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Risk assessment of methylsulfonylmethane (MSM)

Opinion of the Norwegian Scientific Committee for Food and Environment

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Risk assessment of methylsulfonylmethane (MSM)

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.

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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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VKM would like to thank Dag Markus Eide (The Norwegian Institute of Public Health) for valuable contribution and discussion on the application of quantitative structure-activity relationship to predict genotoxicity.

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

NFSA requested VKM to perform a risk assessment of daily intake of 3 g methylsulfonylmethane (MSM) for the general Norwegian population, both sexes, in the age groups: 3-<10 years, 10-<14 years, 14-<18 years and adults ≥18 years. If 3 g MSM/day is not safe, NFSA requested VKM to identify the amount less than 3 g MSM/day that is safe.

MSM is present in small quantities in a large variety of fruits, vegetables, grains, meat, eggs and fish, and is consumed in trace amounts in humans on a normal diet (AECOSAN, 2014; Brien et al., 2008; Crawford et al., 2019a). MSM is found at concentrations about 0.2 mg/kg in the circulation of the adult male body (Hansen et al., 2006), likely derived from the dietary sources, and endogenous and bacterial production (He and Slupsky, 2014).

The hazard identification and characterisation were based on data from studies identified in literature searches. In human studies, no serious adverse health effects of MSM were identified. In animal studies, adverse effects reported included decrease in body weight and organ weights and decrease in bone mineral density. Note that VKM considered the data to be insufficient and that the confidence in the evidence ranged from moderate to very low.

- No ADME (absorption, distribution, metabolism and elimination) data for children and adolescents were available. MSM is rapidly absorbed in adult humans, evenly distributed throughout the body and crosses the blood-brain barrier. A pathway for endogenous MSM production has been suggested, however, the level of the endogenous production is not known. No data on MSM metabolism are available. Urine is the most common excretory pathway.
- VKM considers that the body of evidence on the genotoxic potential of MSM is of sufficient quality and relevance and concludes that there is no concern for genotoxicity.
- A point of departure (PoD) of 6 g/day of MSM (NOAEL in a 16 week study in human adults) was derived for adults (≥18 years). Several factors contributed to uncertainty in the PoD, and to account for the uncertainties a margin of exposure (MoE) of 30 was identified by expert judgement in order to ensure safety.
- No PoD could be established for the age groups 3-<10 years, 10-<14 years and 14-<18 years due to data insufficiency.

For a food supplement dose of 3 g MSM/day in adults (≥18 years) the MoE from the identified PoD of 6 g/day is 2, which is less than the identified acceptable MoE of 30. A daily single dose of 0.2 g yields an acceptable MoE of 30.

VKM concludes:

- A daily dose of 3 g MSM from food supplements may represent a risk of adverse health effects in adults ≥18 years.

- It is unlikely that a daily dose of 0.2 g MSM from food supplements causes adverse health effects in healthy adults ≥18 years.
- As limited data are available, VKM cannot conclude on a daily safe dose of MSM for children and adolescents.

Note that MSM sources other than food supplements have not been taken into consideration in the conclusion that a daily dose of 0.2 g MSM from food supplements is unlikely to cause adverse health effects in adults ≥18 years.

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

Sammendrag på norsk

På oppdrag fra Mattilsynet har Vitenskapskomiteen for mat og miljø (VKM) vurdert risiko ved daglig inntak av 3 g metylsulfonylmetan (MSM) fra kosttilskudd. Risikovurderingen inkluderer den generelle norske befolkningen, begge kjønn, og aldersgruppene 3-<10 år, 10-<14 år, 14-<18 år og voksne ≥18 år. Hvis daglig inntak av 3 g MSM ikke vurderes å være trygt, ba Mattilsynet VKM om å identifisere hva som vil være en trygg dose.

MSM finnes i små mengder i en rekke matvarer som frukt, grønnsaker, korn, kjøtt, egg og fisk, og vi får derfor i oss små mengder MSM fra et normalt kosthold (AECOSAN, 2014; Brien et al., 2008; Crawford et al., 2019a).

Litteraturen som ble brukt i fareidentifiseringen og karakteriseringen ble funnet i systematiske litteratursøk. Det ble ikke identifisert alvorlige negative helseeffekter av MSM i de tre inkluderte humane studiene. I dyreforsøkene ble det rapportert om reduksjon i kroppsvekt og organvekt og reduksjon i bentetthet. Merk at VKM anså dataene som utilstrekkelige, og at tiltro til evidensen varierte fra moderat til veldig lav.

- Det ble ikke funnet data på ADME (absorpsjon, distribusjon, metabolisme og eliminasjon) for barn og ungdom. Hos voksne blir MSM absorbert raskt, det fordeles jevnt i kroppen og krysser blod-hjerne-barrieren, og skilles ut via urin. Det er foreslått en mulig vei for endogen MSM-produksjon, det vil si hvordan MSM produseres i kroppen, men det er ikke kjent hvilke mengder som produseres endogent. Det ble ikke funnet data som beskrev MSMs metabolisme.
- VKM vurderte at tilgjengelig kunnskap om gentoksisitet var av tilstrekkelig kvalitet og relevans, og konkluderte med at det ikke er bekymring for at MSM er gentoksisk.
- 6 g MSM per dag ble satt som et utgangspunkt (PoD) (NOAEL i en 16 ukers studie i en gruppe voksne) for å utlede en trygg MSM-dose for voksne ≥18 år. Det var flere faktorer som bidro til usikkerhet i PoD, og VKM vurderte at det er behov for en eksponeringsmargin (MoE) på 30 for å ta hensyn til denne usikkerheten når det skal vurderes hva som er en trygg MSM-dose.
- For aldersgruppene 3-<10 år, 10-<14 år, 14-<18 år var det ikke mulig å fastsette en PoD på grunn av mangel på data.

Beregnet MoE for et daglig inntak av 3 g MSM i kosttilskudd hos voksne (≥18 år) og en PoD på 6 g ble 2. For å få en eksponeringsmargin på 30 må den daglige MSM-dosen fra kosttilskudd være på 0,2 g.

VKM konkluderer:

- En daglig MSM-dose fra kosttilskudd på 3 g kan utgjøre en risiko for negative helseeffekter for voksne (≥18 år).
- Det er usannsynlig at en daglig MSM-dose fra kosttilskudd på 0,2 g utgjør en risiko for negative helseeffekter for voksne (≥18 år).

- VKM har for lite data til å kunne konkludere på hva som er en trygg MSM-dose fra kosttilskudd for barn og unge.

Abbreviations and glossary

Abbreviations

ADME	absorption, distribution, metabolism and elimination
AUC	area under the curve
bw	body weight
C_{max}	maximum concentration
DMSO	dimethyl sulfoxide
EFSA	European Food Safety Authority
GLP	good laboratory practice
HBGV	Health based guidance value
MSM	methylsulfonylmethane
MoE	margin of exposure
NFSA	Norwegian Food Safety Authority
NOAEL	no observed adverse health effect
OECD	Organisation for Economic Co-operation and Development
PoD	point of departure
RCT	randomized controlled trial
RoB	risk of bias
t_{max}	time to maximum concentration
QSAR	quantitative structure-activity relationship
UF	uncertainty factor
VKM	Norwegian Scientific Committee for Food and Environment
WHO	World Health Organization

Glossary

Absorption, distribution, metabolism and elimination (ADME)
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).

Good laboratory practice
A standardised way of planning, performing and reporting laboratory-based studies to ensure a high standard of quality and reliability (EFSA glossary).

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).

Margin of exposure
The margin required between the PoD and the estimated exposure in order to ensure safety.

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

OECD Guidelines for the testing of chemicals
A tool for assessing the potential effects of chemicals on human health and the environment. The Guidelines are elaborated with the assistance of experts from regulatory agencies, academia, industry, environmental and animal welfare organisations.

"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.

Quantitative structure-activity relationship
The quantitative/qualitative structure activity relationships are a set of methods by which the effects of different compounds are related to their molecular structures. It allows the likely adverse or beneficial effects of a particular chemical to be predicted by comparing it with others which have similar structures (EFSA Glossary).

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 methylsulfonylmethane (MSM) (CAS No. 67-71-0) in food supplements that is covered by the national supplementary provisions might constitute a health risk in the Norwegian population at the following dose of: 3 g/day. If this amount of 3 g MSM/day is not safe, the NFSA will need to know which amount less than 3 g MSM/day that is safe. The risk assessment shall include the Norwegian population, both sexes in the following age groups: 3-<10 years, 10-<14 years, 14-<18 years and adults ≥18 years.

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 dose(s) and age groups given above.
    - Describe uncertainty related to the exposure estimates.
- Characterise health risks associated with exposure to the substance (methylsulfonylmethane (MSM)), 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.

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 3 g methylsulfonylmethane (MSM) in food supplements might constitute a health risk for the Norwegian population, both sexes, in the age groups 3-<10 years, 10-<14 years, 14-<18 years and adults ≥18 years. If 3 g MSM/day constitute a health risk, NFSA need to know which amount less than 3 g MSM/day that is safe.

MSM is a water soluble, highly stable organic sulphur-containing compound (AECOSAN, 2014). It is present in small quantities in a large variety of fruits, vegetables, grains, meat, eggs and fish, and is consumed in trace amounts in humans on a normal diet (AECOSAN, 2014; Brien et al., 2008; Crawford et al., 2019a).

MSM is found at concentrations of about 0.2 mg/kg in the circulation of the adult male body (Hansen et al., 2006), likely derived from the dietary sources, endogenous metabolism and bacterial metabolism (He and Slupsky, 2014).

1.1 Limitations

- The assessment is performed for MSM, and only for the dose(s) 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 3-<10 years, 10-<14 years, 14-<18 years and adults ≥18 years.
- Exposure from other sources of MSM, such as e.g. food, is not estimated.
- Documentation of any claimed beneficial effects is not evaluated.
- Stability of MSM in a product is not addressed.
- Interaction with other components in a product is not addressed.
- Potential impurities are not addressed.

2 MSM specifications

Name and other identifiers of the MSM, and physical and chemical properties, are presented in Table 2-1 and 2-2 (Chemspider; PubChem).

Table 2-1. Name and other identifiers.

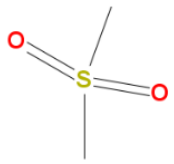
Substance name	Methylsulfonylmethane (MSM)
Synonyms	Dimethyl sulfone, dimethylsulfone, methyl sulfone
CAS number	67-71-0
EINECS number	200-665-9
Molecular formula	$C_2H_6O_2S$
Molecular weight	94.14 g/mol
Structural formula	
Smiles	CS(=O)(=O)C

Table 2-2. Physical and chemical properties.

Physical state	Crystalline, solid
Stability	Stable. Combustible. Incompatible with strong oxidizing agents.
Boiling point (liquids), melting point (solids)	Melting point: 109 °C
Density	1.1±0.1 g/cm^3
Vapor pressure	Not found.
Water solubility	150 g/L (20 ºC)
Partition coefficient (LogP)	-1.41

3 Exposure

Exposure of MSM was estimated from the daily intake of 3 g MSM in food supplements for the Norwegian population, both sexes, in the age groups 3-<10 years, 10-<14 years, 14-<18 years and adults ≥18 years. The default body weights (bw) determined by EFSA (Table 3-1), the median and the 5th percentile, was used for the exposure calculations (EFSA, 2012).

Daily exposure for individuals with the 5 percentile body weight

From a daily dose of 3 g MSM, the exposure is 214.3 mg/kg bw per day for children aged 3-<10 years, 102.0 mg/kg bw per day for children aged 10-<14 years, 66.7 mg/kg bw per day for adolescents aged 14-<18 years, and 57.7 mg/kg bw per day for adults ≥18 years (Table 3-1).

Daily exposure for individuals with the median body weight

From a daily dose of 3 g MSM, the exposure is 138.3 mg/kg bw per day for children aged 3-<10 years, 71.4 mg/kg bw per day for children aged 10-<14 years, 50.0 mg/kg bw per day for adolescents aged 14-<18 years, and 41.7 mg/kg bw per day for adults ≥18 years (Table 3-1).

Table 3-1. Daily exposure from 3 g MSM in food supplements.

Population group	5th percentile body weight	Median body weight	Daily exposure (individuals with the 5th percentile bw)	Daily exposure (individuals with the median bw)
Children 3-< 10 years	14 kg	21.7 kg	214.3 mg/kg bw	138.3 mg/kg bw
Children 10-<14 years	29.4 kg	42 kg	102.0 mg/kg bw	71.4 mg/kg bw
Adolescents 14-<18 years	45 kg	60 kg	66.7 mg/kg bw	50.0 mg/kg bw
Adults ≥18 years	52 kg	72 kg	57.7 mg/kg bw	41.7 mg/kg bw

3.1 Other sources

MSM is a naturally occurring sulphur-containing compound found in some green plants and mammals and is found in trace amounts in a normal human diet (Brien et al., 2008; Crawford et al., 2019a). Foods containing MSM includes e.g. cow's milk (6-8 µg/g), coffee (1.6 µg/g), tomato (0.86 µg/g), tea (0.3 µg/g), and maize (0.11 µg/g) (AECOSAN, 2014).

Hansen et al. (2006) reported that MSM is found naturally at concentrations of about 0.2 mg/kg in the circulation of the adult male body. AECOSAN (2014) reported that MSM levels

have been detected in the range of 0-25 µmol/l in the plasma and cerebrospinal fluid from healthy individuals.

4 Hazard identification and characterisation

The questions for the hazard identification and characterisation for oral intake of MSM are given 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.

Hazard identification	1	Is there a concern for genotoxicity?
	2	Is exposure to MSM associated with other adverse health effects?
Hazard characterisation	3	What is the dose-response relationships between exposure to MSM and the adverse effects?
	4	Can a health-based guidance value be established or a point of departure be identified?

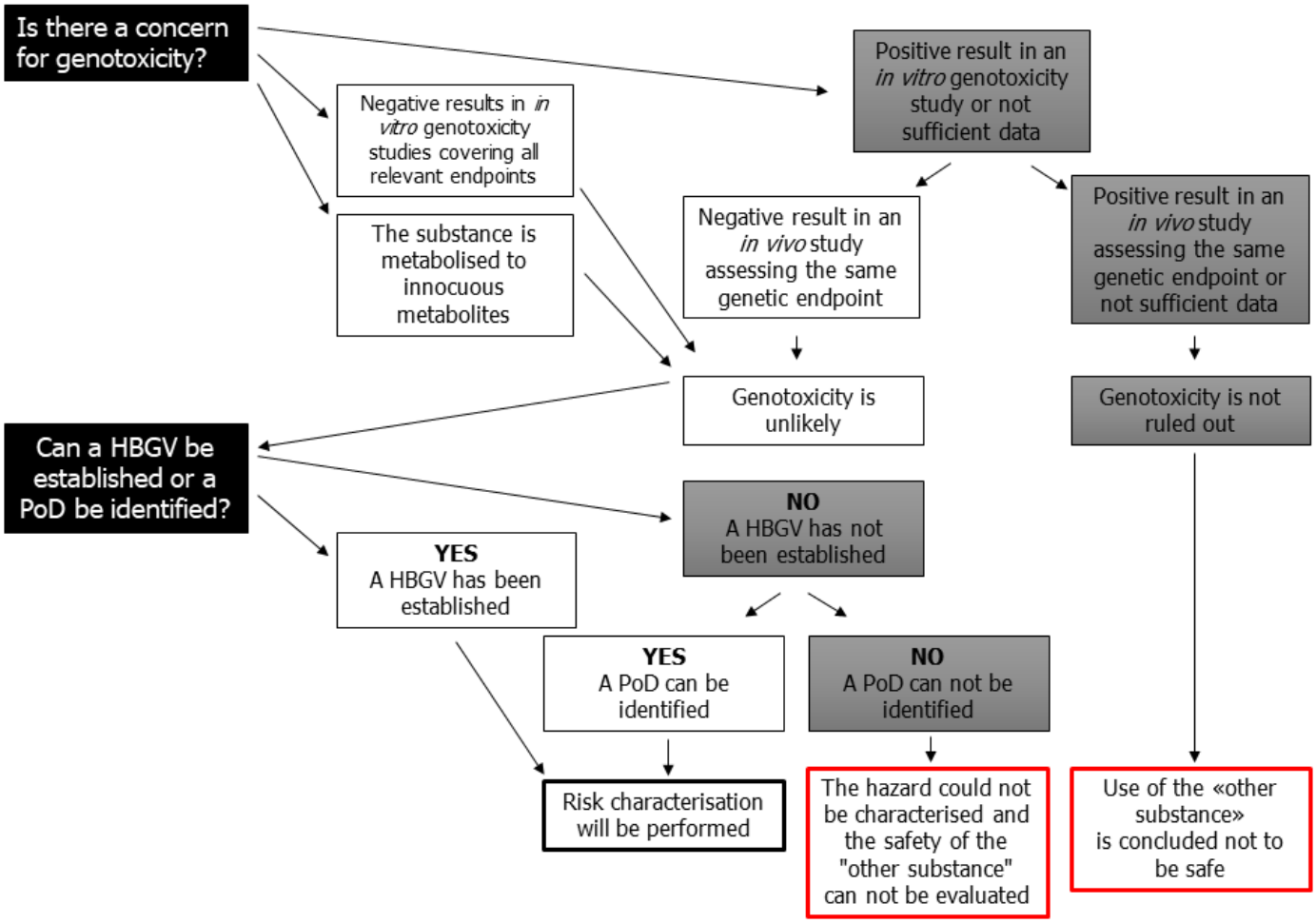


Figure 4-1. Flowchart for the hazard identification and characterisation. HBGV = health-based guidance value; PoD = point of departure.

4.1 Absorption, distribution, metabolism and elimination

For absorption, distribution, metabolism and elimination (ADME) of MSM, we aimed to answer the following questions:

1. What is the ADME of MSM in humans? Is human and animal (rodent) ADME similar?
2. Is MSM metabolised to innocuous metabolites?
3. Is MSM 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?

The electronic databases from MEDLINE (Ovid) and Embase (Ovid) were searched to identify relevant data on ADME of MSM (see Section 10.1 for search terms). We identified 35 publications that were screened for relevance by two of the authors independently, followed by full-text assessment of 11 relevant publications by the same reviewers. Seven publications were included. In addition, five publications were identified through searches for publications

not indexed in the major databases and screening of reference lists (handsearching). An overview of the publication selection is given in Figure 4.1-1, and an overview of the included studies is given in Table 4.1-1 and 4.1-2. Detailed description of the studies is available in Section 10.3.

