the mid and high dose, the EFSA Panel decided that the lowest dose level of 5 mg/kg bw per day should be considered as the NOAEL (EFSA, 2015). The original study and included data thererin was not available and has not been reviewed by VKM.

Simultaneously, the Panel overruled JECFAs adoption of the NOAEL of 20 mg/kg bw per day from 2006 (JECFA, 2006a), and concluded that the original study by Bhat and Chandrasekhara (1986a) was not appropriate for deriving a NOAEL. Based on the MSDI approach of 6.2 µg *per capita* per day compared to the NOAEL for piperine, an adequate margin of safety of more than $4.8 \times 10\,000$ can be calculated for piperine (EFSA, 2015).

Scientific Opinion on the Safety and efficacy of pyridine and pyrrole derivatives belonging to chemical group 28 when used as flavourings for all animal species - EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). The European Food Safety Authority (EFSA, 2016)

The FEEDAP Panel supported the NOAEL of 5 mg/kg bw per day of piperine established in the Scientific Opinion on Flavouring Group Evaluation 86, Revision 2 (EFSA, 2015).

2.1.2 Summary of previous risk assessments

In the present opinion, VKM uses the NOAEL of 5 mg piperine/kg bw per day, established by EFSA (2015), for the risk characterisation of piperine as an ingredient in food supplements.

2.1.3 Literature search

2.1.3.1 Search strategy

Literature searches were performed in Embase and Medline in order to retrieve publications on adverse effects caused by piperine. These databases were chosen to ensure comprehensive study retrieval. The literature searches were performed in January 2016. The strategy for the search is included in Appendix 1.

2.1.3.2 Publication selection

The literature search identified 44 articles. In the primary screening titles and abstracts of all unique publications retrieved were independently screened against the inclusion criteria checklist.

Inclusion criteria checklist:

- Adverse effects in relation to the substance alone are addressed
- Route of exposure for humans is oral
- Route of exposure for animals is oral, in addition, subcutaneous exposure is included if the toxicokinetic is equal to oral exposure
- Human studies are performed in apparently healthy individuals or patient groups assumed to have normal absorption and metabolism of the assessed substance
- Animal model studies address adverse effects relevant to human health

The inclusion criteria checklist was developed by members of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food and Cosmetics and the Panel on Nutrition, Dietetic Products, Novel Food and Allergy. Articles that did not appear to meet the inclusion criteria were excluded from further analysis. In situations where it was unclear whether the publication was of relevance to the study, it was retained for further screening. The primary screening was performed independently by two persons.

The full text of articles (14) that passed the primary screening was retrieved for secondary screening. In this screening, the full text articles were reviewed and compared against the inclusion criteria checklist. The secondary screening was performed by one person.

The secondary screening resulted in 2 full text articles. Additionally, 6 studies from manual search/retrieval of relevant literature cited in the full-text papers have been identified and are included. A final total of 8 publications were identified and included in the results in this report (see Figure 2.1.3.2-1).

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│          Main search            │
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        │ Titles and abstracts │───▶│ Publications not fulfilling the │
        │      n = 44      │        │ inclusion criteria were excluded │
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        │    Full text     │───▶    │ Publications not fulfilling the │
┌───────────────────┐ n = 14 │     │ inclusion criteria were excluded │
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│ 6 publications were │             └────────────────────────────────┘
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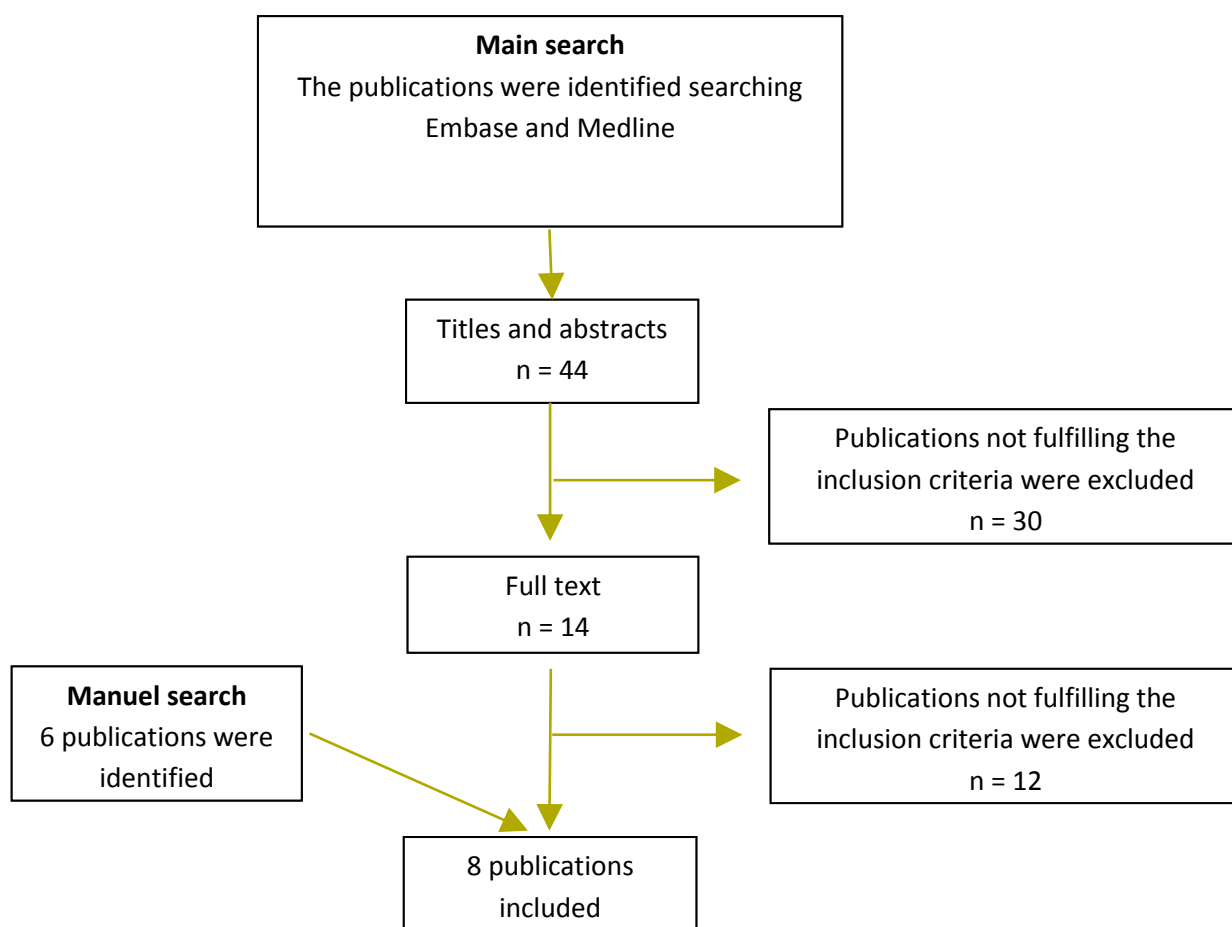
Figure 2.1.3.1-1   Flow chart for the literature search for piperine and the subsequent publication selection.