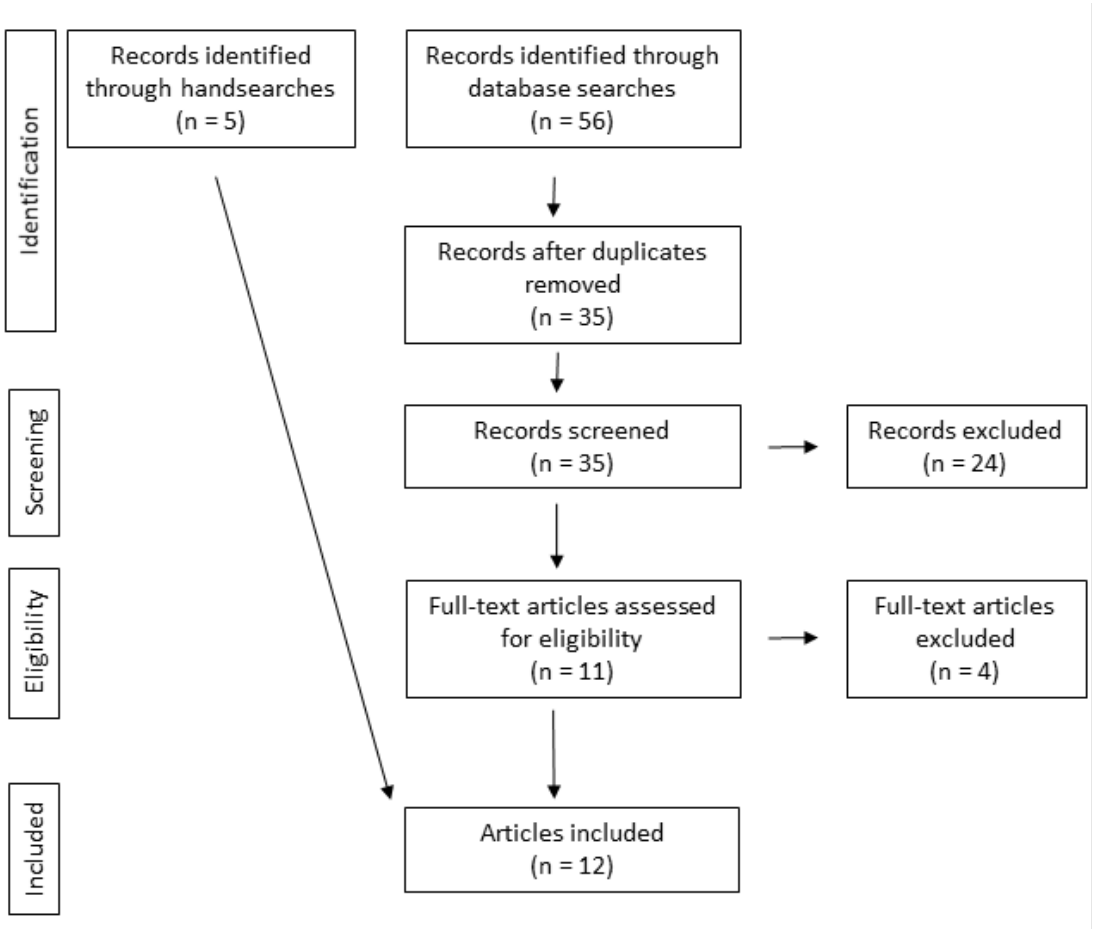


Figure 4.1-1. Flow diagram illustrating the process of selecting relevant publications (modified from Moher et al. (2009)).

Table 4.1-1. Animal ADME studies with MSM.

Route of exposure Time period	Outcome assessed OECD Guidelines for the testing of chemicals * GLP**	Species, strain, sex No/group	Substance Dose(s)	Result	Reference
Oral gavage, a single oral dose.	Absorption, excretion, distribution, metabolism. No guideline, non-GLP.	Rat, Sprague-Dawley, male. N=8 rats (N=5 rats group 1; blood group, and N=3 rats group 2; urine and faeces group).	^{35}S labelled MSM. A single dose of 500 mg/kg and 50 µCi/rat.	Oral [^{35}S]-MSM was rapidly and efficiently absorbed with a mean t_{max} of 2.1 hours. The half-life was 12.2 hours. Some of the administered radioactivity were found in all tissues analysed 48 hours post-dose, but undetectable in tissues after 120 hours. Approximately 85.8 % of the dose was recovered in the urine after 120 hours, 3% was in the faeces. No quantifiable levels of radioactivity were found in any tissues after 120 hours.	Magnuson et al. (2007a)
Oral gavage administration, once per day for 7 days.	Absorption, excretion, distribution, metabolism. No guideline, non-GLP.	Rat, Wistar, male. N=4 rats per group.	^{35}S labelled MSM. 470 mg/kg bw/day.	Levels of radioactivity tended to be highest in blood, spleen, and hair. Over 80 % was excreted the same day, the majority in urine (~70%) and faeces (~10%). The entire radioactivity was not recovered.	Otsuki et al. (2002)
Diet, for 2, 5 and 8 days.	Absorption of MSM by the small intestine and accumulation of the associated sulphur moiety in selected tissues.	Mouse, C57B6, male N=4-5 mice/group for 2, 5 and 8 days	^{35}S labelled MSM. 10 µCi (0.6 µM; 5.5 µg)/day.	[^{35}S]-MSM accumulated in the homogenates in all of the tissues after two days of dosing, and serum had higher levels than solid tissues. The amount of [^{35}S]-MSM activity did not increase in serum or tissue homogenates between days 2 and 8, suggesting an establishment of a	Wong et al. (2017)

	No guideline, non-GLP.			possible stable equilibrium between intake and elimination.	
Oral gavage, for one (study 1) and 21 days (study 2).	Distribution, toxicity. No guideline, non-GLP.	Chicken, Ross 308 broiler, male. Study 1: N=432; 6 treatment groups with 6 replicates of 12 birds per replicate. Study 2: N=168; assigned to either control or test group.	MSM (Cat. No.: 41,867; Sigma Aldrich, St. Louis, MO) in the form of white crystalline powder. Study 1: a single oral dose of MSM at 0, 50, 100, 300, 1000, or 2000 mg/kg bw. Blood and all tissues were collected 48 h post treatment. Study 2: a daily oral gavage of either 0 or 1500 mg/kg bw/day of MSM for 21 days.	Oral MSM at either acute (single dose at 1000 to 2000 mg/kg bw for 48 hours) or subchronic (1500 mg/kg bw daily for 21 days) concentrations appeared to be absorbed and distributed throughout the body. Detection of MSM in plasma and all tissue types at all time-points suggest that MSM is well absorbed and widely distributed across body tissues.	Abdul Rasheed et al. (2019)
Intra-peritoneal administration.	Absorption, excretion, distribution, metabolism. No guideline, non-GLP.	Rat, not specified; sex, strain. Not specified: number of animals/group.	^{35}S labelled MSM. 21 mg/kg.	Around 64% [^{35}S]-MSM was excreted apparently unchanged in the urine within 24 hours. No further details of the study were given.	Hucker et al. (1966)

*GLP: good laboratory practice, a standardised way of planning, performing and reporting laboratory-based studies to ensure a high standard of quality and reliability; ** OECD Guidelines for the testing of chemicals: A tool for assessing the potential effects of chemicals on human health and the environment; t_{max}: time to maximum concentration.

Table 4.1-2. Human ADME studies with MSM.

Participants (sex, number, age, bw)	**Dose** **Duration**	**Outcome assessed** **Result** **Method**	**Reference**
Male and female (n=7), four patients with memory loss, three healthy volunteers.	Daily administration of 1.5 - 6.0 g MSM. Patients: 1.5 – 6 g MSM (equivalent to 40 – 100 mg/kg bw). Healthy volunteers: 2 – 3 g MSM (equivalent to 30 – 50 mg/kg bw). Duration: from 5 weeks to >2 years.	MSM level in brain was analysed using magnetic resonance spectroscopy *in vivo*. MSM was evenly distributed throughout the brain, with similar MSM levels in white and grey matter. No adverse clinical or neurochemical effects were reported.	Lin et al. (2001)
5-year-old child (n=1), bw=20 kg.	MSM dietary supplement, 1250 mg/day for approximately 1 year (equivalent to 62,5 mg/kg bw/day)	MSM level in brain was analysed. MSM concentrations within the basal ganglia was 0.93 mM and 1.24 mM in the white matter. No adverse clinical, structural, or neurochemical effects were reported. Magnetic resonance spectroscopy.	Cecil et al. (2002)
Male (n=1), 62 years.	MSM dietary supplement of 2000 mg capsules per day (equivalent to 182 mg/kg bw per day) for 7 days, followed by 2000 mg MSM capsules per day as a maintenance dosage for 30 days.	MSM level in brain was analysed. MSM detected in the brain and cerebrospinal fluid. Estimated the half-life of MSM in brain was ~7.5 days. No adverse effects were reported. Magnetic resonance spectroscopy	Rose et al. (2000)
Healthy volunteers (n=3); 24–79 years, bw=73–82 kg.	MSM dietary supplement of 2000 mg pills (equivalent to 27.4 mg/kg bw), three repeated experiments of one subject. i. 2000 mg MSM ii. 45 days after first visit; 2000 mg MSM	MSM level in brain was analysed. MSM detected in the brain, and stable signal persisted for at least 4 hours after MSM intake. Estimated half-life time of MSM in the brain was ~72 hours. Magnetic resonance spectroscopy.	Kaiser et al. (2020)

	iii. 6 months after second visit; 2000 mg MSM		
Males, n=40, average age=25.1 years, mean bw = 84.6 kg.	MSM supplementary pills, 3 g/day (equivalent to 35.5 mg/kg bw/day) for a period of four weeks (n=20). Placebo, identical appearance (n=20).	Blood serum collection at three time points (baseline, week 2, week 4). MSM level in blood serum was analysed. Serum MSM concentrations were elevated in all men following ingestion of MSM, in a time-dependent manner. Mean serum MSM concentration increased from ~1.68 mM at week 2 to ~1.91 mM at week 4. Serum MSM concentration of baseline samples was below the limit of quantification for the NMR assay (0.002 mM), except one sample with serum MSM concentration of 0.028 mM. Nuclear Magnetic Resonance spectroscopy.	Bloomer et al. (2015)
Male (n=6), mean bw=90.1 kg.	A single oral dose of MSM supplementary pills of 1, 2 or 3 grams of OptiMSM® in healthy male volunteers (equivalent to 11.1, 22.2 and 33.3 mg/kg bw). Duration: three acute test visits, seven days apart.	Pilot study. MSM levels in blood serum and 24-hour urine were analysed. The half-life time of MSM was roughly estimated to be ½ hour and estimated elimination was around 8 hours.	Kalman and Hewlings (2018)
Male (n=31) & female (n=16), mean age range = (23.3 – 25.5 years) mean bw range = (71.8 – 78.6 kg). 45 completed the 16 weeks intervention study.	MSM supplementary pill, 1, 2 or 3 g/day for up to 16 weeks; (equivalent to 12.7, 26.4 or 41.8 mg/kg bw/day, respectively). Subjects were randomly grouped into 3 groups (each group consisted of 10 males and 5 females in total n=15/dose group). Duration: 16 weeks.	Intervention study. Blood plasma collection at 5 time points (baseline 4, 8, 12, and 16 weeks). Plasma MSM at baseline levels were very low and reached a dose-dependent steady-state by week 8 and were maintained through week 16 with continuous supplementation. Further, the steady-state plasma MSM concentrations increased linearly with increasing dosages, and women had higher overall plasma MSM levels (~1082 µM) than men (~845 µM), but values displayed a very similar pattern across time and dependent upon dose. LC-MS/MS method.	Bloomer et al. (2019)

4.1.1 Summary of ADME

We aimed to answer the following questions:

1. What is the ADME of MSM in humans? Is human and animal (rodent) ADME similar?
2. Is MSM metabolised to innocuous metabolites?
3. Is MSM 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?

The answers to these questions are given in Table 4.1.1-1, which shows an overview and a comparison of the human and animal ADME data available for MSM.

Table 4.1.1-1. ADME findings summary.

ADME	Human findings	Animal findings	Common findings for ADME in humans and animals
Absorption	MSM taken orally is rapidly absorbed within an hour, and serum MSM levels are dose and time-dependent.	Orally administered MSM in rodents is rapidly and efficiently absorbed by the small intestine with a mean t_{max} of ~2.1 hours (Magnuson et al., 2007a; Wong et al., 2017).	In both humans and animals, MSM is rapidly absorbed. Humans (adults, no data for children): Within an hour. Rodents: Mean t_{max} of ~2.1 h.
Distribution	MSM taken orally appeared to be widely distributed and has been detected in brain, cerebrospinal fluid, serum or plasma. MSM crosses the blood-brain-barrier and is distributed in all brain parts. An MSM signal was detected in the brain ~10 minutes after intake of a single dose of MSM (27.4 mg/kg) and the signal was a relatively long-lived, stable signal that persisted for at least 4 hours after the intake (Kaiser at al. 2020).	Orally administered MSM appeared to be widely distributed throughout the body, and measurable levels of MSM were found in plasma and all tissues analysed including brain tissues (Magnuson et al., 2007a; Otsuki et al., 2002; Wong et al., 2017; Rasheed et al., 2019).	In both humans (adults, no data for children) and animals, MSM is widely distributed throughout the body and it crosses blood-brain barrier.
Metabolism	There are no data on MSM metabolism. DMSO studies suggest that some DMSO, a common drug carrier, is metabolised to MSM (Hucker et al., 1966; Wong et al., 2017).	There are no data on MSM metabolism, but the available studies on DMSO (the parent compound of MSM) indicate that a fraction of DMSO is metabolised to MSM in the body and then excreted in urine.	A pathway for endogenous MSM production has been suggested, however, the level of the endogenous production is not known. The suggested pathway for endogenous MSM production for mammalians in general: Methionine is converted to methanethiol by microbiota in the gastrointestinal tract, and mammalian metabolism converts methanethiol to dimethyl sulphide which

ADME	Human findings	Animal findings	Common findings for ADME in humans and animals
			is converted to dimethyl sulfoxide, which is converted to MSM (He and Slupsky, 2014). It is not possible to conclude whether the dose given in the mandate from NFSA results in body levels within the MSM range normally metabolised and eliminated, as no data on endogenous production level of MSM are available. In both humans (no data for children) and animals, most MSM was excreted unchanged in urine. As not all MSM is recovered in urine and faeces, some MSM may be metabolised to other compounds. However, there are no data on MSM metabolism and whether MSM is metabolised to other S-containing metabolites in human or rats. It is therefore not possible to know if MSM metabolites are innocuous.

ADME	Human findings	Animal findings	Common findings for ADME in humans and animals
Elimination	There are few MSM elimination studies. In a pilot study, the half-life time of MSM was roughly estimated to be ½ hour (Kalman and Hewlings, 2018). The half-life of MSM in brain was found to be ~72 hours for a single dose of MSM (27.4 mg/kg bw) (Kaiser at al. 2020), or ~ 7.5 days for MSM dose of 182 mg/kg bw for seven days (Rose et al., 2000). The estimated orally taken MSM elimination time was around 8 hours (Kalman and Hewlings, 2018).	Orally administrated MSM is excreted mainly via the urine (Magnuson et al., 2017; Otsuki et al., 2002; Hucker et al., 1966). Around 64% - 85% of the administered [^{35}S]-MSM dose was recovered in the urine after 24 h - 120 h, whereas only ~3% - 10% was found in the faeces (Magnuson et al., 2017a; Otsuki et al., 2002). The calculated half-life of MSM in blood was found to be ~12.2 hours for a single dose of MSM (500 mg/kg bw) in rats (Magnuson et al., 2007a).	In both humans and animals, urine is the main route of elimination and MSM was eliminated mostly unchanged. Humans (adults, no data for children): The half-life time was roughly estimated to be ½ hour and estimated elimination was around 8 hours. The half-life of MSM in brain was found to be ~72 hours for a single dose of MSM (27.4 mg/kg bw), or ~ 7.5 days for MSM dose of 182 mg/kg bw for seven days. Rodents: The half-life from rat studies was ~12.2 hours. In addition to elimination in urine, small amount of MSM have been found also in rat faeces.

4.2 Genotoxic potential

Gene mutations and structural and numerical chromosomal alterations should be addressed to evaluate genotoxic potential (Klimisch et al., 1997). To identify relevant data of sufficient quality to answer question 1 (Table 4-1), we searched for publications addressing the endpoints gene mutations and structural and numerical chromosomal alterations, published in electronic databases and outside traditional publishing channels. To ensure that the included data were of sufficient quality, internal validity and compliance with respective OECD test guideline was evaluated for studies fulfilling the eligibility criteria.

4.2.1 Identification of relevant literature of sufficient quality

Literature searches in MEDLINE (Ovid) and Embase (Ovid) was performed. The search strategy is reported in Appendix (Section 11.1). Websites of international risk assessment organisations were also searched to identify opinions, risk or safety assessments of MSM. One report was identified (AECOSAN, 2014), however, no additional studies on genotoxicity were included in this report.

4.2.1.1 Publication selection

Four scientific studies were identified in the literature search. The search result was screened based on predefined eligibility criteria presented in Table 4.2.1.1-1.

Table 4.2.1.1-1. Eligibility criteria for studies on genotoxicity.

Exposure	MSM
Outcome of interest	Genotoxicity
Publication type	Primary studies

First, pairs of reviewers screened titles and abstracts independently, and two publications were included. Next, pairs of reviewers screened the full-text articles independently, and two publications were included. A flowchart for the publication selection is available in Figure 4.2.1.1-1. An overview of the study designs and outcomes addressed in the eligible studies is given in Table 4.2.1.1-2. Detailed data extraction forms for Kantor et al. (2013) and Lee et al. (2006) are included in the Appendix (Section 11.4).

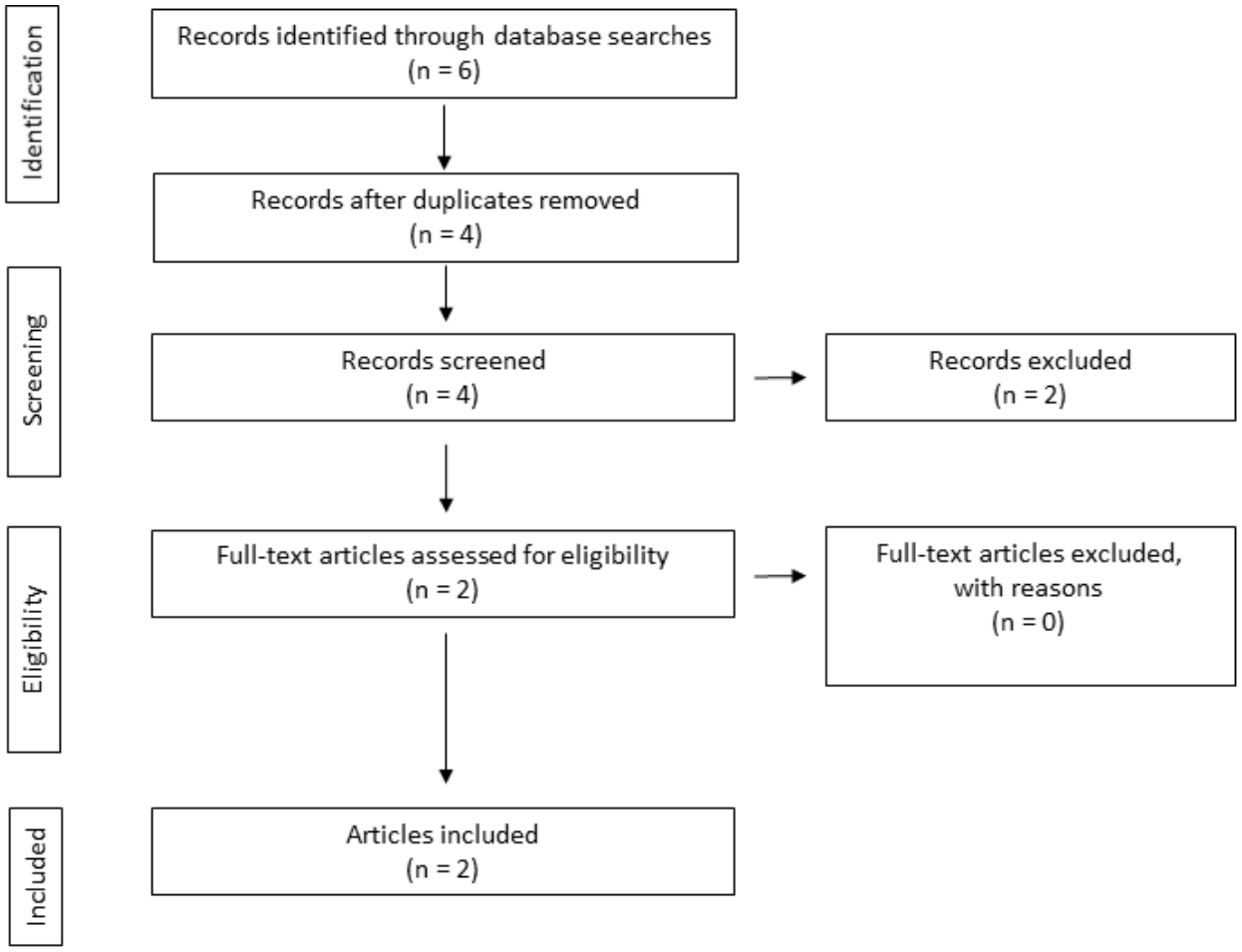


Figure 4.2.1.1-1. Flowchart for the selection of eligible publications on genotoxicity (modified from Moher et al. (2009)).

Table 4.2.1.1-2. An overview of the eligible genotoxicity studies.

Reference	Study design	Method
Kantor et al. (2013)	Cohort, human study	*In vivo* mammalian alkaline comet assay (human lymphocytes)
Lee et al. (2006)	Animal experimental and *in vitro* study	Mammalian *in vivo* micronucleus test Bacterial reverse mutation assay *In vitro* chromosome aberration assay

4.2.1.2 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, not *in vitro* studies.

The RoB questions 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 4.2.1.2-1. Tier 1 represents low RoB, tier 3 represents high RoB. Tier 2 studies did not meet the criteria for tier 1 or 3. Questions 1, 2, 4 and 5 were defined as key questions for cohort studies, whereas questions 3, 4, 6 and 7 were defined as non-key questions (Table 4.2.1.2-2). The key questions address the elements selection bias (appropriate comparison groups), confounding bias, and detection bias (confidence in the exposure characterisation and the outcome assessment). The non-key questions address the elements attrition/exclusion bias and selective reporting bias.

Questions 3, 4, 6 and 7 were defined as key questions for animal studies, whereas questions 1, 2, 5, 8 and 9 were defined as non-key questions (Table 4.2.1.2-3). The key questions address the elements performance bias (experimental conditions and blinding of research personnel) and detection bias (confidence in the exposure characterisation and the outcome assessment). The non-key questions address the elements selection bias (randomisation of exposure and allocation concealment), attrition/exclusion bias and selective reporting 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 4.2.1.2-1. 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. For the human cohort study (Kantor et al., 2013), the overall RoB was classified as tier 3 (Table 4.2.1.2-2), and for the animal study (Lee et al., 2006), the overall RoB was classified as tier 2 (Table 4.2.1.2-3). The detailed evaluation for each RoB question is included in the Appendix (Section 11.3).

Table 4.2.1.2-2. RoB rating and the classification into tier for the human cohort study. *Key question.

Reference	1. Did selection of study participants result in appropriate comparison groups?*	2. Did the study design or analysis account for important confounding and modifying variables?*	3. Were outcome data complete without attrition or exclusion from analysis?	4. Can we be confident in the exposure characterisation?*	5. Can we be confident in the outcome assessment?*	6. Were all measured outcomes reported?	7. Were there no other potential threats to internal validity?	Tier
Kantor et al. (2013)	+	++	-	-	-	++	+	3

Table 4.2.1.2-3. RoB rating and the classification into tier for the animal study. *Key question.

Reference	1. Was administered dose or exposure level adequately randomized?	2. Was allocation to study groups adequately concealed?	3. Were experimental conditions identical across study groups?*	4. Were the research personnel blinded to the study group during the study?*	5. Were outcome data complete without attrition or exclusion from analysis?	6. Can we be confident in the exposure characterisation?*	7. Can we be confident in the outcome assessment?*	8. Were all measured outcomes reported?	9. Were there no other potential threats to internal validity?	Tier
Lee et al. (2006)	+	+	+	+	++	+	-	+	-	2

4.2.1.3 Evaluation of reliability and compliance with OECD test guideline

To evaluate the quality of the studies, we compared the studies with the OECD test guidelines. We used the Klimisch scoring system to assess the reliability of the studies (Table 4.2.1.3-1) (Klimisch et al., 1997).

Table 4.2.1.3-1. An overview of the genotoxicity tests included in the two eligible studies.

Test name and OECD number	Genotoxic endpoint and test model	Non-compliance with the OECD test guideline	Evaluation of quality and reliability
Kantor et al. (2013)			
In vivo mammalian alkaline comet assay, 489	Primary DNA damage were measured in viable human lymphocytes isolated from semi-fasting (more than 6 hours) blood.	The OECD guideline study is based on the use of animals (rodents) in an experimental study. Kantor et al. (2013) used lymphocytes from humans participating in a cohort study, thus, sufficient factors related to the exposure was not included/reported (no vehicle controls or positive controls, the doses were not reported, the time of day for the intake of the supplement was not reported).	The use of MSM supplement was not associated with baseline DNA damage. It was reported that there was an association between use of MSM supplements and reduced DNA repair capacity at 60 minutes. **Klimish score: 3 – not reliable.** The study has severe deficiencies, with the most severe being inappropriate model, study design and uncontrolled exposure. The study will therefore only be used as supporting evidence.
Lee et al. (2006)			
Bacterial reverse mutation assay (Ames test), 471	Gene mutation, strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1538).	The number of strains included (four) was lower than recommended (at least five). The included strains covered base pair substitution and frameshift, not base substitutions, small deletions, cross-linking and oxidizing agents. The number of doses tested (three) was lower than the recommended number (at least five).	MSM did not show mutagenic activity in the strains tested. **Klimish score: 2 - reliable with restriction.** The study has a few deficiencies, but VKM considers that overall, the study is acceptable for the types of

Test name and OECD number	Genotoxic endpoint and test model	Non-compliance with the OECD test guideline	Evaluation of quality and reliability
		The results were reported as mean ±SD, whereas the guideline suggests to report individual plate counts, the mean number of revertant colonies per plate and the standard deviation should be presented for the test substance and positive and negative (untreated and/or solvent) controls.	mutations that are covered by the included strains. However, base substitutions, small deletions, cross-linking agents and oxidizing agents are not covered.
In vitro chromosome aberration assay, 473	Structural chromosome aberrations, Chinese Hamster Lung cell line.	Cytotoxicity and cell proliferation was not measured, as suggested in the guideline. 100 cells per concentration/ control were scored, whereas the guideline suggests that at least 300 well-spread metaphases should be scored per concentration and control to conclude a test chemical as clearly negative.	MSM did not cause structural chromosome aberrations. **Klimish score 3 – not reliable.** The study has severe deficiencies and will only be used as supporting evidence.
Mammalian *in vivo* micronucleus test, 474	Structural and numerical chromosome aberrations, mouse bone marrow.	It was not reported that it was verified that MSM reached the bone marrow.	MSM did not cause structural or numerical chromosome aberrations. **Klimish score 2 – reliable with restrictions.** There are deficiencies concerning the reporting of methods and results. Also, there is a lack of proof that MSM reached the general circulation or target tissue exposure. However, following oral administration two subsequent days of ^{35}S labelled MSM in mice, ^{35}S activity were detected in serum, liver, small intestine and skeletal muscle from hind limbs (Wong et al. 2017). The physical and chemical properties of MSM,

Test name and OECD number	Genotoxic endpoint and test model	Non-compliance with the OECD test guideline	Evaluation of quality and reliability
			such as low molecular weight, high solubility and low lipophilicity, are also indicative that MSM is likely to reach general circulation. On the basis of the abovementioned information, VKM concludes that there is sufficient evidence for target tissue exposure.

4.2.2 Application of quantitative structure-activity relationship to predict genotoxicity

4.2.2.1 Gene mutations

Bacterial *in vitro* mutagenicity (Ames test) of MSM was estimated using the VEGA decision rule system. Four different models, *CAESAR, SARpy, ISS and KNN*, which are based on different methodologies (i.e., statistical and expert rule-based) were used.

All four models were in agreement providing negative predictions concerning the mutagenic potential of MSM. However, the ISS prediction was deemed not reliable and was not taken into account in the expert judgement assessment.

4.2.2.2 Structural and numerical chromosomal alterations

Clastogenic and aneugenic potential of MSM were estimated by using two predictors: VEGA (version 1.1.5) and Toxtree (version 3.1.0) decision rule systems. For Toxtree the decision tree for *Structure alerts for in vivo micronucleus assay in rodents* were applied. No alerts were detected. For VEGA, the models Chromosomal aberration, *in vitro* micronucleus and *in vivo* micronucleus were not applicable.

The Chromosomal aberration and *in vitro* micronucleus had low reliability and MSM was out of the applicability domain of the model and was therefore not included in the expert judgement assessment. For the *in vivo* micronucleus model, there was moderate reliability and there was a positive prediction, meaning MSM was predicted to be genotoxic. However, the similar compounds to MSM identified to have genotoxic potential and used as the basis for the prediction, all had structural alerts that is not present in MSM. VKM considers that based on the QSAR results, MSM is not likely to be genotoxic.

4.2.2.3 Read-across on genotoxicity

Read across with OECD QSAR Toolbox 4.4.1 (QTB) initially referred to 11 experimental study results from the Toxnet, Genotoxicity OASIS and IUCLID databases. They were all negative for gene mutation. Results from experimental studies will weigh heavily in the read across verdict. There were no structural alerts relevant for genotoxicity on substructures in MSM (sulphone). Read-across were all negative when evaluating between 110 and 8748 structurally similar molecules, depending on the search strategy chosen in QTB. The read across verdicts were based on the five nearest neighbors among molecules with >50% similarity to MSM. No metabolites were known from experiments nor suggested by the three metabolism simulators in QTB. Thus, metabolites were not considered in this analysis.

4.2.2.4 QSAR on genotoxicity

69 pre-defined QSARs in QTB reported seven negative and five positive predictions on genetic toxicity. The five positive predictions were based on the similarity between the sulphone and the sulphonic acid substructure. Substances with the sulphonic acid substructure have tested positive in some genotoxoicity assays.

4.2.2.5 Conclusions from read-across and QSAR

MSM can with high reliability be regarded as not genotoxic according to read-across and QSAR.

4.2.3 Evidence synthesis and evaluation confidence in evidence

4.2.3.1 Gene mutations

We identified two experimental tests that are relevant for assessing the potential of MSM to induce gene mutations. In addition, application of QSAR and read-across to predict mutagenicity was performed.

The bacterial reverse mutation test (Lee et al. (2006)) were assessed as reliable with restrictions (Klimisch score 2), however the test did not cover all types of mutations. QSAR models predicted MSM to be non-mutagenic. The comet assay assessing primary damage (Kantor et al., 2013) were assessed as not reliable (Klimisch score 3) and to have high RoB and will not be included in the confidence of evidence. QSAR models and read-across predicted MSM to be non-mutagenic.

There is consistency between experimental and *in silico* results. VKM considers that the body of evidence is of sufficient quality and relevance to be able to conclude on the mutagenicity of MSM. There is consistency between experimental and *in silico* results.

4.2.3.2 Structural and numerical chromosomal alterations

We identified three experimental tests that are relevant for assessing the potential of MSM to induce chromosomal aberrations. In addition, application of QSAR to predict aneugenicity and clastogenicity was performed.

The mammalian *in vivo* micronucleus test assessing structural and numerical chromosome aberrations were assessed as reliable with restrictions (Klimisch 2) and to have moderate RoB. The *in vitro* mammalian chromosome aberration test (Lee et al., 2006) were assessed as not reliable (Klimisch score 3) and was only used as supporting evidence. The comet assay assessing primary damage (Kantor et al., 2013) were assessed as not reliable (Klimisch score 3) and to have high RoB and was not included in the confidence of evidence. QSAR model predicted MSM to be positive for the induction of micronuclei. The positive prediction was based on structural alerts not found in MSM and VKM therefore considered this prediction as not relevant. In addition, both VEGA and QTB read-across analyses calculated that the information from these substances did not indicate the combined negative prediction for genotoxicity.

4.2.4 Conclusion on genotoxic potential

VKM considers that the body of evidence on the genotoxic potential of MSM is of sufficient quality and relevance and concludes that there is no concern for genotoxicity.

4.3 Adverse health effects

To identify relevant data of sufficient quality to answer question 2, 3 and 4 (Table 4-1), we searched for publications published in electronic databases and outside traditional publishing channels. In addition, websites of international risk assessment organisations were also searched to identify opinions, risk or safety assessments of MSM. One report was identified (AECOSAN, 2014), however, no additional studies on adverse health effects were included based in this report.

4.3.1 Literature searches in electronic databases

Literature searches in MEDLINE (Ovid) and Embase (Ovid) was performed. The search strategy is reported in Appendix (Section 12.1).

4.3.1.1 Publication selection

Literature retrieved from the searches were screened based on the eligibility criteria presented in Tables 4.3.1.1-1 (animal studies) and 4.3.1.1-2 (human studies).

Table 4.3.1.1-1. Hazard: eligibility criteria for animal studies.

Study design	Animal studies testing more than one dose of the substance
Animal models	Mammalian animals
Exposure	MSM 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 associated with the substance assessed
Language of the full-text	English, Norwegian, Swedish, Danish, German
Publication type	Scientific publications

Table 4.3.1.1-2. Hazard: eligibility criteria for human studies.

Study design	Human experimental studies (RCTs and other controlled studies) Human observational studies (cross-sectional studies, case-control studies and cohort studies)
Population	All age groups, males and females
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 119 publications. First, pairs of reviewers screened titles and abstracts independently, and 30 publications were included. Next, pairs of reviewers screened the full-text articles independently, and 12 were included (Figure 4.3.1.1-1).

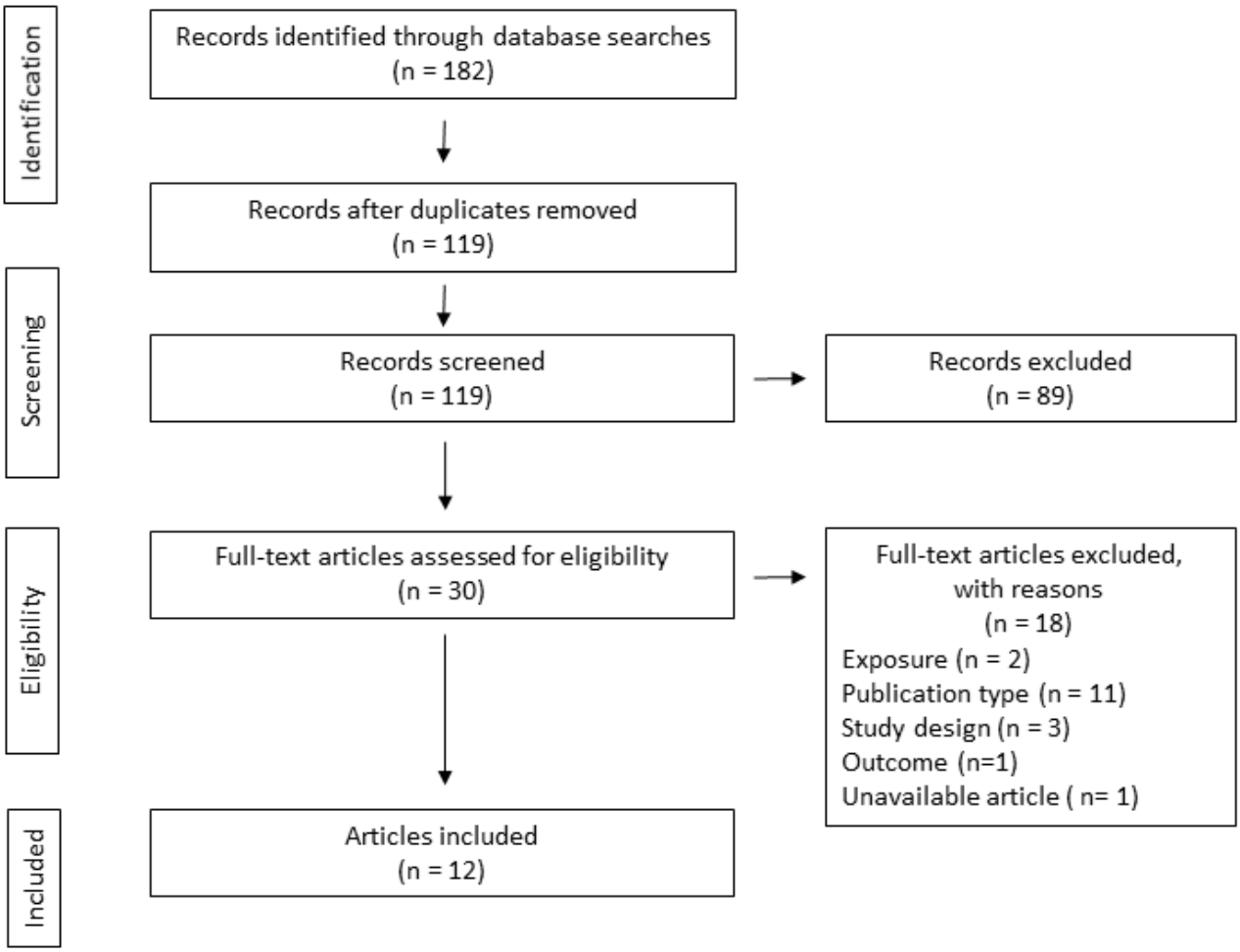


Figure 4.3.1.1-1. Flowchart for the selection of publications on adverse health effects (modified from Moher et al. (2009)).