2.2 General information

2.2.1 Chemistry

Piperine is a naturally occurring alkaloid. The molecular formula of piperine (CAS No. 94-62-2; EINECS no. 202-348-0) is $C_{17}H_{19}NO_3$ and the molecular weight is 285.34 g/mol. EFSA commented that the registered name is changed to (*E,E*)-piperine (EFSA, 2015). The IUPAC name is (2E,4E)-5-(1,3-benzodioxol-5-yl)-1-(1-piperidinyl)-2,4-pentadien-1-one. Different piperine isomers occur in black pepper, with the *trans-trans* form of (*E,E*)-piperine being the most pungent and abundant isomer (normally >70%). Structurally-related isomers with less hot taste include isopiperine (*cis-trans*) , chavicine (*cis-cis*) and isochavicine (*trans-cis*) (Attokaran, 2011). *Piper nigrum* is the major natural source for extraction and production of piperine for use as food additive, but it can also be produced by chemical synthesis (EFSA, 2016). Black pepper contains oleoresin (3.5 to 12%) as a mixture of essential oils and piperine alkaloids, which can be extracted by supercritical fluid or solvent extraction. The solvent-free oleoresin contains ≥30% piperine and is often used instead of pepper in processed foods. The structural formula of piperine is shown in Figure 2.2.1-1.
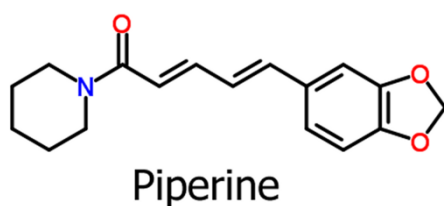

Piperine

Figure 2.2.1-1   The structural formula of piperine.

2.2.2 Occurrence

Piperine is the major pungent compound found in spices like black pepper (*Piper nigrum* L.) and long pepper (*Piper longum* L.) (Srinivasan, 2007), but also occurs in the spice Grains of Paradise (*Aframomum melegueta* K. Schum.) from West Africa (EFSA, 2012a). Black pepper is one of the most widely traded and used spices in the world. Black pepper is obtained from immature or almost ripe berries after drying/fermentation. Green pepper is produced from immature pepper fruits under controlled conditions retaining the green colour, while white pepper is obtained from fully ripe or black pepper upon removal of the pericarp (skin). Piperine content of commonly consumed dried black pepper ranges between 4-6%, with contents up to 10%, with slightly higher levels in white pepper (Attokaran, 2011). Other potential sources of piperine include piperine (pepper)-flavoured finished food products, including beverages and spirits. Piperine is also used in cosmetics as a perfuming agent (CosIng, 2016). Based on the MSDI approach, the estimated exposure to piperine from natural sources when consuming black pepper as flavouring ingredient is 6.2 μg/day and 0.07 μg/day in the EU and USA, respectively (EFSA, 2015).

2.3 Absorption, distribution, metabolism and excretion (ADME)

2.3.1 In humans

Piperine dissolves very slowly in water, but is quickly absorbed across the intestinal border and assumed to be readily metabolised (please see 2.3.2 Animal studies). According to a pharmacokinetic study in humans, no accumulation after daily oral doses of 20 mg piperine for 7 days was observed in blood serum (Wang et al., 2010).

2.3.2 Animal studies

About 97% of piperine was absorbed in the intestine when administered to male albino rats by gavage (170 mg/kg bw per day), while the remaining 3% was excreted as piperine in the feces. Piperine was not detectable in urine (limit of detection was unknown), indicating a complete metabolic conversion of this compound. Examination of the passage of piperine through the gut indicated that the highest concentration in the stomach and small intestine was attained at about 6 h. Only traces (less than 0.15%) of piperine were detected in serum, kidney and spleen from 30 min to 24 h. For intraperitoneally administered piperine (85 mg/kg bw per day), about 1–2.5% of the dose was detected in the liver 0.5–6 h after administration, in contrast to 0.1–0.25% of the orally administered dose (Bhat and Chandrasekhara, 1986b).

After oral administration of piperine (170 mg/kg bw) to rats, the metabolites in urine (0–96 h) were identified to be piperonylic acid, piperonyl alcohol, piperonal and vanillic acid in the free form, whereas only piperic acid was detected in 0–6 h bile (Bhat and Chandrasekhara, 1987). The kidney appears to be the major excretion route for piperine metabolites in rats as no metabolite could be detected in feces. In a more recent study, a new major urinary metabolite was detected in rat urine and plasma and characterized as 5-(3,4-methylenedioxy phenyl)-2,4-pentadienoic acid-N-(3-yl propionic acid)-amide (Bajad et al., 2003).

2.4 Toxicological data/Adverse effects

2.4.1 Human studies

There were no studies on toxicity of piperine in humans in the included literature, when used orally in amounts which are commonly found in piperine-containing spices such as pepper (*Piper nigrum*, *Piper longum*), Grains of Paradise (*Aframomum melegueta*), and pepper-seasoned finished food products, beverages and spirits. High acute oral consumption through administration of a test meal containing 1.5 g black pepper led to gastric mucosal injury (Myers et al., 1987).

2.4.1.1 Interactions

Several studies have reported the potential of piperine to influence drug metabolising enzyme systems and affect the bioavailability of drugs. Piperine shows inhibitory interaction with drug metabolising cytochrome P450 enzymes of the liver and small intestine, namely CYP1A2, CYP1A1, CYP2D6, CYP3A4 and P-gp efflux transporter, thus supporting drug bioavailability-enhancing effects studied in humans *in vivo* (Chinta et al., 2015; Srinivasan, 2007; Srinivasan, 2013). Inhibitory activity has been mainly studied *in vitro* and in animal models, reporting effects in drug metabolism due to inhibition or decrease in activity of aryl hydroxylation, N-demethylation, O-deethylation, glucuronidation, UDP-glucose dehydrogenase and UDP- glucuronyl transferase. Interactions in humans were observed using piperine doses between 5 to 20 mg/kg bw per day, either applied as a single dose or continous dosage (7-21 days), and in combination with single/double doses of respective drugs or phytochemicals. The improved bioavailability is reflected in various pharmacokinetic parameters, among others t_{max}, C_{max}, half-life and area under curve (AUC) values. Enhanced drug bioavailability might be explained as an effect of facilitated absorption from the intestine (due to inhibition of xenobiotic transporters) and/or by protection from being metabolised in the liver (due to CYP enzyme inhibition).

Clinical studies have shown the effect of piperine to increase plasma levels of pharmaceutical drugs and bioactive phytochemicals in humans including other substances, e.g. the antiretroviral drug nevirapine against HIV-1 infection and AIDS (Kasibhatta and Naidu, 2007), β-blocker propranolol and asthma drug theophylline (Bano et al., 1991), the anti-epileptic drug phenytoin (Velpandian et al., 2001), the antiinflammatory phytochemical curcumin (Shoba et al., 1998) and coenzyme Q10 (Badmaev et al., 2000). The latter is the only long-term study (21 days) which tested the bioavailability of this nutritional compound based on a continuous and concurrent daily ingestion with piperine at levels of 5 mg/kg bw per day. The statistically significant increase in bioavailable coenzyme Q10 indicated that chronic ingestion of relatively high levels of piperine might potentially interact with the metabolism of drugs, phytochemicals and other substances (see also 2.4.2.2 Interactions).

Recent reports also point to the potential of piperine to enhance the bioefficacy and potency of ingested substances (resveratrol) without altering bioavailability (Wightman et al., 2014), possibly due to thermogenic and heat-proffering properties of piperine affecting resveratrol activity, neuronal vasculature and cerebral blood flow. It is also worth mentioning that sex-dependent variability of CYP3A activity might lead to different effects of drug co-administered piperine, as observed in male and female volunteers pre-treated with piperine and subsequent application of the sedativum and muscle-relaxing drug midazolam (Rezaee et al., 2014). The inhibitory potential for piperine conjugates has not yet been evaluated, but it is unlikely that they are more potent enzyme inhibitors than the original piperine molecule (Volak et al., 2013).

In both *in vivo* and *in vitro* studies, herb-drug interactions and modulation of the digestive system have been widely studied and are briefly discussed in Section 2.4.2.2. Pepper (*Piper*

nigrum and *Piper longum*) with its major active ingredient piperine has traditionally been used in folk and Ayurvedic medicine for thousands of years to cure gastric disturbances, stimulate digestion and relieve diarrhea (Srinivasan, 2013). In this context it should be emphasized that ingestion of piperine in excess of 10 mg doses due to high consumption of pepper (food flavouring) or intake of dietary supplements containing *P. nigrum* or *P. longum* above common dietary levels, might lead to clinically significant drug interactions (Gurley et al., 2012).

In view of the dichotomous effects of piperine on CYP enzymes and P-gp, i.e. inhibitory activity on the one hand, and enhanced gene and protein expression on the other hand, as described in animals (see 2.4.2.2 Interactions), further studies are required in order to reveal long-term effects of orally applied piperine on the pharmacokinetics of drugs, phytochemicals and other substances in humans.

2.4.1.2 Allergic sensitisation (including adjuvant effects)

There was no information concerning allergic sensitisation or allergy adjuvant effects in the literature reviewed on human studies in the present risk assessment. The absence of information in the selected literature does not document an absence of allergic sensitisation or allergy adjuvant effects.

2.4.2 Animal studies

An overview of the included studies on adverse effects of piperne in animals is given in Table 2.4.2-1.

Table 2.4.2-1   An overview of animal studies on adverse effects of orally-applied piperine.