4.3.1.2 Internal validity

RoB was evaluated as described in Section 4.2.1.2, and an overview of the scoring and classification into tiers is given in Table 4.3.1.2-1, 4.3.1.2-2 and 4.3.1.2-3.

For human cohort studies and animal experimental studies, the definitions of key and non-key questions are described in Section 4.2.1.2. For human intervention studies, we defined questions 1, 2, 3, 5 and 6 as key questions, whereas questions 4, 7 and 8 were defined as non-key questions (Table 4.3.1.2-1). 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.

Two reviewers independently assessed RoB. The human intervention studies were classified as follows: two tier 1 and four tier 3 (Table 4.3.1.2-1). The human cohort studies were classified as tier 1 and tier 3 (Table 4.3.1.2-2). The animal study experimental studies were classified as follows: two tier 1 and two tier 2 (Table 4.3.1.2-3). The detailed evaluation for each RoB question is included in the appendix (Section 12.3).

Table 4.3.1.2-1. RoB rating and the classification into tier for human intervention studies *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
Barrager et al. (2002)	– –	– –	– –	+	-	-	-	-	3
Crawford et al. (2019b)	++	++	++	+	+	++	+	++	1
Hewlings and Kalman (2018)	+	+	++	++	-	-	+	-	3
Kim et al. (2006)	++	++	++	-	+	+	+	++	1
Tennent et al. (2017)	++	++	++	-	-	+	++	– –	3
Usha and Naidu (2004)	++	++	++	+	-	+	– –	– –	3

Table 4.3.1.2-2. RoB rating and the classification into tier for human cohort studies. *Key question.

	1. Did selection of study participants result in appropriate comparison groups?*	2. Did the study design or analysis account for important confounding and modifying variables?*	3. Were outcome data complete without attrition or exclusion from analysis?	4. Can we be confident in the exposure characterisation?*	5. Can we be confident in the outcome assessment?*	6. Were all measured outcomes reported?	7. Were there no other potential threats to internal validity?	Tier
Lin et al. (2001)	– –	-	+	-	++	+	+	3
Satia et al. (2009)	+	++	++	+	++	++	++	1

Table 4.3.1.2-3. RoB rating and the classification into tier for animal experimental studies. *Key question.

	1. Was administered dose or exposure level adequately randomized?	2. Was allocation to study groups adequately concealed?	3. Were experimental conditions identical across study groups?*	4. Were the research personnel blinded to the study group during the study?*	5. Were outcome data complete without attrition or exclusion from analysis?	6. Can we be confident in the exposure characterisation? *	7. Can we be confident in the outcome assessment? *	8. Were all measured outcomes reported?	9. Were there no other potential threats to internal validity?	Tier
Ezaki et al. (2013) (subacute toxicity, rat study)	+	+	+	+	++	-	+	++	++	2
Ezaki et al. (2013) (subacute toxicity, mice study)	+	+	+	+	++	-	+	++	++	2
Horvath et al. (2002) (acute toxicity)	+	+	++	+	+	+	-	-	+	2
Horvath et al. (2002) (subchronic toxicity)	+	+	++	+	+	+	-	-	+	2
Kamel and El Morsy (2013) (acute toxicity)	+	+	++	+	++	+	+	++	++	1

	1. Was administered dose or exposure level adequately randomized?	2. Was allocation to study groups adequately concealed?	3. Were experimental conditions identical across study groups?*	4. Were the research personnel blinded to the study group during the study?*	5. Were outcome data complete without attrition or exclusion from analysis?	6. Can we be confident in the exposure characterisation?*	7. Can we be confident in the outcome assessment?*	8. Were all measured outcomes reported?	9. Were there no other potential threats to internal validity?	Tier
Magnuson et al. (2007b) (maternal and developmental toxicity)	+	+	++	+	+	++	+	++	++	1

4.3.1.3 Study characteristics

As publications classified as tier 3 (high RoB) are not included in the evidence synthesis due to the high concern for bias on key element(s), only study characteristics for the eligible publications classified as tier 1 (low RoB) or tier 2 (moderate RoB) are reported. A brief overview is given in Table 4.3.1.3-1 and 4.3.1.3-2, whereas detailed descriptions and data extraction forms are available in Section 12.4 (Appendix).

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

Reference	Aim	Participant characteristics	Dose and duration	Endpoints	Result (adverse health effects)
Randomised controlled trials					
Crawford et al. (2019b)	To ensure that 16 weeks of MSM does not cause adverse effects in patients with the musculoskeletal disorders of osteoarthritis and back pain.	Participants were active duty military members and retired military members and their families all having symptoms of low back pain lasting greater than 12 weeks. Age: 18 to 65 years.	6 g MSM/day (n=46; 34.8% female and 65.2% male) or placebo (n=40; 37.5% female and 72.5% male). The dose was equivalent to about 73 mg/kg bw/day. All participants received naproxen. 16 weeks study duration.	Hematology, including white blood cells, platelets and haemoglobin. Clinical biochemistry, including glucose, total bilirubin, alanine aminotransferase, aspartate aminotransferase, and creatinine. Physiologic parameters including weight, diastolic and systolic blood pressure.	No significant difference between MSM and placebo.
Kim et al. (2006)	Evaluate efficacy of MSM in osteoarthritis pain of the knee.	50 participants, 40-76 years with osteoarthritis pain of the knee. 25 participants received MSM, 25 participants received placebo. Of the 50 patients enrolled, 40 completed the study: 21 (84%) in the MSM group and 19	A stepwise approach to the full dose was applied. 2 g/day in two divided doses for 3 days. 4 g/day in two divided doses for 4 days. 6 g/day in two divided doses for 11 weeks.	At baseline and 12 weeks: Hematology, including complete blood counts and differential white blood cells. Clinical biochemistry, including renal and hepatic functions. Questionnaires including GI symptoms and modified neurotoxic symptoms. Physiologic parameters, including blood pressure, weight, body mass index.	No major adverse events reported. Side effects reported included bloating, constipation, indigestion, fatigue, concentration issues, insomnia, and headache. Patients in the MSM and placebo groups reported the symptoms in comparable frequency.

		(76%) in the placebo group.	21 receiving MSM completed the study (68.4% female and 31.6 male). 19 receiving placebo completed the study (57.1% female and 42.9 % male). 40-76 years.	Weekly and biweekly phone calls to patients.	
Cohort study					
Satia et al. (2009)	Examine associations of various herbal/specialty supplements with lung and colorectal cancer risk.	77,125 participants (the VITAL study), 50 to 76 years. 76,460 controls (52% female and 48% male), 665 lung cases (45% female; 55% male). 76,084 controls (45% female and 55% male), 428 colorectal cancer cases (49% female; 51% male). Recruitment was conducted from October 2000 to December 2002.	A closed-ended format was used to inquire about current versus past use of supplements, duration of use (1-2, 3-5, 6+ years), and frequency (1-2, 3-5, 6+ days per week) over the previous 10 years. Questions on dose were not included because of lack of accurate information on potency.	Participants were followed for lung and colorectal cancers occurring from baseline through December 31, 2006, by linking the cohort to the Seattle-Puget Sound SEER registry.	No increase in cancer risk reported.

Table 4.3.1.3-2. Animal studies on adverse effects.

Reference	Aim	Study design	Exposure	Outcomes assessed and results (adverse effects)
Ezaki et al. (2013) (subacute toxicity)	To assess safety and efficacy of MSM on bone and knee joints in osteoarthritis animal model; cartilage formation.	Cartilage formation: 6-week-old growing male Wister rats were used and assigned to 4 groups; 7 animals/group. Study duration was 4 weeks. Cartilage formation: Body and tissue weights were measured, and the right femur and tibia were removed for measurement of bone mineral density. The left legs were removed for measurement of cartilage thickness. The kidney and liver were subjected to histomorphological analysis.	MSM (pure MSM 100 %). Doses of 0.06, 0.6, and 6 g/kg bw/day. 0.06 g/kg bw/day was supposed to be equal to the highest recommended dose of the so-called health foods, i.e., 3 g/day for an adult of body weight 50 kg.	Intake of 0.6 and 6 g/kg bw/day decreased liver weight, lean body mass, and bone mineral density. Serum biochemical markers: triglyceride and serum calcium were significantly reduced. Body weight and spleen weights in the 6 g/kg bw/day group were significantly lower, whereas the kidney weight was significantly higher than the control group.
Ezaki et al. (2013) (subacute toxicity)	To assess safety and efficacy of MSM on bone and knee joints in osteoarthritis animal model; cartilage degradation.	Cartilage degradation: 10-week-old male STR/OrtCrlj mice were used and assigned to 3 groups. Study duration was 13 weeks. For cartilage degradation, 10-week-old male STR/OrtCrlj mice were used and assigned to 3 groups. Study duration was 13 weeks.	MSM (pure MSM 100 %). Doses of doses of 0.06 and 0.6 g/kg bw/day. 0.06 g/kg bw/day was supposed to be equal to the highest recommended dose of the so-	Cartilage degradation: Body weight and total food intake did not differ among the three groups, and total bone mineral density of the femur and tibia in control, low and middle dosed MSM groups was not significantly different. The spleen weight in the 0.6 g/kg bw/day group was significantly lower than the control group. MSM intake

Reference	Aim	Study design	Exposure	Outcomes assessed and results (adverse effects)
			called health foods, i.e., 3 g/day for an adult of body weight 50 kg.	decreased total liver score including fat vacuole score, glycogen area, and focal necrosis score in a dose-dependent manner. Mice: the spleen weight in the 0.6 g MSM/kg bw/day group was significantly lower than the control group. MSM intake decreased total liver score including fat vacuole score, glycogen area, and focal necrosis score in a dose-dependent manner.
Horvath et al. (2002) (acute toxicity)	To evaluate the acute toxicity of MSM in rats at a dose five to seven times the maximum recommended dose in humans.	Animals were assigned randomly to their respective treatment groups based on body weight. After randomisation, 10 male and 10 female rats were assigned to the treatment groups	Each rat received a single oral dose by gavage of 2 g/kg bw of MSM or a single dose by gavage of the vehicle.	MSM administered in a single gavage dose of 2 g/kg resulted in no adverse events or mortality. It was concluded that MSM is well tolerated in rats at an acute dose of 2 g/kg.
Horvath et al. (2002) (subchronic toxicity)	To evaluate the subchronic toxicity of MSM in rats at a dose five to seven times the maximum recommended dose in humans.	Animals were randomly assigned to each respective treatment group based on body weight. An equal number of males and females were assigned to each treatment group.	Each rat in the MSM treatment group received a daily dose of 1.5 g/kg bw of MSM by gavage in a volume of 10 ml/kg distilled water for 90 days. Rats in the control group received 10 ml/kg bw distilled water daily for 90 days.	MSM administered as a daily dose of 1.5 g/kg for 90 days by gavage resulted in no adverse events or mortality. Necropsy did not reveal any gross pathological lesions or changes in organ weights. Renal histology of treated animals was normal. No hematological or blood chemistry alterations were measured. It was concluded that MSM is well tolerated in

Reference	Aim	Study design	Exposure	Outcomes assessed and results (adverse effects)
				rats at a subchronic dose of 1.5 g/kg. No data were shown.
Kamel and El Morsy (2013) (acute toxicity)	To investigate the effect of MSM on carbon tetrachloride (CCl_4)- induced acute liver injury in rats.	Fifty female Sprague–Dawley rats, divided into 5 groups, 10 per group. Group 1 was the control group, group 3 was the MSM group.	Control group received 10 % tween 80 solution orally by gavage for 5 days. 1 h after the last dose, they received an intraperitoneal injection of corn oil (0.1 ml/100 g body weight) on the 5th day. Rats received MSM (400 mg/kg bw) dissolved in 10 % tween 80 solution orally by gavage for 5 days. 1 h after the last dose, they received an intraperitoneal injection of corn oil (0.1 ml/100 g body weight) on the 5th day.	No histopathological changes were recorded in liver sections of rats treated with MSM for 5 days. No effect on serum biochemical markers including alanine aminotransferase and aspartate aminotransferase.
Magnuson et al. (2007b)	To determine the developmental toxicity potential of MSM when administered orally to pregnant rats during the period of major organogenesis and histogenesis.	A preliminary dose-finding study was performed. MSM was administered as microspherical pellets to 8–9 sperm-positive female Sprague–Dawley rats/group/day on gestation days 6–20. Definitive developmental study: MSM was administered via gavage to four groups of 24–25 timed bred	Maternal and developmental toxicity: The purity of MSM was 99.9% and the formulations were stable for 32 days for concentrations ranging from 10 to 100 mg/ml when stored at room temperature. For the preliminary dose-finding study, MSM was administered by oral gavage at dose levels of 0	Dose-finding study: Maternal feed consumption, body weight, body weight gain, uterus weight and corrected body weight/body weight gain were unaffected by treatment. Developmental toxicity study: No evidence of maternal toxicity, and no significant differences in litter viability, litter size, or litter body weight were detected. Foetal evaluations showed no

Reference	Aim	Study design	Exposure	Outcomes assessed and results (adverse effects)
		primiparous female rats on gestation days 6–20.	(vehicle control), 50, 250, 500, and 1000 mg/kg bw/day. The purity of MSM was 99.9% and the formulations were stable for 32 days for concentrations ranging from 10 to 100 mg/ml when stored at room temperature. For the definitive developmental study, MSM was administered at dose levels of 0 (vehicle control), 50, 500, or 1000 mg MSM/kg bw/day. The study was conducted according to OECD guideline 414, although immunotoxic and endocrine endpoints were not included.	biologically significant increase in the incidence of anomalies in the MSM treated groups, and no malformations were seen in any of the foetuses. No evidence of foetal mortality, alterations to growth, or structural alterations were observed in the foetuses of dams administered 50–1000 mg/kg/day.

4.4 Evidence synthesis and evaluation of confidence in the body of evidence

The confidence in the body of evidence was assessed for each outcome according to OHAT (2019) as shown in Table 4.4-1. Four reviewers calibrated themselves once to ensure similar evaluation. Then pair of reviewers independently evaluated the confidence in evidence for each outcome. A more detailed evaluation of the confidence in evidence is given in Section 12.5 (Appendix).

Table 4.4-1. Confidence of evidence for the different lines of evidence for MSM. The lines of evidence are sorted by the outcomes.

Outcome, line of evidence (n) and initial rating	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
Blood pressure								
2 RCTs Initial rating:++++	Not serious	Not serious	Not serious	Serious	Not large	No	-	Moderate
Body weight and organ weight								
2 RCTs Initial rating:++++	Not serious	Not serious	Not serious	Serious	Not large	No	-	Moderate
4 animal studies Initial rating:++++	Serious	Serious	Not serious	Not serious	Not large	No	No	Low
Cancer (lung and colorectal)								
1 cohort study Initial rating: +++	Not serious	-	Not serious	Serious	Not large	No	-	Low
Developmental toxicity								
1 animal study Initial rating:++++	Not serious	-	Not serious	Serious	Not large	No	-	Moderate
Hematology and clinical biochemistry								
2 RCT Initial rating:++++	Not serious	Not serious	Not serious	Serious	Not large	No	-	Moderate
3 animal studies Initial rating:++++	Serious	Not serious	Not serious	Serious	Not large	No	No	Low
Kidney toxicity								

2 RCTs Initial rating:++++	Not serious	Not serious	Not serious	Serious	Not large	No	-	Moderate
3 animal studies Initial rating:++++	Serious	Serious	Not serious	Serious	Not large	No	No	Very low
Liver toxicity								
2 RCTs Initial rating:++++	Not serious	Not serious	Not serious	Serious	Not large	No	-	Moderate
4 animal studies Initial rating:	Serious	Serious	Not serious	Not serious	Not large	No	No	Low
Oxidative stress								
1 RCT Initial rating:++++	Not serious	-	Not serious	Serious	Not large	No	-	Moderate
1 animal study Initial rating:++++	Not serious	-	Not serious	Serious	Not large	No	-	Moderate
"Other side effects"								
1 RCT Initial rating:++++	Not serious	-	Not serious	Serious	Not large	No	-	Moderate

4.5 Summary and conclusions of hazard identification and characterisation

Literature searches were performed to identify relevant studies to answer the following ADME questions:

- What is the ADME of MSM in humans? Is human and animal (rodent) ADME similar?
- Is MSM metabolised to innocuous metabolites?
- Is MSM 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?

The studies reported that, in both humans and animals, MSM is rapidly absorbed, widely distributed throughout the body, and urine is the main route of excretion. A pathway for endogenous MSM production has been suggested, however, the level of the endogenous production is not known and it is therefore not possible to conclude whether the dose given in the mandate from NFSA results in body levels within the MSM range normally metabolised and eliminated. As no data on MSM metabolism were available, it is not possible to know if MSM metabolites are innocuous.

Literature searches were performed to identify relevant studies to answer the following questions:

- Is there a concern for genotoxicity?
- Is exposure to MSM associated with adverse health effects?
- What is the dose-response relationships between exposure to MSM and the adverse effects?
- Can a health-based guidance value be established or a point of departure be identified?

For genotoxicity, two experimental tests assessing the genotoxic potential of MSM were included (Kantor et al., 2013; Lee et al., 2006). For other adverse effects, two RCTs with osteoarthritis patients (Crawford et al., 2019b; Kim et al., 2006), one cohort study (Satia et al., 2009), and four publications on animal experimental studies (Ezaki et al., 2013; Horvath et al., 2002; Kamel and El Morsy, 2013; Magnuson et al., 2007b) were included.

A brief overview of the studies included in the evidence synthesis for other adverse effects is given in Table 4.5-1 (more detailed study characteristics in Section 4.3.1.3 and 12.4). A brief overview of the overall confidence in the body of evidence for the different outcomes is given in Table 4.5-2 (more detailed information in Section 4.4 and 12.5).

Table 4.5-1. An overview of the studies included in the evidence synthesis for adverse effects.

Reference	Study design	Dose and duration	Intervention/control (number that completed the study)	Outcomes	Adverse effects
Human studies					
Crawford et al. (2019b)	Clinical trial	6 g MSM/day for 16 weeks. All participants received naproxen	46 (34.8% female; 65.2% male) /40 (37.5% female; 72.5% male) 18-65 years	Blood pressure, body/organ weight, hematology, clinical biochemistry, kidney effects, liver effects	No significant difference between MSM and placebo
Kim et al. (2006)	Clinical trial	2 g/day for three days; 4 g/day for four days; 6 g/day for eleven weeks	21 receiving MSM completed the study (68.4% female and 31.6 male). 19 receiving placebo completed the study (57.1% female and 42.9 % male) 40-76 years	Blood pressure, body/organ weight, hematology, clinical biochemistry, kidney effects, liver effects, oxidative stress, "other side effects"	No major adverse events reported. "Other side effects" reported in comparable frequency by both groups
Satia et al. (2009)	Cohort	Users (no specific doses reported)	Lung cancer: 665 (45% female; 55% male)/76,460 (52% female; 48% male) Colorectal cancer: 428 (49% female; 51% male)/76,084 (52% female; 48% male) 50-76 years	Cancer (lung, colorectal)	No increased risk for lung or colorectal cancer
Animal experimental studies					

Reference	Study design	Dose and duration	Intervention/control (number that completed the study)	Outcomes	Adverse effects
Ezaki et al. (2013)	Animal experimental, cartilage formation (rats) and degradation (mice)	Rats: 0.06, 0.6, and 6 g/kg bw/day for four weeks Mice: 0.06 and 0.6 g/kg bw/day for 13 weeks	Rats: 7/7 Mice: 6/6	Body/organ weight, clinical biochemistry, kidney effects, liver effects	Rats: Intake of 0.6 and 6 g/kg bw/day: decreased liver weight, lean body mass, and bone mineral density. Serum triglyceride and serum calcium were significantly reduced. Body weight, spleen and kidney weights in the 6 g/kg bw/day group were significantly lower and higher than those in the control group, respectively Mice: The spleen weight in the 0.6 g/kg bw/day group was significantly lower than the control group. MSM intake decreased total liver score including fat vacuole score, glycogen area, and focal necrosis score in a dose-dependent manner
Horvath et al. (2002)	Animal experimental, acute and subchronic toxicity (OECD, 408)	Rat, acute toxicity: 2 g/kg bw (one dose, gavage) Rat, subchronic toxicity: 1.5 g/kg bw/day for 90 days	Acute toxicity: 10/10 Subchronic toxicity: 40/40	Body/organ weight, hematology, clinical biochemistry, kidney, liver	Acute toxicity: no adverse events or mortality Subchronic toxicity: no adverse events or mortality

Reference	Study design	Dose and duration	Intervention/control (number that completed the study)	Outcomes	Adverse effects
Kamel and El Morsy (2013)	Animal experimental, effect of MSM on liver injury (induced by CCl_4)	0.4 g/kg bw/day for five days	10/10	Liver, oxidative stress	No histopathological changes were recorded in liver sections of rats treated with MSM
Magnuson et al. (2007b)	Animal experimental, developmental toxicity (OECD, 414)	0.05, 0.5, 1.0 g/kg bw/day on gestation days 6–20 (gavage)	24-25/24-25	Body/organ weight, fetal toxicity	No evidence of maternal or fetal toxicity A NOAEL of 1000 mg/kg bw/day was identified

Table 4.5-2. Summary of integration of confidence across lines of evidence for each outcome.

		Blood pressure	**Body weight/organ weight**	**Cancer (lung and colorectal)**	**Developmental toxicity**	**Hematology and clinical biochemistry**	**Kidney toxicity**	**Liver toxicity**	**Oxidative stress**	**«Other side effects»**
Human studies	Significant effects reported (yes/no)	No	No	No	-	No	No	No	No	No
	Confidence (High, moderate, low, very low)	Moderate	Moderate	Low	-	Moderate	Moderate	Moderate	Moderate	Moderate
Animal studies	Significant effects reported (yes/no)	-	Yes and no	-	No	Yes and no	Yes and no	Yes and no	No	-
	Confidence (High, moderate, low, very low)	-	Low	-	Moderate	Low	Very low	Low	Moderate	-

Based on two experimental tests relevant for assessing the potential of MSM to induce gene mutations, and application of QSAR and read-across to predict mutagenicity, VKM considers that the body of evidence on the genotoxic potential of MSM is of sufficient quality and relevance and concludes that there is no concern for genotoxicity.

In short term human studies, no serious adverse health effects of MSM were identified. In animal studies, adverse effects reported included decrease in body weight and organ weights and decrease in bone mineral density. Note that VKM considered the data to be insufficient and that the confidence in the evidence ranged from moderate to very low.

We assessed the confidence in evidence for the outcomes blood pressure, body and organ weight, cancer, maternal and developmental toxicity, hematology and clinical biochemistry, kidney toxicity, liver toxicity, oxidative stress and other side effects (e.g. stomach pain, nausea). No overall dose-response relationships were identified for the body of evidence across studies for any outcome. For the rating of confidence in evidence for the different outcomes, none of the evidence was judged as high, but were moderate to very low. Overall, the confidence in evidence from human studies was higher than from animal experimental data. None of the identified studies on adverse effects of MSM assessed immunotoxicity or endocrine effects.

A total of six experimental animal studies were included in the weight of evidence approach. An acute study assessing liver effects following exposure to 0.4 g/kg bw/day for 5 days did not show any adverse effects (Kamel and El Morsy, 2013). Another acute animal study did not reveal any adverse effects following MSM exposure (Horvath et al., 2002). Significant dose-dependent decrease in liver weight and bone mineral density was observed in a rat subacute study at doses of MSM of 0.06 and 6 g/kg bw/day. Decrease in body weight, spleen and kidney weights, serum triglycerides and serum calcium concentrations were observed at 6 g/kg bw/day compared to the control group. In mice, liver effects were observed in a dose-dependent manner and decrease in spleen weight was observed at 0.6 g MSM/kg bw/day (Ezaki et al., 2013). In one subchronic study, no adverse effects were observed at a dose of 1.5 g/kg bw/day (Horvath et al., 2002). However, a NOAEL could not be established due to poorly reported data. VKM identified a NOAEL of 1 g/kg bw/day MSM (the highest dose) for maternal and developmental toxicity based on a rat study (Magnuson et al., 2007b).

No serious adverse health effects of MSM were identified at the doses (2-6 g/day for 11-16 weeks) reported in the two included RCTs (Crawford et al., 2019b; Kim et al., 2006). One of the RCTs, assessed liver function following use of 6 g MSM/day for 16 weeks and did not identify adverse effects on the liver (Crawford et al., 2019b). One cohort study assessing the association between MSM use and cancer risk, did not find an association (Satia et al., 2009).

VKM concludes that for adults (≥ 18 years), a point of departure (PoD) of 6 g/day of MSM could be identified based on the RCT with exposure length of 16 weeks (Crawford et al., 2019b).

VKM identified several uncertainties concerning the available data and the identified PoD (Table 4.5-3).

Table 4.5-3. Identified uncertainties in the available data for the safety evaluation of MSM.

Identified uncertainty	Description
Exposure length	• PoD is derived from a short-time study and is not representative for chronic exposure
Data insufficiency (toxicokinetic and toxicodynamic)	• Scarce information on ADME of MSM in humans • Few studies and the human studies have small numbers of participants • Lack of data on immunotoxicity and endocrine effects • Lack of data on skeletal effects
Data quality	• The confidence in evidence is very low to moderate for all outcomes

As there is a possibility of identifying a lower PoD (a more sensitive effect), the identified uncertainties need to be accounted for in the evaluation of the margin of exposure (MoE) in the risk characterisation (Table 4.5-4). MoE is the margin required between the PoD and the estimated exposure in order to ensure safety, and uncertainty factors (UFs) are used to determine the acceptable MoE. Derivation of UFs should be considered on a case-by-case basis and justified (EFSA, 2012). The derivation of an UF for MSM:

- The PoD is derived from a short-term human study. For animal experiments there are definitions of which exposure length that equals an acute, subacute, subchronic and chronic exposure length. In addition, established guidelines for extrapolation from short-term to chronic exposure exists (EFSA, 2012; ECHA, 2012). For human studies, no established guidelines exist. For mice and rats a 2-year exposure length is considered to be chronic exposure, whereas an exposure length of 90 days or 13-weeks are considered to be subchronic exposure. Considering a much longer life span in humans compared to rodents, VKM considers the exposure length of 16 weeks in humans to be a subacute exposure. VKM considers that a factor of 6 is sufficient to extrapolate from subacute to chronic exposure, as recommended by ECHA in a REACH guidance document (Table R8-5 in ECHA (2012)).
- Only studies with low to moderate RoB were included in the evidence synthesis, and in general, the human studies (RCTs) had higher quality than the experimental animal studies. The identified PoD of 6 g/day of MSM is derived from a RCT with low risk of bias. However, the confidence in evidence was not high for any of the outcomes but varied from very low to moderate. VKM considers a factor of 2 is sufficient to cover the uncertainties due to data quality.
- Data for ADME of MSM in humans are scarce and there is no information on metabolism. Only a few studies of low to moderate risk of bias that assessed adverse effects following MSM exposure were identified. The human studies included small numbers of participants. The RCT used to identify the PoD for adults (Crawford et al., 2019b) included only a limited number of participants, with a bias on sex (more men than women represented), all with musculoskeletal disorders of osteoarthritis and back pain and all receiving naproxen in addition to MSM, and VKM therefore considers that these participants are likely not representative for the whole adult

population. There is a lack of data concerning immunotoxicity or endocrine effects following MSM exposure. Moreover, one subchronic study in rats showed significant reduction in bone mineral density and for serum calcium concentrations. There is a need for more data on skeletal effects of MSM. To adjust for human interindividual variation VKM uses the default uncertainty factor of 10, consisting of the subfactors 3.16 each for toxicokinetic and toxicodynamic variation (EFSA, 2012). These subfactors are meant to cover all age groups. As the PoD is only set for adults and no serious adverse effects were identified for the dose range of the PoD, including maternal or fetal toxicity, VKM considers that the default subfactors divided by two (3.16/2) is sufficient to cover potential differences in toxicokinetic and toxicodynamic in the adult population.

VKM concludes that the uncertainty related to data insufficiency, data quality and the extrapolation from short-term to long-term exposure is adequately covered by a factor of 30, which is the acceptable margin of exposure.

Table 4.5-4. Uncertainties that need to be accounted for in the margin of exposure for adults.

Area of uncertainty		**Factor**	
Short-term to long-term exposure		6	Adjusts for the possibility of identifying a lower PoD for chronic toxicity when extrapolating from a study of shorter duration (EFSA, 2012).
Data quality		2	Adjusts for very low to moderate confidence in evidence. The UF is derived based on expert judgement.
Data insufficiency	Toxicokinetic	1.58	Adjusts for human interindividual variation (only adults). The UF is derived based on expert judgement based on default values (EFSA, 2012).
	Toxicodynamic	1.58	
Acceptable margin of exposure for adults (≥ 18 years)		30	Adjusts for overall uncertainty

No studies assessing the safety of MSM in children or adolescents were identified. Moreover, ADME data on MSM is lacking. It is therefore not possible to elucidate whether body weight is the only decisive factor for the tolerance level of MSM in children and adolescents. When there is sufficient data of adequate quality, this uncertainty can be adjusted for by applying a UF. However, as mentioned, there are several uncertainties concerning the identified PoD. One uncertainty is lack of data on immunotoxicity and endocrine effects following MSM exposure. Endocrine effects can be seen at very low doses and children and adolescents are considered to be vulnerable groups since altered endocrine function could impact development (WHO, 2012). VKM considers that the uncertainties related to the evidence for possible adverse effects of MSM in children and adolescents is higher than for adults. Based on these considerations, VKM concludes that a PoD for the age groups 3-<10 years, 10-<14 years and 14-<18 years cannot be identified.

5 Risk characterisation

NFSA requested VKM to perform a risk assessment of daily intake of 3 g MSM for the general Norwegian population, both sexes, in the age groups: 3-<10 years, 10-<14 years, 14-<18 years and adults ≥18 years. If 3 g MSM/day is not safe, NFSA requested VKM to identify the amount less than 3 g MSM/day that is safe.

VKM was not able to identify a PoD for the age groups: 3-<10 years, 10-<14 years, 14-<18 years (Section 4.5). Therefore, it is not possible to characterise the risk related to daily intake of 3 g MSM for these age groups.

For adults ≥18 years, VKM identified a PoD of 6 g/day from a short-term human study. A MoE of 10 was identified to account for the uncertainties related to extrapolation from short-term to long-term exposure, for the data quality, and the data insufficiency.

For a daily single dose in adults (≥18 years) of 3 g/day of MSM in food supplements the MoE is 2, which is less than the identified acceptable MoE of 30 (Table 5-1).

Table 5-1. The margin between PoD and MSM dose in food supplements (adults ≥18 years).

PoD (adults ≥18 years)	MSM dose given by the NFSA	Calculated MoE
6 g/day	3 g/day	2

A single dose of 0.2 g/day yields an acceptable MoE of 30.

VKM concludes:

- A daily dose of 3 g MSM from food supplements may represent a risk of adverse health effects in adults ≥18 years.
- It is unlikely that a daily dose of 0.2 g MSM from food supplements causes adverse health effects in healthy adults ≥18 years.
- As limited data are available, VKM cannot conclude on a daily safe dose of MSM for children and adolescents.

6 Uncertainty

There is uncertainty related to MSM metabolism, both the level of endogenous production and whether MSM is metabolised to innocuous metabolites.

It is uncertain to what degree other sources than food supplements of MSM contribute to the exposure. MSM from sources other than food supplements have not been taken into consideration in the conclusion that a daily dose of 0.2 g MSM from food supplements is unlikely to cause adverse health effects in adults ≥18 years.

Several uncertainties were identified concerning the derived PoD for adults. Therefore, a MoE of 30 was identified by expert judgement to account for the uncertainties arising from extrapolation from subchronic to chronic exposure and data insufficiency (Table 4.5-3 and 4.5-4), in order to ensure safety.

7 Summary, discussion and conclusions

NFSA requested VKM to perform a risk assessment of daily intake of 3 g MSM for the general Norwegian population, both sexes, in the age groups: 3-<10 years, 10-<14 years, 14-<18 years and adults ≥18 years. If 3 g MSM/day is not safe, NFSA requested VKM to identify the amount less than 3 g MSM/day that is safe.

MSM is a water soluble, highly stable organic sulphur-containing compound (AECOSAN, 2014). It is present in small quantities in a large variety of fruits, vegetables, grains, meat, eggs and fish, and is consumed in trace amounts in a normal human diet (AECOSAN, 2014; Brien et al., 2008; Crawford et al., 2019a). MSM is found at concentrations about 0.2 mg/kg in the circulation of the adult male body (Hansen et al., 2006), likely derived from the dietary sources, endogenous metabolism and bacterial metabolism (He and Slupsky, 2014).

No ADME data for children and adolescents were available. MSM is rapidly absorbed, within an hour in adult humans, and with mean t_{max} of ~2.1 h in rodents. In both humans and animals, MSM is evenly distributed throughout the body and it crosses blood-brain barrier. A pathway for endogenous MSM production has been suggested, however, the level of the endogenous production is not known. In both adult humans and animals, most MSM is excreted unchanged in urine. Some MSM may be metabolised to other compounds, as several studies show that not all MSM is recovered in urine and faeces. However, no data on MSM metabolism and whether MSM is metabolised to other S-containing metabolites in adult human or rats are available. In both adult humans and animals, urine is the most common excretory pathway and MSM was mostly excreted unchanged. The half-life for elimination was roughly estimated to be ½ hour and estimated total elimination was around 8 hours. In rat studies, the half-life was ~12.2 hours.

The hazard identification and characterisation were based on data from studies identified in literature searches. For the evaluation of genotoxicity, VKM considers that the body of evidence on the genotoxic potential of MSM is of sufficient quality and relevance and concludes that there is no concern for genotoxicity. For adults (≥18 years) a PoD of 6 g/day of MSM was derived, based on a RCT with exposure length of 16 weeks. Several factors contributed to uncertainty in the PoD, including extrapolation from short-term to long-term exposure, moderate to very low confidence in the evidence (data quality), and data insufficiency including scarce information on metabolism of MSM, few studies with a small number of participants that are likely not representative for the whole adult population, and the lack of data on immunotoxicity and endocrine effects. To account for the uncertainties, a MoE of 30 was identified by expert judgement.

Due to the lack of ADME data for MSM, it was not possible to elucidate whether body weight is the only decisive factor for the tolerance level of MSM in children and adolescents compared to adults. Moreover, no studies assessing the safety of MSM in children or adolescents were identified, including lack of data on immunotoxicity and endocrine effects following MSM exposure. Endocrine effects can be seen at very low doses and children and

adolescents are considered to be vulnerable groups since altered endocrine function could impact development (WHO, 2012). Overall, VKM considers that the uncertainties related to the evidence for possible adverse effects of MSM in children and adolescents is higher than for adults. Based on these considerations, VKM concludes that a PoD for the age groups 3-<10 years, 10-<14 years and 14-<18 years cannot be identified.

For a daily single dose in adults (≥18 years) of 3 g MSM in food supplements the MoE is 2, which is less than the identified acceptable MoE of 30 (Table 7-1). A daily single dose of 0.2 g yields an acceptable MoE of 30 (Table 7-1).

Table 7-1. An overview of the factors used to characterise the risk.

Uncertainties and the derivation of an acceptable MoE for MSM	PoD adults ≥18 years	MSM dose	Calculated MoE
• Short-term to long-term exposure; a factor of 2 adjusts for the possibility of identifying a lower PoD for chronic toxicity when extrapolating from a study of shorter duration. • Data insufficiency; a factor of 2.5 (1.58 for toxicokinetics ax 1.58 for toxicodynamics) adjusts for human interindividual variation (only adults). • Data quality; a factor of 2 adjusts for very low to moderate confidence in evidence. • Total adjustments: **MoE = 6 x 1.58 x 1.58 x 2 = 30**	6 g/day	3 g/day	2
	6 g/day	0.2 g/day	30

VKM concludes:

- A daily dose of 3 g MSM from food supplements may represent a risk of adverse health effects in adults ≥18 years.
- It is unlikely that a daily dose of 0.2 g MSM from food supplements causes adverse health effects in healthy adults ≥18 years.
- As limited data are available, VKM cannot conclude on a daily safe dose of MSM for children and adolescents.

Note that MSM sources other than food supplements have not been taken into consideration in the conclusion that a daily dose of 0.2 g MSM from food supplements is unlikely to cause adverse health effects in adults ≥18 years.

8 Data gaps

Data gaps related to ADME:

- No ADME data for children and adolescents were identified, and VKM can therefore not rule out that there are differences with regard to ADME in adults, and children and adolescents.
- No data on MSM metabolism were available from human or animal studies, and it is therefore not possible to know if MSM metabolites are innocuous.

Data gaps related to hazard identification and characterisation

- Human studies investigating the effect of several MSM doses and exposure for longer time periods are needed. More data from high quality studies will reduce the uncertainty in the hazard characterisation.
- Animal chronic toxicity studies are needed. More data from high quality studies will reduce the uncertainty related to the extrapolation to chronic exposure.
- Data on immunotoxicity and endocrine effects are needed. More data from high quality studies including immunotoxic and endocrine endpoints with equal gender and age distribution in the study population will contribute to reduce the uncertainty related to the hazard characterisation.