Reference	Study	Dose and number in treatment group		Conclusion with regard to adverse effects
		Piperine	Control	
Rao et al. (2015)	Effect on liver function in CF-1 albino mice on a high-cholesterol diet Duration: 3 weeks	5 mg piperine/kg bw per day; n=20 animals	Control group, no piperine; n=10 animals	Hepatic dysfunction, significant increase of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in serum, and decrease of total protein; potential hepatocyte damage due to altered membrane permeability.
Thiel et al. (2014)	Genotoxicity study *in vivo* in NMRI BR mice Duration: 2 days	143.5, 287.0 and 574.0 mg/kg bw per day; n=10 animals/sex/group	Control groups with vehicle, chlorpromazine and cyclo-phosphamide; n=5 animals/sex/group	Not genotoxic; no increase in frequency of micronuclei in the *in vivo* micronucleus test (MNT).
Bauter (2013), as quoted by EFSA (2015)	Dietary toxicity study in Crl:Sprague-Dawley® CD® IGS rats Duration: 90 days	5, 15 or 50 mg/kg bw per day for males (M) and females (F); n=10 animals/sex/group	Control group, no piperine (vehicle); n=10 animals/sex	Dose-dependent increase in plasma cholesterol levels at doses of 15 and 50 mg/kg bw per day. NOAEL of 5 mg/kg bw per day.

Reference	Study	Dose and number in treatment group		Conclusion with regard to adverse effects
Da Silva Cardoso et al. (2009) [Portuguese]	Dietary toxicity study in 7-day old male chicks (Cobb Avian 48) Duration: 14 days	1.12, 2.25 and 4.50 mg/kg bw per day; n=15 animals/group	Control group, no piperine (vehicle)	Liver histopathological changes were noticed in a dose-dependent manner; increase in hepatocyte degeneration, steatosis, bile duct proliferation (2.25 and 4.50 mg/kg bw per day) and bile duct necrosis (4.50 mg/kg bw per day). The reported dose-response effects were not always consistently statistically significant, conclusive or were partly contrasting regarding the highest dose at 4.5 mg. No specific clinical or serum markers of hepatotoxicity were assessed (only visual observation).
Dogra et al. (2004)	Immunotoxicological study in 6-week old male mice Duration: 5 days	1.12, 2.25 and 4.50 mg/kg bw per day; n=6 animals/group	Control group, no piperine (vehicle); n=6 animals	Reduction in total leucocytes, increase in % neutrophils; suppression of mitogenic response of B-lymphocyte to lipopolysaccharide; number of IgM forming cells in spleen and level of primary antibody in serum was decreased (2.25 and 4.50 mg/kg bw per day). Suggested NOAEL of 1.12 mg/kg bw per day. The reported dose-response effects were not always consistently statistically significant, conclusive or were partly contrasting regarding the highest dose at 4.5 mg.

Reference	Study	Dose and number in treatment group		Conclusion with regard to adverse effects
Daware et al. (2000)	Reproductive toxicity study in Swiss albino female mice, 10-13-week old Duration: 14 days of treatment, 3 consecutive cycles	10 and 20 mg/kg bw per day; n=6 animals/group	Control group, no piperine (vehicle)	Interference with crucial reproductive events: increase of diestrous phase (both doses) with decreased mating performance (10 mg) and decreased fertility index (both doses); anti-implantation activity after 5 days post-mating piperine treatment (both doses).
Malini et al. (1999)	Effect on male reproductive organs and spermatogenesis in 3-4 months old albino rats Duration: 30 days	5 and 10 mg/kg bw per day; n=10 animals/group	Control group, no piperine (vehicle)	Partial degeneration of germ cell types (5 mg/kg bw per day); severe damage to seminiferous tubule, decrease in seminiferous tubular and Leydig cell nuclear diameter, desquamation of spermatocytes and spermatids, decrease in caput and cauda epididymal sperm concentrations, increase in serum gonadotropins, and decrease in intratesticular testosterone (10 mg/kg bw per day).
Bhat and Chandrasekhara (1986a)	Dietary toxicity study in 4-week old weanling, male Wistar rats Duration: 8 weeks	5, 10 and 20 mg/kg bw per day of piperine from black pepper oleoresin, and 10 mg/kg bw per day piperine; n=6 animals/group	Control group, no piperine (basal diet)	No effect on food intake, feed efficiency, organ weights, hematological parameters or clinical chemistry values; non-dose-dependent variations were observed in nitrogen and fat absorption and retention of nitrogen in some groups of treated rats. NOAEL of 20 mg/kg bw per day.

In a recent study by Rao et al. (2015), the effect of piperine on liver function of CF-1 albino mice was investigated. A total of 30 CF-1 albino mice were fed high cholesterol diet and divided into two groups, 20 mice were administered piperine at a dose of 5 mg/kg bw per day, while the control group (n=10) did not receive any piperine. Blood was drawn from each mouse before the study and after 3 weeks by cardiocentesis. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total protein were measured. Serum ALT was significantly elevated (P=0.0002) in mice administered piperine for 3 weeks compared to the control. Serum AST (P=0.046) and ALP (P= 0.0001) was both significantly increased after the administration of piperine. Serum total protein (P= 0.011) values were significantly decreased in the treatment group. The study indicated a potential damage to liver with piperine extract, and suggests further research to prove these findings.

Based on a recent 90-days study in Crl: Sprague-Dawley® CD® IGS rats (Bauter, 2013) submitted to EFSA by the Flavour Industry, EFSA evaluated the dietary toxicity of orally administered piperine (EFSA, 2015). The study was performed according to OECD Guideline (TG 408). Four groups of adult rats (10/sex/group) were given average daily intake levels of 0 (vehicle), 5, 15 or 50 mg/kg bw per day for males and females for at least 90 days. Piperine was shown to be homogeneously distributed, stable and considered to have met target concentrations in the diet for all intake levels. No mortalities, clinical or ophthalmological changes could be attributed to piperine administration. Decreased male body weight gain (20%) and male (15%) and female (12%) reduction of food consumption at 50 mg/kg bw per day intake were considered the result of decreased food intake related to administration of high dietary concentrations of piperine, since no effect on food efficiency was found. No effect was observed on the final body weights. No gross and microscopic changes or clinical pathology or organ weight changes attributed to piperine administration were observed. Only minor statistically significant and not dose-dependent changes in hematology, coagulation and clinical chemistry parameters were found. A statistically significant and dose-dependent increase in cholesterol levels in males with approximately 30% and 55% was observed at piperine levels of 15 and 50 mg/kg bw per day, respectively, while no effect was observed in females.