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10 Appendix ADME

10.1 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: December 18, 2020

Result: 30

1	((("methylsulfonylmethane" or "67-71-0" or "dimethyl sulfone" or "methyl sulfone" or "dimethylsulfone").mp.) AND (absorption/ or absorption, physicochemical/ or Metabolism/ or Biotransformation/ or (Absorption or distribution or metabol* or elimination or excretion or degradation or biotransformation? or bioconversion? or "biological transformation?" or toxicokinetic? or clearance or detoxification or detoxication or adme).tw,kf)) NOT (comment or editorial or letter).pt.	30

Database: Embase 1974 to date of the search

Date of search: December 18, 2020

Result: 26

1	((((("methylsulfonylmethane" or "dimethyl sulfone or 67-71-0 or methyl sulfone or dimethylsulfone*").mp.) AND (absorption/ or metabolism/ or excretion/ or degradation/ or biotransformation/ or toxicokinetics/ or clearance/ or detoxification/ or metabolite/ or (Absorption or distribution or metabol* or elimination or excretion or degradation or biotransformation? or bioconversion? or "biological transformation?" or toxicokinetic? or clearance or detoxification or detoxication or adme).tw,kw)) NOT (conference abstract* or letter* or editorial*).pt.) AND Elsevier.cr.	26

10.2 Studies excluded after full-text evaluation

Four studies were excluded after full-text evaluation for relevance (Crawford et al., 2019a; Li et al., 2016; Magnuson et al., 2007b; Rasheed et al., 2020). The reason for exclusion was the outcome, as these not were relevant for answering the questions on ADME of MSM in Section 3.

10.3 Description of the included studies.

10.3.1 ADME in animal studies

The pharmacokinetic profile and distribution of radiolabelled MSM was investigated in rats (Magnuson et al., 2007a). Male Sprague-Dawley rats were administered a single oral dose of [^{35}S]-MSM (500 mg/kg), and blood radioactivity levels were determined at different time points for up to 48 hours (0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 12, 24, and 48h). Further, tissue radioactivity levels were determined at 48 and 120h, while urine and fecal radioactivity levels were measured at different time points for up to 120 h following [^{35}S]-MSM administration to rats. Oral [^{35}S]-MSM was rapidly and efficiently absorbed with a mean time to maximum concentration (t_{max}) of 2.1 h, with a mean maximum concentration (C_{max}) of 622 µg equiv/ml, and with a mean calculated area under the curve (AUC) from zero to infinity ($AUC_{(0\text{-inf.})}$) of 15124 h·µg equiv/ml. The half-life of MSM in blood was calculated at 12.2 hours. Total radioactivity appeared to be widely distributed throughout the body, and measurable levels of total ^{35}S were found in all tissues analysed at 48 h post-dose. The observed soft tissue (liver, heart, kidneys, spleen, testes, brain, and eye) distribution of radioactivity indicated a consistent distribution throughout the body with relatively lower concentrations in skin and bone. Approximately 85.8 % of the administrated dose was recovered in the urine after 120 h, whereas only 3% was found in the faeces. No quantifiable levels of radioactivity were found in any tissues after 120 h, indicating complete elimination of [^{35}S]-MSM. These results indicate that MSM is rapidly absorbed, well distributed, and completely excreted.

Otsuki et al. (2002) investigated the distribution of oral MSM using a ^{35}S radioisotope tracer method in rats of different ages fed standardized diets. Male Wistar rats (n = four per group) were fed standardized diets for 2, 43, 83, and 96 days (Groups: G1, G2, G3, and G4, respectively) followed by a daily oral gavage administration of [^{35}S]- dimethylsulfone for 7 days by oral gavage at a dose level of 470 mg/kg/day. Urine and faeces were collected daily for 7 days, and at the end of dosing, tissues were collected for the determination of radioactivity. The total radioactivity yield in urine, faeces, and tissues for the groups G1, G2, G3, and G4 were 100.6, 94.9, 89.4 and 68.5% of the administered ^{35}S, respectively. Levels of radioactivity tended to be highest in blood, spleen, and hair. The majority of the ^{35}S radioactivity was excreted into the urine (~70%) and the faeces (~10%), and the increase in the radioactivity yields of each group levelled off throughout the 7-day administration, indicating that the dimethylsulfone metabolism rate was relatively high. The uptake of radioactivity is observed in the blood, spleen and hair, and over 80% of the administered [^{35}S]-dimethylsulfone is excreted the same day. Meanwhile, the distribution of the ^{35}S concentration in the rat system indicates that the administrated dimethylsulfone might have been metabolized to yield certain sulfur-containing compounds, since not all of the radioactivity was recovered.

Wong et al. (2017) investigated the absorption of MSM by the small intestine and accumulation of the associated sulfur moiety in selected tissues with chronic (8 days)

administration using juvenile male mice. To determine the accumulation of the sulfur moiety of MSM, ^{35}S labelled MSM at a dose of 10 µCi (0.6 µM; 5.5 µg) per day was provided to mice for 2 (n = 4), 5 (n = 5) and 8 days (n = 5) using pureed sweet potato as the vehicle. Blood cell and serum, liver, small intestine and skeletal muscle from both hind limbs were collected at 18–24 h following the last dose. They observed accumulation of ^{35}S activity measured in the homogenates in all of the tissues after two days of dosing. The serum had higher ^{5}S activity than the solid tissues. The amount of ^{35}S activity did not increase in serum or tissue homogenates between days 2 and 8, suggesting an establishment of a possible stable equilibrium between intake and elimination. Further, they calculated the percentage of ^{35}S activity measured in the homogenate that was recovered in the gels, and a 3-fold increase in the percentage of liver homogenate ^{35}S activity recovered in the gel between days 2 and 8 of dosing was observed. The serum proteins had a comparable increase in the percentage of homogenate ^{35}S that was recovered in the gel. The increase in percentage recovery between days 2 and 8 was less for the blood cell fraction and small intestine but both were statistically significant. The different magnitudes of increase for the ^{35}S isolated by gel electrophoresis of the serum and tissue homogenates are of interest but unexplained. The specific proteins that accumulated the ^{35}S were not identified and it was not determined if the ^{35}S was associated with incorporation of labelled methionine or cysteine or was a product of posttranslational sulfation.

Abdul Rasheed et al. (2019) investigated the toxicity and tissue distribution of MSM following oral gavage in broilers. In study 1, 15-day-old male broilers (n = 432) were assigned to 6 treatment groups with 6 replicates of 12 birds per replicate and administered a single oral dose of MSM at 0, 50, 100, 300, 1000, or 2000 mg/kg bw. MSM concentrations were analysed from blood and tissue samples collected over a 48 h. In study 2, 3-day-old chicks (N = 168) were assigned to either control or test group and administered a daily oral gavage of either 0 or 1500 mg/kg bw/day of MSM for 21 days. MSM concentrations were analysed from blood and tissue samples collected at day 7, 14 and 21, respectively.

Toxicity was assessed through changes in hematology and clinical blood chemistry. In study 1, plasma MSM concentrations were below 167 µg/mL at all time-points in birds receiving up to 300 mg/kg bw, and were significantly higher in birds receiving 1000 or 2000 mg/kg bw. Significant increase in lymphocyte and decrease in heterophil counts at 8 h and decrease in hematocrit at 48 h were observed in birds receiving 1000 or 2000 mg/kg bw. Except for liver and brain, no difference in tissue MSM concentrations were observed at 24 h post administration in birds receiving 50, 100, or 300 mg/kg bw doses. however, tissue MSM concentrations were different between the above doses at 48 h. Birds dosed with a single gavage of 2000 mg/kg bw exhibited a trend of increasing plasma MSM level with a peak concentration of 2357 µg/mL at 8 h post administration, and then decreasing to a concentration of 340 µg/mL at 48 h. A similar pattern of plasma MSM distribution was observed in 1000 mg/kg bw dose.

In study 2, they observed that measurable MSM was recovered from plasma and tissue samples after daily dosing for 21 days. Birds receiving a daily dose at 1500 mg/kg bw/day had consistently higher plasma and tissue MSM concentrations compared with control birds.

MSM concentration appeared to be highest at day 21 in all tissues and plasma samples. Furthermore, MSM was detected in plasma and tissues of control groups, but mean values were often not statistically different from zero. Growth performance variables were unaffected by MSM, and birds that were dosed with MSM had decreased liver enzyme concentrations at day 7 and 21 and decreased glucose and phosphorus at day 7.

Oral MSM at either acute (single dose at 1000 to 2000 mg/kg BW) or sub-chronic (1500 mg/kg bw daily for 21 days) concentrations appeared to be absorbed and distributed throughout the body. Detection of MSM in plasma and all tissue types at all time-points suggest that MSM is well absorbed and widely distributed across body tissues.

Hucker et al. (1966) investigated excretion of DMSO and dimethyl-sulphone (MSM) in rats. In the dimethyl-sulphone investigations, rats were intra-peritoneal injected with 21 mg/kg of ^{35}S- dimethyl-sulphone and urinary excretion of ^{35}S- dimethyl-sulphone was measured. Around 64% of the dose was excreted as apparently as unchanged sulphone in the urine within 24 hours. No further details of the study were not given. In the DMSO investigations, a single intraperitoneal administration of 0.5 mg/ml ^{35}S-DMSO to 24 rats (sex, strain not specified) resulted in urinary excretion of 15% of the ^{35}S-DMSO dose as MSM. In this same report, results of human excretion of dimethyl-sulphone following DMSO administration were described. One human subject (sex, age not mentioned) receiving a daily dose of 21g of DMSO. The 24 hours urine analysis showed that MSM represented approximately 3% of the daily dose.

10.3.2 ADME in human studies

Lin et al. (2001) investigated levels of MSM in brains of individuals (both healthy and with memory loss) following daily administration of 1.5 - 6 g MSM for various periods (from 5 weeks to > 2 years). Magnetic resonance spectroscopy was used to detect brain levels of MSM. In this study, four patients (two male, two female) and three controls (two male, one female) participated. MSM was detected in brains of all subjects at concentrations of 0.42–3.40 mmole/kg brain and was equally distributed between grey and white matter. The imaging results revealed an even distribution of MSM throughout the brain, including brainstem, with similar concentrations in grey and white matter. In the patient subjects, intra-cerebral steady state levels of MSM were observed at 1.67 mM for two patients. The lowest MSM level was observed in one patient, who received MSM for only 24 hours prior to magnetic resonance scanning. While the highest MSM levels (3.39 mM) were observed in one patient, who received MSM for over two years and had evidence of a prior stroke.

The time course of accumulation of MSM in the brain was evaluated in one normal subject, receiving the average daily dose of ~ 44 mg/kg. Cerebral MSM concentrations increased rapidly and reached steady state of 2.33 mM, within two weeks. The concentrations of the intrinsic cerebral metabolites (NAA, Cr, Cho, mI, and Glx) remained normal throughout the trial. The remaining normal volunteers demonstrated the characteristic resonance at 3.15 ppm at somewhat lower intensity with cerebral MSM of 0.70 mM. No systematic effects of

MSM on brain metabolites were observed. The results of this study suggest that MSM crosses the blood-brain-barrier.

Cecil et al. (2002) investigated brain distribution of MSM in a five-year-old child. Magnetic resonance imaging and scanning analysis of the child's brain suggested accumulation of MSM in basal ganglia (caudate, putamen, internal capsule) and parietal matter. According to his parent, the child received 1250 mg/day MSM hypoallergenic powder (corresponding to a MSM dose of 62.5 mg/kg bw/day) for one year. The MSM concentration within the basal ganglia was determined to be 0.93 mM and 1.24 mM in the white matter. They did not observe any adverse clinical, structural, or neurochemical effects following MSM use, however, no toxicity assessment was performed.

Rose et al. (2000) quantified MSM levels in a 62-year-old male subject with Alzheimer's disease. The subject received "MSM complex" dietary supplement (1000 mg) in capsule form was taken at an initial dose of 2 capsules per 11 kg of body weight for 7 days corresponding to MSM concentration of 182 mg/kg/day, and followed by 2000 mg/day as a maintenance dosage for 30 days. MSM was detected in the brain, and the measured concentration of MSM was initially 2.36 mM. The washout half-life of dimethyl sulfone in the brain was calculated to be approximately 7.5 days.

Kaiser et al. (2020) investigated the potential of a common dietary supplement, MSM, to act as a chemical shift reference for *in vivo* ^{1}H MR spectroscopy (MRS). Three normal volunteers were healthy males: subject 1 (69 years, 79 kg), subject 2 (50 years, 73 kg), and subject 3 (24 years, 82 kg) and given 1.0 g MSM OPTI MSM tablet from Nature's Way. The consistent concentration of 1.0 g of MSM per tablet was verified by constructing aqueous phantoms that included a concentration standard of sodium acetate. A solution of 2000 mg of MSM dissolved in ~ 50 mL of water (MSM dose of 27.4 mg/kg bw) was given to one subject, and the spectra by ^{1}H-MRS were acquired following MSM consumption and for the next 255 minutes. A retest of the same experiment was performed 45 days later (using a 2000 mg dose of MSM), after sufficient time to ensure complete elimination of MSM from the first test. To assess the washout kinetics of MSM, the subject consumed 2000 mg of MSM six months after the uptake retest. Spectra were recorded as before, over 18 minute periods at 4, 8, 12, 52, 168, and 300 hours after ingestion. The intake/washout experiments in normal brain demonstrate an ability to cross the blood-brain barrier with a signal visible in ~10 minutes after intake and a relatively long-lived, stable signal that persists for at least 4 hours after MSM intake. The half-life time of MSM in the brain was determined to be ~ 72 hours following an acute 2000 mg dose.

Bloomer et al. (2015) investigated the impact of daily ingestion of MSM at 3 g (35.5 mg/kg bw) for a period of four weeks on serum MSM concentrations of healthy men. A total of 20 men were assigned to consume 3 g of MSM daily for a period of four weeks, while control group (N = 20) received an identical-appearing placebo. Blood samples were obtained from all 40 subjects at each of the three time points (baseline, week 2, and week 4) and blood serum was analysed for MSM concentration using Nuclear Magnetic Resonance (NMR) spectroscopy. The MSM levels for placebo group were below the limit of detection. The

baseline serum MSM levels from subjects assigned to the MSM condition were below the limit of quantification for the NMR assay, except one subject with baseline MSM level of 0.028 mM. Serum MSM levels increased across time to a mean (± SD) of 1.68 ± 0.60 mM at week 2 and 1.91± 0.81 mM at week 4. The serum MSM levels at week 2 and week 4 were greater than at baseline, but not different from one another.

Serum MSM concentrations were elevated in all men following ingestion of MSM, in a time-dependent manner. The pattern of MSM level increase varies somewhat from subject to subject.

In a follow up study, Bloomer et al. (2019) evaluated plasma MSM levels in men and women (n= 45) following 16 weeks of oral MSM supplementation at dosages of 1, 2 or 3 grams daily (corresponding to 12.7, 26.4 or 41.8 mg/kg bw/day, respectively). Plasma MSM levels were measured by LC-MS/MS method. Plasma MSM levels of the 26.4 and 41.8 mg/kg bw/day dose groups were significantly higher compared to the 12.7mg/kg bw/day dose group, while the 41.8 mg/kg bw/day dose group were also significantly higher compared to the 26.4 mg/kg bw/day dose group ($p<0.05$). Further, plasma MSM levels at weeks 4, 8, 12, and 16 were higher as compared to baseline ($p<0.05$) but no differences were noted between weeks 4-16 ($p>0.05$). At weeks 4, 8, 12, and 16, plasma MSM levels were higher for the 41.8 mg/kg bw/day dose groups as compared to the 12.7mg/kg bw/day dose group ($p<0.05$). A gender effect was observed ($p=0.01$), with higher overall plasma MSM levels in women (1082 ± 1006 µM) as compared to men (845 ± 805 µM). Both men and women respond to MSM supplementation in a similar manner as related to plasma MSM concentration. A higher dose of supplement results in a greater plasma MSM level. MSM levels reach peak concentration within the initial 8 weeks of supplementation and do not increase further during subsequent weeks of treatment. If higher plasma MSM levels are desired, longer use of lower dosages do not seem to be effective, as bioaccumulation is minimal.

In a pilot study, Kalman and Hewlings (2018) investigated the pharmacokinetic and pharmacodynamics behaviour of MSM along with impacts on sulfate following a single oral dose of 1, 2 or 3 grams of OptiMSM® in healthy male volunteers. Six males aged 42.5 ± 17.5 years and a mean weight of 90.1 ± 18.3 kg, received a dose of 1, 2 or 3 grams of OptiMSM, corresponding to MSM doses of 11.1, 22.2 or 33.3 mg/kg bw, a randomly assigned sequence at three acute test visits spaced seven days apart. Blood serum were collected and analysed at 0, 45, 90, 135 and 240 minutes, and subjects provided a pooled 24-hour post-dose urine collection. Blood serum MSM levels displayed the rise and fall pattern consistent with rapid absorption within an hour, followed by slower elimination from the bloodstream over the course of one or two days. The C_{max} for MSM doses of 1, 2 and 3 g MSM were ~100, 152 202 uM/ml, respectively. While the t_{max} for MSM doses of 1, 2 and 3 g MSM were ~ 68, 90 and 115 minutes, respectively. The four-hour AUC score was 1 g = 346 ± 141 uM/ml*hours, 2 g = 496 ± 170 uM/ml*hours and 3 g = 653 ± 135 uM/ml*hours. The 24-hour urinary excretion was ~116, 164 and 140 mM for doses of 1, 2 and 3 g MSM, respectively. MSM had a dose effect for MSM concentrations, by the C_{max} value and AUC

score but not t_{max}, the 24-hour urinary excretion shows no dose dependence. The half-life time of MSM was roughly estimated to be ½ hour and estimated elimination was around 8 hours. Further, no sulfate pharmacokinetic parameters were dose dependent. MSM absorption appears to be dose-dependent with rapid uptake with less predictable impacts on sulfate metabolism.

11 Appendix Genotixicity

11.1 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: December 18, 2020

Result: 3

1	((("methylsulfonylmethane" or "67-71-0 " or "dimethyl sulfone" or "methyl sulfone" or "dimethylsulfone").mp.) AND (Mutation/ or Mutagens/ or Mutagenesis/ or Mutagenicity Tests/ or DNA damage/ or dna breaks/ or dna breaks, double-stranded/ or dna breaks, single-stranded/ or Comet Assay/ or Chromosome Aberrations/ or Cytogenetics/ or Aneugens/ or Micronucleus Tests/ or Sister Chromatid Exchange/ or DNA Adducts/ or Frameshift Mutation/ or Point Mutation/ or Chromosome Duplication/ or Gene Duplication/ or Chromosome Breakage/ or Aneuploidy/ or Noxae/ or (Mutation? or mutagen* or (gene? adj2 alteration?) or mutator? or Genotoxi* or "Genetic Toxicity Test?" or "Ames test*" or "ames salmonella assay?" or "mouse lymphoma tk assay?" or "mouse lymphoma assay?" or "mouse spot test" or mutamouse or (Muta adj2 Mouse) or "Big Blue" or "LacZ mouse" or "LacI mouse" or "cII gene" or "gpt delta" or (("deoxyribonucleic acid" or DNA) adj (damage* or injur* or lesion? or break* or adduct? or reactivity)) or "strand break*" or "doublestrand break*" or "singlestrand break*" or "comet assay*" or "single cell gel electrophoresis" or "singlecell gel electrophoresis" or SCGE or "alkaline elution" or "unscheduled DNA synthesis" or "unscheduled deoxyribonucleic acid synthesis" or "Rec assay? with Bacillus subtilis" or "SOS test with Escherichia coli" or ((chromosom* or autosom*) adj (aberration? or abnormalit* or anomal* or defect? or error? or duplication? or break* or endoreduplication?)) or cytogen* or clastogen* or aneugen* or "Aneuploidyinducing Agent?" or "Polyploidy Inducing Agent?" or "Polyploidyinducing Agent?" or "micronucleus assay?" or "micronucleus test*" or "MN assay?" or "SOS chromotest*" or "sister chromatid exchange*" or ((Frameshift or "Frame Shift" or "reading frame" or point) adj Mutation?) or "reading frame shift" or ((OutofFrame or "Out of Frame") adj (Mutation? or Insertion? or Deletion?)) or gentox* or "gene duplication?" or "gene doubling?" or Aneuploidy or aneuploid* or (toxic adj (substance? or agent? or chemical? or compound?)) or noxae).tw,kf)) NOT (comment or editorial or letter).pt.	3

Database: Embase 1974 to date of the search

Date of search: December 18, 2020

Result: 3

1	((((("methylsulfonylmethane" or "dimethyl sulfone or 67-71-0 or methyl sulfone or dimethylsulfone*").mp.) AND (gene mutation/ or mutation/ or mutagenic agent/ or mutagenic activity/ or mutagenesis/ or mutagenicity/ or mutagen testing/ or Ames test/ or genotoxicity/ or DNA damage/ or dna strand breakage/ or double stranded dna break/ or single stranded dna break/ or comet assay/ or unscheduled DNA synthesis/ or chromosome aberration/ or cytogenetics/ or clastogen/ or aneugen/ or micronucleus test/ or SOS chromotest/ or sister chromatid exchange/ or DNA adduct/ or Frameshift Mutation/ or point mutation/ or toxic substance/ or aneugen/ or chemical mutagen/ or (Mutation? or mutagen* or (gene? adj2 alteration?) or mutator? or Genotoxi* or "Genetic Toxicity Test?" or "Ames test*" or "ames salmonella assay?" or "mouse lymphoma tk assay?" or "mouse lymphoma assay?" or "mouse spot test" or mutamouse or (Muta adj2 Mouse) or "Big Blue" or "LacZ mouse" or "LacI mouse" or "cII gene" or "gpt delta" or (("deoxyribonucleic acid" or DNA) adj (damage* or injur* or lesion? or break? or adduct? or reactivity)) or "strand break*" or "doublestrand break*" or "singlestrand break*" or "comet assay?" or "single cell gel electrophoresis" or "singlecell gel electrophoresis" or SCGE or "alkaline elution" or "unscheduled DNA synthesis" or "unscheduled deoxyribonucleic acid synthesis" or "Rec assay? with Bacillus subtilis" or "SOS test with Escherichia coli" or ((chromosom* or autosom*) adj (aberration? or abnormalit* or anomal* or defect? or error? or duplication? or break* or endoreduplication?)) or cytogen* or clastogen* or aneugen* or "Aneuploidyinducing Agent?" or "Polyploidy Inducing Agent?" or "Polyploidyinducing Agent?" or "micronucleus assay?" or "micronucleus test*" or "MN assay?" or "SOS chromotest*" or "sister chromatid exchange*" or ((Frameshift or "Frame Shift" or "reading frame" or point) adj Mutation?) or "reading frame shift" or ((OutofFrame or "Out of Frame") adj (Mutation? or Insertion? or Deletion?)) or gentox* or "gene duplication?" or "gene doubling?" or Aneuploidy or aneuploid* or (toxic adj (substance? or agent? or chemical? or compound?)) or noxae).tw,kw)) NOT (conference abstract* or letter* or editorial*).pt.) AND Elsevier.cr.	3

11.2 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
Dell'Edera et al. (2017)	Outcome
Kowalska et al. (2020)	Outcome

11.3 Evaluation of internal validity for the outcome genotoxicity

The seven questions considering aspects relevant for RoB evaluation of human cohort studies in the OHAT tool (OHAT, 2015; OHAT, 2019) were used to evaluate RoB. 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 - -

Kantor et al. (2013), human cohort study.

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Did selection of study participants result in appropriate comparison groups?	There is indirect evidence that subjects (both exposed and non-exposed) were similar (e.g., recruited from the same eligible population, recruited with the same method of ascertainment using the same inclusion and exclusion criteria, and were of similar age and health status), recruited within the same time frame, and had the similar participation/response rates.	+
Confounding bias	2	2. Did the study design or analysis account for important confounding and modifying variables?	There is direct evidence that appropriate adjustments or explicit considerations were made for primary covariates and confounders in the final analyses through the use of statistical models to reduce research-specific bias including standardization, matching, adjustment in multivariate model, stratification, propensity scoring, or other methods that were appropriately justified.	++

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Attrition/exclusion bias	3	Were outcome data complete without attrition or exclusion from analysis?	There is indirect evidence that loss of subjects (i.e., incomplete outcome data) was unacceptably large and not adequately addressed.	-
Detection bias	4	Can we be confident in the exposure characterisation?	There is insufficient information provided about the exposure assessment.	-
	5	Can we be confident in the outcome assessment?	There is insufficient information provided about blinding of outcome assessors.	-
Selective reporting bias	6	Were all measured outcomes reported?	There is direct evidence that all of the studies measured outcomes (primary and secondary) outlined in the methods, have been reported.	++
Other sources of bias	7	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	Statistical methods were appropriate.	+

Lee et al. (2006), animal experimental study.

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	There is no information about randomisation. However, it is anticipated that lack of randomisation would not significantly affect the results.	+
	2	Was allocation to study groups adequately concealed?	No information. However, it is anticipated that this would not affect the results.	+

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Performance bias	3	Were experimental conditions identical across study groups?*	There is direct evidence that same vehicle was used in control and experimental animals and identical non-treatment-related experimental conditions are assumed as the authors did not report differences in housing or husbandry.	+
	4	Were the research personnel blinded to the study group during the study?	No information. However, it is anticipated that this would not affect the results.	+
Attrition/exclusion bias	5	Were outcome data complete without attrition or exclusion from analysis?	Yes	++
Detection bias	6	Can we be confident in the exposure characterisation?*	It is stated that highly purified MSM was used, however the specific purity and origin is not reported.	+
	7	Can we be confident in the outcome assessment?*	There is insufficient information provided about blinding of outcome assessors	-
Selective reporting bias	8	Were all measured outcomes reported?	All outcome data are reported, but not in sufficiently detail.	+
Other sources of bias	9	Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	No information.	-

11.4 Data extraction

Study characteristics	Title	Specialty Supplement Use and Biologic Measures of Oxidative Stress and DNA Damage
	Author(s)	E.D. **Kantor**, C.M. Ulrich, R.W. Owen, P. Schmezer, M.L. Neuhouser, J.W. Lampe, U. Peters, D.D. Shen, T.L. Vaughan, E. White
	Year of publication	**2013**
	Country	USA
	Funding	Grants from the National Cancer Institute and Office of Dietary Supplements, and institutional support from the National Center for Tumor Diseases and German Cancer Research Center.
	Reported conflict of interest	No potential conflicts of interest were disclosed
Methods/ intervention	Study design	Cohort, study participants were drawn from the overall VITAL cohort, a prospective study of 77,719 western Washington residents.
	Blinding	No information
	Randomisation	No information
	Exposure	Supplement use was ascertained by a supplement inventory/interview conducted at the time of home visit. Persons reporting use of a given supplement within the 2 weeks before interview were classified as current users, whereas those reporting no use or last use more than 2 weeks prior were classified as non-users.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	Of the 290 persons contacted, 220 (76%) agreed to participate and completed the study protocol. The sample was stratified to obtain an equal sex distribution, and the final sample included 149 randomly selected VITAL study respondents and 71 oversampled high users of vitamin C (n ¼ 26), vitamin E (n ¼ 23), or calcium (n ¼ 22).
	Doses	Not reported, only given as use or not use.
	Inclusion/exclusion criteria for participants	Persons living outside the Seattle metropolitan area were excluded from the biomarker study, as were persons with Alzheimer's disease, insulin-dependent diabetes, or any conditions preventing the collection of fasting blood.
	Gender	Female=102; male=107 (includes participants using other supplements than MSM).
	Age	50 to 70+ years (includes participants using other supplements than MSM).
	Number of exposed/non-exposed	MSM and DNA damage: MSM use n=9; no MSM use n=110.

	Confounders and other variables as reported	A priori, all regression analyses were adjusted for age, sex, and pack-years smoked. Additional covariates were selected for inclusion by assessing their association with each outcome in this minimally adjusted model. The broad set of potential confounding variables evaluated included various demographic (race/ethnicity, education), lifestyle/anthropometric [body mass index (BMI), physical activity, and alcohol consumption], medical [aspirin use, baby aspirin use, non-aspirin nonsteroidal anti-inflammatory drug (NSAID) use, use of cholesterol-lowering drugs, history of cardiovascular disease, cancer, diabetes, and sunburns], and dietary/supplementary factors (multivitamin use and intake of the following vitamins and minerals from supplements and from diet and supplements combined: b-carotene, vitamin C, a-tocopherol, iron, selenium, and zinc). Additional dietary factors considered include: energy intake, fiber intake, saturated fat intake, and dietary g-tocopherol intake. In evaluating associations involving dietary variables, energy intake was included in the model. Because baseline DNA damage may reflect long-term exposures from an accumulation of insults, we also tested a selected subset of variables representing long-term exposure for this outcome, including BMI at age 45 years, 10-year physical activity, as well as 10-year supplemental intake of b-carotene, vitamin C, and a-tocopherol.
	Health and socioeconomic status of participants	-
Results	Reported outcome (including measures of variance)	Oxidative stress, primary DNA damage, and DNA repair capacity.
	Parameters measured and methods used	Oxidative stress was measured by urinary 8-Isoprostane and PGF2α. Primary DNA damage (DNA strand breaks) and DNA repair capacity were assessed by the Comet assay. DNA strand breaks were expressed as Olive tail moment (Subtracting the head mean from the tail mean, after which this difference was then multiplied by the percentage of DNA in the tail/100). To evaluate DNA repair, DNA damage was induced by a dose rate of 0.54 Gy/min.
	Measurement time points	Samples were taken during interviews. Time point for intake of the supplements is not reported.
	Power analysis	Not reported

Statistical analysis	Statistical test	Linear regression was used to evaluate the associations between specialty supplements and measures of oxidative stress and DNA damage. Analyses were conducted using Stata (version 12).
Comments		It is not reported whether the participants used more than one supplement.

Study characteristics	Title	Evaluation of Genotoxicity on Plant-Derived Dietary Sulfur
	Author(s)	Y.-I. **Lee**, Lee, Y.-S., Park, J.-C., Lee, K.B., You, K.-H.
	Year of publication	**2006**
	Country	Korea
	Funding	Supported by grant from the Basic Research Program of the Korea Science and Engineering Foundation.
	Reported conflict of interest	Not reported
Type of study	Good laboratory practice	Not reported
	Guideline study (yes/no; if yes, specify)	No
	Study design (including number of groups/ number of animals per group)	Mammalian *in vivo* micronucleus test: 6 mice per group, five groups (control, positive control and three treatment groups). Bacterial reverse mutation assay (Ames test): Four bacterial strains included. *In vitro* chromosome aberration assay: Chinese hamster lung cell line.
Animal model	Species/(sub)strain/line	Mice
	Disease models (e.g. allergy)	NR
	Sex and age	NR

Study design and exposure	Feed (name, source)	NR
	Compound purity	Highly purified methylsulfonylmethane.
	Vehicle used	Carboxymethylcellulose
	Dose regimen and frequency	Mammalian *in vivo* micronucleus test: Single doses of MSM: 1250, 2500, and 5000 mg/kg. Positive control group received an i.p. dose of mitomycin C at 4 mg/kg. Bacterial reverse mutation assay (Ames test): 10000, 5000 and 2500 µg/plate. *In vitro* chromosome aberration assay: 5, 2.5 and 1.24 mg/ml.
	Route of administration	Mammalian *in vivo* micronucleus test: Oral gavage
	Period of exposure (e.g. pre-mating, mating, gestation, lactation, adult)	Mammalian *in vivo* micronucleus test: NR
	Exposure duration	Mammalian *in vivo* micronucleus test: All mice were sacrificed 48h after the treatment. Bacterial reverse mutation assay (Ames test): 48 hours. *In vitro* chromosome aberration assay: 24 hours.
Results and statistical analysis	Main outcome(s)	Micronucleus formation.
	Period of outcome assessment (premating, mating, gestation, lactation, adult)	Mammalian *in vivo* micronucleus test: NR

	Parameters measured and methods used	Mammalian *in vivo* micronucleus test: The frequency of micronucleated polychromatic erythrocytes (MNPCE) in the methylsulfonylmethane-treated and vehicle control groups was compared using a mouse bone marrow micronucleus assay. Bacterial reverse mutation assay (Ames test): Number of revertant colonies per plate. *In vitro* chromosome aberration assay: One-hundred metaphase cells were observed, and the chromosomal aberrations recorded were chromatid and chromosome gap, chromatid break, chromatid exchange, chromosome break, chromosome exchange, and fragmentation.
	Statistical test(s)	Chi-square test
Comments		

12 Appendix Adverse effects

12.1 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: December 18, 2020

Result: 93

1	((("methylsulfonylmethane" or "dimethyl sulfone" or "67-71-0" or "methyl sulfone" or "dimethylsulfone").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.	93

Database: Embase 1974 to date of the search

Date of search: December 18, 2020

Result: 89

1	((((("methylsulfonylmethane" or "dimethyl sulfone or 67-71-0 or methyl sulfone or dimethylsulfone*").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 "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.	89

12.2 Studies excluded after full-text evaluation

An overview of the publications considered not to fulfil the eligibility criteria is given in Table 12.2-1.

Table 12.2-1. Publications considered not eligible.

Reference	Reason for exclusion
Abdul Rasheed et al. (2019)	Study design
Brien et al. (2008)	Publication type
Butawan et al. (2017)	Publication type
Crawford et al. (2019a)	Publication type
Cronin and Ballen (1999)	Publication type
Desideri et al. (2020)	Publication type
(Ely and Lockwood, 2002)	Publication type
Karabay et al. (2014)	Study design
Kowalska et al. (2018)	Study design
Liu et al. (2018a)	Publication type
Liu et al. (2018b)	Publication type

Reference	Reason for exclusion
McCabe et al. (1986)	Exposure
Moore and Morton (1985)	Publication type
Ong et al. (2020)	Publication type
Rasheed et al. (2019)	Not available
Stuber et al. (2011)	Publication type
Tant et al. (2005)	Exposure

12.3 Evaluation of internal validity

The detailed RoB evaluations for the eligible human intervention studies, cohort studies, and animal studies are shown in Section 12.3.1, 12.3.2 and 12.3.3, respectively.

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 - -

12.3.1 Human intervention studies

Barrager et al. (2002)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	All participants were allocated to the same study groups. There was no control group.	– –
	2	Was allocation to study groups adequately concealed?	All participants were allocated to the same study groups. There was no control group.	– –
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	All participants were allocated to the same study groups. There was no control group.	– –
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Loss of subjects was adequately addressed and reasons were documented.	+
Detection bias	5	Can we be confident in the exposure characterisation?	NR; there is insufficient information provided about the validity of the exposure assessment method.	–
	6	Can we be confident in the outcome assessment?	NR; there is insufficient information provided about blinding of outcome assessors.	–

Selective reporting bias	7	Were all measured outcomes reported?	NR; there is insufficient information provided about selective outcome reporting.	–
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	No control group was included.	–

Crawford et al. (2019b)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	There is direct evidence that subjects were allocated to any study group including controls using a method with a random component.	++
	2	Was allocation to study groups adequately concealed?	There is direct evidence that at the time of recruitment the research personnel and subjects did not know what study group subjects were allocated to, and it is unlikely that they could have broken the blinding of allocation until after recruitment was complete and irrevocable.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	There is direct evidence that the subjects and research personnel were adequately blinded to study group, and it is unlikely that they could have broken the blinding during the study.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	It is deemed that the proportion lost to follow-up would not appreciably bias results (not exceeding 20% in each group, and reasons described).	+
Detection bias	5	Can we be confident in the exposure characterisation?	There is indirect evidence that the exposure (including purity and stability of the test substance and compliance with the treatment, if applicable) was independently characterised and purity confirmed generally as ≥99%.	+
	6	Can we be confident in the outcome assessment?	There is direct evidence that the outcome was assessed using well-established methods and subjects had been followed for the same length of time in all study groups.	++
Selective reporting bias	7	Were all measured outcomes reported?	There is indirect evidence that all of the study's measured outcomes (primary and secondary) outlined in the protocol, methods, abstract,	+

			and/or introduction (that are relevant for the evaluation) have been reported	
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	The statistics were appropriate.	++

Hewlings et al. 2018

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Indirect evidence that animals were allocated to any study group including controls using a method with a random component.	+
	2	Was allocation to study groups adequately concealed?	Indirect evidence that the research personnel and subjects did not know what study group subjects were allocated to and it is unlikely that they could have broken the blinding of allocation until after recruitment was complete and irrevocable.	+
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	There is direct evidence that the subjects and research personnel were adequately blinded to study group, and it is unlikely that they could have broken the blinding during the study.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	There is direct evidence that there was no loss of subjects during the study and outcome data were complete.	++
Detection bias	5	Can we be confident in the exposure characterisation?	NR; there is insufficient information provided about the validity of the exposure assessment method.	–
	6	Can we be confident in the outcome assessment?	Indirect evidence that the outcome assessment method is an insensitive instrument.	–
Selective reporting bias	7	Were all measured outcomes reported?	It is deemed that the unplanned analyses were appropriate and selective reporting would not appreciably bias results.	+
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	Statistical analysis were not described for adverse outcomes.	–