A 14-days dietary toxicity study in 7-days old male chicks (Cobb Avian 48) was carried out by oral administration at 0.00, 1.12, 2.25 and 4.50 mg/kg bw per day of piperine for 14 days (Da Silva Cardoso et al., 2009). Sixty male chicks were randomly allocated to four experimental groups (n=15). Parameters such as body weight gain, liver relative weight, hematological, anatomical and pathological alterations were recorded. The oral route administration did not influence on weight gain or liver relative weight, and no modification on size and organs' colour and/or parenchyma/mucous membranes injuries were observed. Piperine did not alter hematological parameters, except for leukocytes counting, which caused a significant increase of heterophils (≥1.12 mg/kg bw per day) and total number of leukocytes (≥2.25 mg/kg bw per day). Liver histopathological changes were noticed in a dose-dependent manner, including an increase in hepatocyte degeneration, steatosis, bile duct proliferation at piperine levels of 2.25 and 4.50 mg/kg bw per day, and bile duct

necrosis at levels of 4.50 mg/kg bw per day. The results suggest that 1.12 mg/kg bw per day of piperine orally administrated was not liver toxic for broiler chicks.

The immunotoxicological effects of piperine were investigated in Swiss male mice, gavaged at a dose of 1.12, 2.25 or 4.50 mg/kg bw per day for five consecutive days (Dogra et al., 2004). No toxic effect at any dose level was observed, and the liver weight gain was normal. Treatment at highest dose (4.50 mg/kg bw per day), however, resulted in significant decrease in the weight of spleen, thymus and mesenteric lymph nodes, but not of peripheral lymph nodes. All dose levels suppressed the cellular population of lymphoid organs, except for the spleen, where the doses of 1.12 and 2.25 mg/kg bw per day caused a significant increase in cell number. Hematologically, doses of 2.25 and 4.50 mg/kg bw per day caused a significant reduction in total leucocyte and differential leucocyte counts. The highest dose of 4.50 mg/kg bw per day suppressed the mitogenic response of B-lymphocyte to lipopolysaccharide (LPS), and significantly reduced the mitogenic response of T-lymphocytes to phytohemagglutinin (PHA) and the nitroblue tetrazolium (NBT) dye reducing activity of peritoneal exudate cells (PECs), while doses of 1.12 and 2.25 mg/kg bw per day induced only non-significant increase of PHA and NBT values. Since the lowest dose of 1.12 mg/kg bw per day of piperine had no immunotoxic effect, it may be considered as the immunological NOAEL dose.

In a 14-days study in Swiss albino female mice (10 to 13-week old), the reproductive toxicity of piperine was studied (Daware et al., 2000). Relevant short-term tests were employed to assess the effect on estrous cycle, mating behaviour, toxicity to male germ cells, fertilization, implantation and growth of pups. Piperine (10 and 20 mg/kg bw per day) increased the period of the diestrous phase (non-significantly) which resulted in decreased mating performance (20 mg/kg bw per day) and fertility index (10 and 20 mg/kg bw per day). Post-partum litter growth was not affected by the piperine treatment. Sperm shape abnormalities were not induced when tested in male mice at doses of 35, 50 and 75 mg piperine/kg bw per day. Significantly increased anti-implantation activity was recorded after 5 days post-mating oral treatment with piperine (10 and 20 mg/kg bw per day). The sex ratio and post-implantation loss were unaffected after treatment with piperine. No histopathological changes were detected in the ovary or the uterus at the cellular level. The authors concluded that the results indicated that piperine might interfere with several crucial reproductive events in a mammalian model showing significantly reduced implantation values.

The effect of orally administered piperine (5 and 10 mg/kg bw per day) on male reproductive organs and spermatogenesis in 3 to 4-month old albino rats was investigated in a 30-day study (Malini et al., 1999). Histological studies revealed that piperine at 5 mg/kg bw per day caused partial degeneration of germ cell types, whereas 10 mg/kg bw per day caused severe damage to the seminiferous tubule, decrease in seminiferous tubular and Leydig cell nuclear diameter and desquamation of spermatocytes and spermatids. Correlated to the structural changes, a fall in caput and cauda epididymal sperm concentrations was also evident. A 10 mg/kg bw per day dose of piperine also caused a marked increase in serum gonadotropins and a decrease in intratesticular testosterone concentration, despite normal serum

testosterone titres. The study indicated that piperine is a putative antispermatogenic agent, however the exact site and mechanism underlying this effect still remain elusive, and further studies are required, according to the authors.

Bhat and Chandrasekhara (1986a), as quoted by WHO (2006a), investigated effects of black pepper, its oleoresin and piperine in weanling rats. In this study, groups of six male Wistar rats (4- week-old weighing 59-65 g) were fed diets containing black pepper oleoresin at a concentration of 110, 220 and 440 ppm, pepper at 2000 ppm, or piperine at 100 ppm for a period of 8 weeks. The dietary levels of pepper and oleoresin were intended to be approximately 5-20 times the normal daily intake of humans. Given that the piperine content of the black pepper oleoresin was approximately 45%, the dietary levels of piperine resulting from feeding of the oleoresin were approximately 50, 100 and 200 ppm. These levels of piperine in pepper oleoresin (45% piperine) were calculated to provide average daily intakes of approximately 5, 10 and 20 mg/kg bw per day of piperine, respectively. The 100 ppm dietary level of piperine was calculated to provide an average intake of approximately 10 mg/kg bw per day. A group of control animals was maintained on basal diet. Ingestion of black pepper oleoresin, pepper or piperine had no effect on food intake, feed use efficiency, organ weights (liver, kidney, spleen and adipose tissue), hematological parameters (hemoglobin, red blood cells, white blood cells, lymphocytes and neutrophils) or clinical chemistry values (total protein, albumin:globulin ratio, glucose, cholesterol, AST, ALT and ALP). Isolated, non-dose-dependent variations were observed in nitrogen and fat absorption and in the retention of nitrogen in some groups of treated rats. The authors concluded that the results of this study showed a no observed adverse effect level (NOAEL) of 20 mg/kg bw per day, the highest dose tested.

2.4.2.1 Genotoxicity in vivo

In order to investigate conflicting results in genotoxicity testing, piperine was administered to mice up to the maximum tolerated dose (MTD) and micronuclei frequency was determined in a recent study (Thiel et al., 2014). Since piperine reduces core body temperature and interferes with blood cells, both known to result in irrelevant positive *in vivo* micronucleus tests (MNTs), mechanistic endpoints were added: core body temperature, hematology, erythropoietin level and organ weights. In addition, an *in vitro* MNT in Chinese hamster ovary cells was performed. Piperine was found negative in the *in vitro* MNT. It caused significant reduction of core body temperature, decrease of white blood cells and spleen weights but no increase in the MNT. Based on the overall weight of evidence it was concluded that piperine had no genotoxic potential.

Piperine consistently gave negative results in a variety of studies *in vivo* (JECFA, 2006b) as noted in EFSAs recent Scientific Opinion on Flavouring Group Evaluation 86, Revision 2 (EFSA, 2015). Piperine did not induce micronuclei in the bone marrow of male Swiss mice given a single dose of 10 or 20 mg/kg bw by gavage, or when administered intraperitoneally to adult male mice at sublethal doses of 1, 2 or 4 mg/kg bw per day on 5 consecutive days. Piperine did not cause mutations in male germ cells, as assessed by sperm shape

abnormality and tests for dominant lethal mutations in mice and hamsters. Mice given piperine at doses of 35, 50 or 75 mg/kg bw per day by gavage, or doses of 1, 2 or 4 mg/kg bw per day by intraperitoneal injection for 5 days showed no sperm shape abnormalities or dominant lethal mutations.

2.4.2.2 Interactions

As already discussed under Section 2.4.1.1, piperine is a strong modulator of the gastrointestinal system, affects drug metabolizing enzymes and shows bioenhancer properties. Numerous *in vivo* animal studies confirm findings in human studies regarding interactions with drugs and phytochemicals due to coadministration of piperine and alteration of pharmacokinetics. Drug interactions in animals were observed in several *in vivo* studies using piperine administered through the diet, normally at doses between 10 to 50 mg/kg bw per day.

Oral administration of piperine (10 and 20 mg/kg bw) has been shown to increase the oral exposure and bioavailability of fexofenadine, an antihistamine drug, through enhanced gastrointestinal absorption in rats likely due to inhibition of P-gp-mediated cellular efflux (Jin and Han, 2010). Maximum concentrations and plasma levels of the isoflavone puerarine were increased when orally coadministering piperine in rats (10 and 20 mg/kg bw), whereas coapplication of piperine-containing white pepper decreased oral puerarine absorption (Liang et al., 2014). In another study in mice, intragastric coadministration of piperine (20 mg/kg bw) was shown to increase the bioavailability of the green tea polyphenol epigallo-catechin-3-gallate (EGCG) by inhibiting glucuronidation in mouse small intestine (by 40%), but not in hepatic microsomes (Lambert et al., 2004).

A recent study showed that piperine might activate the transcriptional activity of human pregnane X receptor (hPXR) in human hepatocytes and intestinal cells at low piperine concentration and subsequently induce the expression of CYP3A4 and P-gp in these cells, as observed in experiments with mice (Wang et al., 2013). Thus, the induction of CYP3A4 and P-gp expression at low piperine levels might counteract the weak inhibitory effects on these two proteins, compared to strong inhibitory activity at higher levels. These findings are supported by an earlier study (Han et al., 2008) reporting that prolonged (14 days) peroral doses of piperine (112 µg/kg bw per day) might increase the expression of intestinal P-gp efflux transport proteins, though not in the liver. These results are in contrast to many human and animal studies reporting inhibitory activity on cytochrome P450 enzymes and P-gp, thus revealing a potential dichotomous effect of piperine.

2.4.2.3 Allergic sensitisation (including adjuvant effects)

There was no information concerning allergic sensitisation or allergy adjuvant effects in the literature reviewed on animal studies in the present risk assessment. The absence of information in the selected literature does not document an absence of allergic sensitisation or allergy adjuvant effects.

2.4.3 *In vitro* studies

2.4.3.1 Genotoxicity in vitro

No mutagenicity was found in the standard Ames assay when various strains of *Salmonella typhimurium* (TA97a, TA98, TA100, TA102, TA1535, TA1537, TA1538, TA1530, TA1531, TA1532 and TA1964) were incubated with up to 10000 µg/plate of piperine (JECFA, 2006b).

2.4.3.2 Interactions

Piperine has been shown to enhance drug bioavailability through inhibition of drug metabolism (Atal et al., 1985), both using *in vitro* and *in vivo* studies in a rat model. Piperine exerts inhibitory activity on P-gp-mediated drug transport in Caco-2 cell monolayers, and drug-metabolizing enzyme CYP3A4 in human hepatic microsomes, indicating that dietary piperine could affect plasma concentrations of P-gp and CYP3A4 substrates in humans (Bhardwaj et al., 2002). More recent studies have investigated the effect of piperine to interfere with metabolism of pharmaceutical drugs. *In vitro* studies using the MCF-7 breast cancer cell line with the anticancer drug paclitaxel indicated synergistic effects by concomitant exposure with piperine, resulting in potentiated antitumor activity of paclitaxel. The synergy interaction potentially involves an epidermal growth factor receptor signaling blockade (Motiwala and Rangari, 2015). However, using a P-gp-dependent ATPase assay in rat jejunal membrane *in vitro*, a biphasic and concentration-dependent response could be observed, i.e. stimulation at low concentration and inhibition at high concentration (Najar et al., 2010), thus supporting the dichotomous effect of piperine as reported by Wang et al. (2013) (see 2.4.2.2 Interactions).

2.4.4 Vulnerable groups

Potential adverse effects might occur due to undesired food-drug interactions caused by the uptake of black pepper or piperine-containing food. Caution should be taken regarding dietary piperine consumption during drug administration in patients (e.g. cancer treatment and chemotherapy), particularly those who favour daily pepper spice or utilise certain pepper remedies (Wang et al., 2013). Excess intake of >10 mg doses of piperine due to high consumption of pepper or intake of dietary supplements containing *P. nigrum* or *P. longum* above common dietary levels, might lead to clinically significant interactions with several drugs (Gurley et al., 2012).

2.5 Summary of hazard identification and characterisation

Piperine is a naturally occurring alkaloid. Piperine is a solid compound, it dissolves very slowly in water, but is quickly absorbed across the intestinal border and assumed to be readily metabolised as indicated by animal studies. The kidney appears to be the major excretion route for piperine metabolites in rats. According to a pharmacokinetic study in

humans, no accumulation after a daily oral dose of 20 mg piperine for 7 days was observed in blood serum.

Available data from *in vivo* and *in vitro* studies indicated that piperine had no genotoxic potential.

Several adverse health effects were identified in animal studies, including enhanced plasma cholesterol, hepatic dysfunction and histopathological changes, immunomodulatory effects and reproductive toxicity. Two dietary toxicity studies carried out in chicks (Da Silva Cardoso et al., 2009) and mice (Dogra et al., 2004), revealed hepatotoxic and immunomodulatory changes, respectively. Both reports suggested a NOAEL of 1.12 mg/kg bw per day, the lowest dose (other doses tested were 2.25 and 4.50 mg/kg bw per day). The reported dose-response effects in these two studies were not always consistently statistically significant, conclusive or were partly contrasting. For that reason, the suggested NOAELs are not used in the risk characterisation of piperine by VKM.

The range of doses reported to cause interactions with drugs and phytochemicals when studied *in vivo*, 5 to 20 mg/kg bw per day in humans and 10 to 50 mg/kg bw per day in animals (Chinta et al., 2015; Srinivasan, 2007; Srinivasan, 2013), exceeded estimated daily intake levels of piperine. Potential interactions of orally coadministered piperine were reported and comprise (a) inhibitory activity on drug metabolising enzyme systems and P-gp for various drugs, and simultaneously, enhanced bioavailability of drugs, and (b) modulation of gene and protein expression of CYP enzymes and P-gp efflux transporters. Provided that the ingestion of piperine via pepper (food flavouring) or intake of dietary supplements containing *P. nigrum* or *P. longum* does not exceed common dietary levels, the risk of adverse piperine-drug and piperine-phytochemical interactions is minimal.

A NOAEL of 5 mg/kg bw per day was set in 2015 by EFSA based on the dose-dependent increase in plasma cholesterol levels in males at the mid and high dose (15 and 50 mg/kg bw per day) in a 90-days toxicity study in rats. The study was performed according to OECD Guideline (TG 408) (Bauter, 2013).

The value used for comparison with the estimated exposure in the risk characterization is the NOAEL of 5 mg/kg bw per day from this rat study.

3 Exposure / Intake

3.1 Food supplements

Exposure to piperine was estimated from the intake of food supplements for the age groups children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years).

NFSA requested VKM to perform a risk assessment of 1.5 mg/day of piperine in food supplements for children above 10 years, adolescents and adults. The default body weights (bw) for these age groups as determined by EFSA were used: children (10 to <14 years); 43.4 kg, adolescents (14 to <18 years); 61.3 kg and adults (≥18 years) 70.0 kg (EFSA, 2012b). From a daily dose of 1.5 mg piperine, the calculated intake levels were 34.6, 24.5 and 21.4 µg/kg bw per day for children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years), respectively (Table 3.1-1).

Table 3.1-1   Estimated daily exposure of piperine in children, adolescents and adults from food supplements.

Age groups	Daily doses (mg)	Body weight (kg)	Exposures (µg/kg bw per day)
Children (10 to <14 years)	1.5	43.4	34.6
Adolescents (14 to <18 years)	1.5	61.3	24.5
Adults (≥18 years)	1.5	70.0	21.4

3.2 Other sources

Dried, ground black pepper (*Piper nigrum*) and its variants is one of the most common spices in European/Western cuisine, and thus, a major source of piperine exposure through the diet. Other potential sources of piperine include the spice Grains of Paradise (*Aframomum melegueta*) from West Africa, and consumption of piperine (pepper)-flavoured beverages and spirits.

Based on the MSDI approach, the estimated exposure to piperine from natural sources when consuming black pepper as flavouring ingredient, is 6.2 µg/day and 0.07 µg/day in the EU and USA, respectively (EFSA, 2015).

Piperine is also used in cosmetics as a perfuming agent (CosIng, 2016).

4 Risk characterisation

4.1 Food supplements

NFSA requested VKM to perform a risk assessment of 1.5 mg/day of piperine in food supplements for the general population, ages 10 years and above. The value used for comparison with the estimated exposure in the risk characterization is the NOAEL of 5 mg/kg bw per day based on a 90-day dietary toxicity study in rats (EFSA, 2015).

The risk characterization is based on the Margin of Exposure (MOE) approach; the ratio of the NOAEL to the exposure. The calculated MOE values are used to determine human health risk. An acceptable MOE value for a NOAEL-based assessment of piperine based on an animal study is ≥100, which includes a factor 10 for extrapolation from animals to humans, and a factor 10 for interindividual human variation (EPA, 2012). A MOE below 100 may also be acceptable; however, such assessment must be based on supporting scientific literature and expert judgement.

From a daily dose of 1.5 mg piperine, the daily exposure is calculated as 34.6, 24.5 and 21.4 µg/kg bw per day for children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years), respectively (Table 3.1-1). Using the MOE approach, for a daily intake of 1.5 mg piperine from food supplements and a NOAEL-value of 5 mg/kg bw per day from a rat study, the margins of exposure are 145, 204 and 234 for children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years), respectively (Table 4.1-1). Thus, for a daily intake of 1.5 mg piperine, the MOE is above 100 for all age groups.

Table 4.1-1   The calculated margins between the NOAEL and the exposure to piperine from food supplements (MOE values) for the various age groups.

Age groups	Margin of exposure (MOE)
Children (10 to <14 years)	145
Adolescents (14 to <18 years)	204
Adults (≥18 years)	234

For a daily intake of 1.5 mg piperine, the MOE values are above 100 for all age groups. VKM concludes that it is unlikely that a daily dose of 1.5 mg piperine from food supplements causes adverse health effects in children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years).

5 Uncertainties

5.1 Hazard identification and characterisation

No studies on children or adolescents were identified. A tolerance as for adults, based on body weight, was assumed for these groups.

Since the rat study used to derive the NOAEL of 5 mg/kg bw per day, was of limited duration (approximately 3 months), there is some uncertainty related to safety of longer duration of exposure to piperine.

5.2 Exposure

With use of the default (mean) body weight of an age (population) group, the variance in all individuals in the group will not be covered.

6 Conclusions with answers to the terms of reference

The Norwegian Scientific Committee for Food Safety (VKM) has, at the request of the Norwegian Food Safety Authority, assessed the risk of piperine (1.5 mg/day) in food supplements. The present risk assessment is based on previous risk assessments and a literature search.

The value used for comparison with the estimated exposure in this risk characterization is the NOAEL of 5 mg/kg bw per day based on a 90-day dietary toxicity study in rats (EFSA, 2015). The risk characterization is based on the Margin of Exposure (MOE) approach; the ratio of the NOAEL to the exposure. The calculated MOE values were used to determine human health risk. An acceptable MOE value for a NOAEL-based assessment of piperine based on an animal study is ≥100, which includes a factor 10 for extrapolation from animals to humans, and a factor 10 for interindividual human variation. Using the MOE approach, for a daily intake of 1.5 mg piperine from food supplements and a NOAEL of 5 mg/kg bw per day from the rat study, the margins of exposure are 145, 204 and 234 for children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years), respectively. Thus, for a daily intake of 1.5 mg piperine, the MOE values are above 100 for all age groups.

VKM concludes that it is unlikely that a daily dose of 1.5 mg piperine from food supplements causes adverse health effects in children (10 to <14 years), adolescents (14 to <18 years) and adults (≥18 years).

The range of reported dose levels causing interactions with drugs and phytochemicals when studied *in vivo* exceed estimated daily intake levels of piperine: 5 to 20 mg/kg bw per day and 10 to 50 mg/kg bw per day in humans and animals, respectively. Compared to the reported piperine levels in humans, it is unlikely that 1.5 mg piperine per day from food supplements might increase the risk of interactions with drugs and phytochemicals. Provided that the ingestion of piperine via pepper (food flavouring) or intake of dietary supplements containing *P. nigrum* or *P. longum* does not exceed common dietary levels, the risk of adverse piperine-drug and piperine-phytochemical interactions is minimal. However, caution should be taken regarding dietary piperine consumption above this level during drug administration in patients (e.g. cancer treatment and chemotherapy), particularly those who favour daily pepper spice or utilise certain pepper remedies.

An overview of the conclusions on food supplements containing 1.5 mg/day of piperine is presented in Table 6.1.

Table 6.1   An overview of the conclusions on daily intake of piperine from food supplement. Green: estimated exposure to piperine (mg/day) that is unlikely to cause adverse health effects.

Food supplement Age groups	Piperine 1.5 mg/day
Children **(10 to <14 years)**	
Adolescents **(14 to <18 years)**	
Adults **(≥18 years)**	

7 Data gaps

There is a lack of human studies that have investigated the effect of varying and high doses of piperine for longer periods.

There is lack of chronic toxicity studies of piperine in animals.

No studies on adverse health effects of piperine in children, adolescents, pregnant women or lactating women were identified.

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9 Appendix

Search Strategy

Database: Embase <1974 to 2016 January 07>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>

1. piperin*.ti. (957)
2. pepper*.mp. [mp=ti, ab, hw, tn, ot, dm, mf, dv, kw, nm, kf, px, rx, an, ui] (16566)
3. 1 and 2 (320)
4. (risk* or safety or adverse or side-effect*1 or hazard* or harm* or eosinophil* or negative or contraindicat* or contra-indicat* or interact* or toxicity or toxic).tw. (9804998)
5. 3 and 4 (72)
6. (conference abstract* or letter* or editorial*).pt. (4883060)
7. 5 not 6 (67)
8. limit 7 to (danish or english or norwegian or swedish) (67)
9. removal of duplicates from 8 (44)