Kim et al. (2006)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Randomised using computer-generated random numbers	++
	2	Was allocation to study groups adequately concealed?	Research personnel and subjects did not know what study group subjects were allocated to at the time of recruitment, and it is unlikely that they could have broken the blinding of allocation until after recruitment was complete and irrevocable.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Subjects and research personnel were adequately blinded to study group, and it is unlikely that they could have broken the blinding during the study. The placebo consisted of inert ingredients and was indistinguishable in color, size and taste compared to the MSM.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Loss of subjects was unacceptably large (greater than 20%).	–
Detection bias	5	Can we be confident in the exposure characterisation?	Purity was independently confirmed as ≥98% and exposure was consistently administered across treatment groups. Stability was not reported.	+
	6	Can we be confident in the outcome assessment?	It is deemed that the outcome assessment methods used would not appreciably bias results, and that lack of adequate blinding of outcome assessors would not appreciably bias results.	+
Selective reporting bias	7	Were all measured outcomes reported?	All of the study's measured outcomes outlined in the methods is reported.	+
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	The intent-to-treat analysis was performed using SPSS (version 11.0) software. Appropriate statistics	++

Tennent et al. (2017)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Randomization was conducted using an unbound random number generator that was maintained by the dispensing pharmacy and blinded from all study investigators until completion of the study.	++
	2	Was allocation to study groups adequately concealed?	At the time of recruitment the research personnel and subjects did not know what study group subjects were allocated to, and it is unlikely that they could have broken the blinding of allocation until after recruitment was complete and irrevocable.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Personnel and participants were blinded, and the MSM and placebo were of identical appearance.	++
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	Loss of subjects was unacceptably large.	–
Detection bias	5	Can we be confident in the exposure characterisation?	NR; there is insufficient information provided about the validity of the exposure assessment method	–
	6	Can we be confident in the outcome assessment?	The outcomes were self-reported. It is deemed that the outcome assessment method used would not appreciably bias results, there is direct evidence that the participants were blinded, and it is unlikely that they could have broken the blinding prior to reporting outcomes	+
Selective reporting bias	7	Were all measured outcomes reported?	All of the study's measured outcomes outlined in the methods were reported.	++
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	The statistics were not appropriate.	– –

Usha and Naidu (2004)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Randomization was conducted using a block design method.	++
	2	Was allocation to study groups adequately concealed?	The allocation to study groups was adequately concealed.	++
Performance bias	3	Were the research personnel and human subjects blinded to the study group during the study?	Research personnel and participants were blinded to the study groups during the study.	+ +
Attrition/exclusion bias	4	Were outcome data complete without attrition or exclusion from analysis?	It is deemed that the proportion lost to follow-up would not appreciably bias results (less than 20% in each group).	+
Detection bias	5	Can we be confident in the exposure characterisation?	NR; there is insufficient information provided about the validity of the exposure assessment method.	–
	6	Can we be confident in the outcome assessment?	The outcome was assessed using acceptable methods, and subjects were followed for the same length of time in all study groups.	+
Selective reporting bias	7	Were all measured outcomes reported?	All of the study's outcomes outlined in the method section were not reported.	– –
Other sources of bias	8	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	The statistics were not appropriate.	– –

12.3.2 Cohort studies

Lin et al. (2001)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Did selection of study participants result in appropriate comparison groups?	There was only one group, no comparison group.	– –
Confounding bias	2	Did the study design or analysis account for important confounding and modifying variables?	NR; there is insufficient information provided about co-exposures in occupational studies or studies of contaminated sites where high exposures to other chemical exposures would have been reasonably anticipated.	–

Attrition/exclusion bias	3	Were outcome data complete without attrition or exclusion from analysis?	Loss of subjects was adequately addressed.	+
Detection bias	4	Can we be confident in the exposure characterisation?	NR; there is insufficient information provided about the exposure assessment, including validity and reliability.	−
	5	Can we be confident in the outcome assessment?	The outcome was assessed using well-established methods.	++
Selective reporting bias	6	Were all measured outcomes reported?	There is indirect evidence that all of the study's measured outcomes outlined in the methods have been reported.	+
Other sources of bias	7	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	The number of participants was low.	+

Satia et al. (2009)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Did selection of study participants result in appropriate comparison groups?	There is indirect evidence that subjects (both exposed and non-exposed) were similar (e.g., recruited from the same eligible population, recruited with the same method of ascertainment using the same inclusion and exclusion criteria, and were of similar age and health status), recruited within the same time frame, and had the similar participation/response rates.	+
Confounding bias	2	Did the study design or analysis account for important confounding and modifying variables?	Numerous covariates were captured, appropriate adjustments were made.	++
Attrition/exclusion bias	3	Were outcome data complete without attrition or exclusion from analysis?	Loss of subjects was adequately addressed and reasons were documented.	++
Detection bias	4	Can we be confident in the exposure characterisation?	Exposure was assessed using a validated questionnaire.	+
	5	Can we be confident in the outcome assessment?	Participants were followed for lung and colorectal cancers occurring from baseline through December 31, 2006, by linking the cohort to the Seattle-Puget Sound SEER registry. Cases were captured through all hospitals in the area, offices of	++

			pathologists, oncologists, and radiotherapists, and from State death certificates. Cancer cases were identified in the cohort using matching algorithms on personal identifiers and human review.	
Selective reporting bias	6	Were all measured outcomes reported?	All outcomes outlined in the methods were reported.	++
Other sources of bias	7	Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol)?	The statistics were appropriate.	++

12.3.3 Animal studies

Ezaki et al. (2013) (rat study)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	The authors state that the animals were randomly assigned to the diet groups. The method for randomisation was not described.	+
	2	Was allocation to study groups adequately concealed?	It is deemed that lack of adequate allocation concealment would not appreciably bias results.	+
Performance bias	3	Were experimental conditions identical across study groups?	We assume that same vehicle was used in control and experimental animals. This was, however, not described by the authors.	+
	4	Were the research personnel blinded to the study group during the study?	There is direct evidence that the research personnel were adequately blinded to study group for the histological analyses. For the other results, it is deemed that lack of adequate blinding during the study would not appreciably bias results.	+
Attrition/exclusion bias	5	Were outcome data complete without attrition or exclusion from analysis?	Outcome data were complete.	++
Detection bias	6	Can we be confident in the exposure characterisation?	There was no information on the stability of MSM in animal feed.	–
	7	Can we be confident in the outcome assessment?	It is deemed that lack of adequate blinding of outcome assessors for some of the experiments would not appreciably bias results.	+

Selective reporting bias	8	Were all measured outcomes reported?	There is direct evidence that all of the study's measured outcomes outlined in the methods have been reported.	++
Other sources of bias	9	Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	There were no other potential threats to internal validity.	++

Ezaki et al. (2013) (mice study)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	The authors state that the animals were randomly assigned to the diet groups. The method for randomisation was not described.	+
	2	Was allocation to study groups adequately concealed?	It is deemed that lack of adequate allocation concealment would not appreciably bias results.	+
Performance bias	3	Were experimental conditions identical across study groups?	We assume that same vehicle was used in control and experimental animals. This was, however, not described by the authors.	+
	4	Were the research personnel blinded to the study group during the study?	There is direct evidence that the research personnel were adequately blinded to study group for the histological analyses. For the other results, it is deemed that lack of adequate blinding during the study would not appreciably bias results.	+
Attrition/exclusion bias	5	Were outcome data complete without attrition or exclusion from analysis?	Outcome data were complete.	++
Detection bias	6	Can we be confident in the exposure characterisation?	There was no information on the stability of MSM in animal feed.	_
	7	Can we be confident in the outcome assessment?	It is deemed that lack of adequate blinding of outcome assessors for some of the experiments would not appreciably bias results.	+
Selective reporting bias	8	Were all measured outcomes reported?	There is direct evidence that all of the study's measured outcomes outlined in the methods have been reported.	++
Other sources of bias	9	Were there no other potential threats to internal validity (e.g., statistical methods	There were no other potential threats to internal validity.	++

	were appropriate and researchers adhered to the study protocol)?		

Horvath et al. (2002) – acute toxicity study

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Indirect evidence that animals were allocated to any study group including controls using a method with a random component	+
	2	Was allocation to study groups adequately concealed?	It is deemed that lack of adequate allocation concealment would not appreciably bias results.	+
Performance bias	3	Were experimental conditions identical across study groups?	There is direct evidence that same vehicle was used in control and experimental animals.	++
	4	Were the research personnel blinded to the study group during the study?	It is deemed that lack of adequate blinding during the study would not appreciably bias results.	+
Attrition/exclusion bias	5	Were outcome data complete without attrition or exclusion from analysis?	Indirect evidence that loss of animals was adequately addressed and reasons were documented when animals were removed from a study.	+
Detection bias	6	Can we be confident in the exposure characterisation?	Indirect evidence that the exposure was independently characterised and purity confirmed generally as ≥99%3.	+
	7	Can we be confident in the outcome assessment?	Indirect evidence that it was possible for outcome assessors to infer the study group prior to reporting outcomes without sufficient quality control measures,	–
Selective reporting bias	8	Were all measured outcomes reported?	NR; insufficient information about outcome reporting.	–
Other sources of bias	9	Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	Indirect evidence that statistical comparisons were appropriate.	+

Horvath et al. (2002) – subchronic toxicity study

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	Indirect evidence that animals were allocated to any study group including controls using a method with a random component	+
	2	Was allocation to study groups adequately concealed?	It is deemed that lack of adequate allocation concealment would not appreciably bias results.	+
Performance bias	3	Were experimental conditions identical across study groups?	There is direct evidence that same vehicle was used in control and experimental animals.	++
	4	Were the research personnel blinded to the study group during the study?	It is deemed that lack of adequate blinding during the study would not appreciably bias results.	+
Attrition/exclusion bias	5	Were outcome data complete without attrition or exclusion from analysis?	Indirect evidence that loss of animals was adequately addressed and reasons were documented when animals were removed from a study.	+
Detection bias	6	Can we be confident in the exposure characterisation?	Indirect evidence that the exposure was independently characterised and purity confirmed generally as ≥99%3.	+
	7	Can we be confident in the outcome assessment?	Indirect evidence that it was possible for outcome assessors to infer the study group prior to reporting outcomes without sufficient quality control measures,	_
Selective reporting bias	8	Were all measured outcomes reported?	NR; insufficient information about outcome reporting.	_
Other sources of bias	9	Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	Indirect evidence that statistical comparisons were appropriate.	+

Kamel and El Morsy (2013)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	It is deemed that allocation without a clearly random component during the study would not appreciably bias results	+
	2	Was allocation to study groups adequately concealed?	It is deemed that lack of adequate allocation concealment would not appreciably bias results	+
Performance bias	3	Were experimental conditions identical across study groups?	The same vehicle was used in control and experimental animals, and non-treatment-related experimental conditions were identical across study groups	++
	4	Were the research personnel blinded to the study group during the study?	It is deemed that lack of adequate blinding during the study would not appreciably bias results	+
Attrition/exclusion bias	5	Were outcome data complete without attrition or exclusion from analysis?	The results reported were complete.	++
Detection bias	6	Can we be confident in the exposure characterisation?	MSM was analytical grade. No information on stability. Only 5 days experiment	+
	7	Can we be confident in the outcome assessment?	The outcome was assessed using well-established methods, assessed at the same length of time after initial exposure in all study groups. It is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results	+
Selective reporting bias	8	Were all measured outcomes reported?	All of the study's measured outcomes outlined in the methods is reported.	++
Other sources of bias	9	Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	Statistics were appropriate.	++

Magnuson et al. (2007b)

Type of bias	No	Question	Risk of bias evaluation	Risk of bias rating
Selection bias	1	Was administered dose or exposure level adequately randomized?	It is deemed that allocation without a clearly random component during the study would not appreciably bias results	+
	2	Was allocation to study groups adequately concealed?	It is deemed that lack of adequate allocation concealment would not appreciably bias results	+
Performance bias	3	Were experimental conditions identical across study groups?	The same vehicle was used in control and experimental animals, and non-treatment-related experimental conditions were identical across study groups	++
	4	Were the research personnel blinded to the study group during the study?	It is deemed that lack of adequate blinding during the study would not appreciably bias results	+
Attrition/exclusion bias	5	Were outcome data complete without attrition or exclusion from analysis?	Indirect evidence that loss of animals was adequately addressed and reasons were documented when animals were removed from a study.	+
Detection bias	6	Can we be confident in the exposure characterisation?	There is direct evidence that the exposure (including purity and stability of the test substance) was independently characterised and purity confirmed generally as ≥99%, and that exposure was consistently administered (i.e., with the same method and time-frame) across treatment groups.	++
	7	Can we be confident in the outcome assessment?	The outcome was assessed using well-established methods, assessed at the same length of time after initial exposure in all study groups. It is deemed that lack of adequate blinding of outcome assessors would not appreciably bias results	+
Selective reporting bias	8	Were all measured outcomes reported?	There is direct evidence that all of the study's measured outcomes outlined in the methods is reported.	++
Other sources of bias	9	Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	Statistics were appropriate. The study followed good laboratory practice.	++

12.4 Study characteristics/data charting

12.4.1 Human studies

Crawford et al. (2019) evaluated the safety of MSM in patients with musculoskeletal disorders of osteoarthritis and back pain. The study was part of a trial approved by Wilford Hall Ambulatory Surgical Center Institutional Review Board. Subjects were a combination of active duty military members and their families and retired military members and their families on one United States Air Force Base installation. All were between the ages of 18 and 65 with symptoms of low back pain lasting greater than 12 weeks. The study was designed as a randomized, double-blind, placebo-controlled trial to determine whether 6 g daily of MSM plus standard of care naproxen improved symptoms of lower back pain versus standard care naproxen plus placebo. The study lasted for 16 weeks. One hundred patients were enrolled in this study; 46 in the MSM + naproxen group and 40 in placebo + naproxen group completed the study. Physiological tests included body weight, systolic blood pressure, and diastolic blood pressure (mmHg) at screening visit time zero, 4 weeks, 8 weeks, 12 weeks, and 16 weeks. Laboratory tests included blood tests analysed for complete blood count, white blood cell count, hemoglobin A1C, platelets, glucose, creatinine, total bilirubin, alanine aminotransferase, and aspartate aminotransferase at the screening visit/time zero, 4 weeks, 8 weeks, 12 weeks, and 16 weeks. Daily consumption of 6 g MSM/day for 16 weeks did not have a significant effect on any of the studied outcomes. The authors specified the following study limitations: people with complex medical conditions were not included; biomarkers were only measured for 16 weeks while patients may take MSM for years.

Kim et al. (2006) performed a pilot randomised double-blind, placebo-controlled clinical trial to evaluate the efficacy of MSM in osteoarthritis pain of the knee. Study inclusion criteria included men and women >40 years diagnosed with knee osteoarthritis, and the participants were assigned to receive MSM (n=25) or placebo (n=25) for 12-weeks. A dosage of 6 g/day was selected based on common clinical and over-the-counter uses of MSM. A stepwise approach to the full dose was undertaken. The first week, 2 g/day was given in two divided doses for 3 days and then increased to 4 g/day for 4 days. Week 2, the dose was increased to 6 g/day. To evaluate potential adverse effects, laboratory tests, questionnaires, blood pressure, weight, body mass index, and other vitals were collected at baseline and 12 weeks. The laboratory tests included hematology (complete blood counts and differential white blood cells), clinical chemistry (renal and hepatic functions), fasting lipid profile, urinalysis, and stool occult blood test. The questionnaires included the standard gastro-intestinal symptoms and modified neurotoxic symptoms. Questions related to changes in blood clotting, cognitive function (fatigue, concentration, slowing, memory, motor coordination and language), peripheral neurological symptoms (sensory disturbance and muscle weakness), and other symptoms (insomnia, headache and

blurred vision) were also included. Side effects reported included bloating, constipation, indigestion, fatigue, concentration issues, insomnia, and headache, and participants in the MSM and placebo groups reported symptoms in comparable frequency. No major adverse events reported, according to the authors.

Associations of supplements, including MSM, with lung and colorectal cancer risk was evaluated by Satia et el. (2009) for participants in the VITAL (vitamins and lifestyle) study. Intake of supplements, duration and frequency, was reported by the participants. Due to the lack of accurate information, supplement exposure was categorized as "no use" or "any use" over the previous 10 years. Recruitment was conducted from October 2000 to December 2002, and 77,125 participants aged 50 to 76 years were included. In the study period from baseline through December 31, 2006, 665 participants were diagnosed with lung cancer and 428 was diagnosed with colorectal cancer. MSM use was not associated with increased cancer risk.

Study characteristics	Title	Methylsulfonylmethane for treatment of low back pain: A safety analysis of a randomized, controlled trial
	Author(s)	P. Crawford, A. Crawford, F. Nielson, R. Lystrup
	Year of publication	2019
	Country	USA
	Funding	AFMSA/SG5 provided funding for research coordinators, and Bergstrom Nutrition provided MSM and placebo through a Cooperative Research and Development Agreement but had no involvement in design of the trial or review of results.
	Reported conflict of interest	Not reported
Methods/ intervention	Study design	Randomised, double-blind, placebo-controlled trial.
	Blinding	Double-blind
	Randomisation	At the beginning of the study, subjects were randomly assigned to two groups. Randomisation was balanced using stratified random sampling with proportionate allocation to ensure that all aspects of the population were represented in the sample. After enrollment, subjects were randomised into 16 weeks of therapy with either six grams of MSM plus standard of care naproxen or placebo plus standard of care naproxen.
	Exposure (including duration of the study	6 g MSM/day (n=46) or placebo (n=40). All participants used naproxen. Study duration: 16 weeks.

Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	One hundred patients were enrolled; 46 in MSM + naproxen group and 40 in the placebo + naproxen group completed the study.
	Inclusion/exclusion criteria for participants	Inclusion criteria: - Between the ages of 18 and 65. - Symptoms of low back pain lasting greater than 12 weeks. Exclusion criteria: - Lower back pain caused by infection, tumor, osteoporosis, ankylosing spondylitis, fracture, deformity, known autoimmune process, or cauda equina syndrome. - Patients who met the criteria for surgery as indicated by progressive motor deficit, sphincter impairment from neurological cause, or who had disabling sciatic pain in the absence of backache lasting 6 weeks or more attributed to a compromised nerve root and demonstrated by magnetic imaging or computer tomography. - Patients with treated or untreated central nervous system impairment; oncologic disease during the previous 5 years, unexplained weight loss, fever, or chills; diagnosed upper urinary tract infection within the last 28 days; history of intravenous drug use; immunocompromised host; sciatica; history of bleeding disorders; history of high blood pressure; history of heart, kidney, liver, or ulcer disease; allergic to analgesics or NSAIDs; pregnant or breastfeeding; initial pain greater than 8/10 on initial intake evaluation; comprehensive metabolic panel with values outside safe range. - Patients with a severe comorbidity such as a detriment to the patient's wellbeing, cirrhosis, or ongoing dialysis.
	Gender	Female: 27; Male: 59.
	Age	18-65 years.

	Confounders and other variables as reported	Ethnicity
	Health and socioeconomic status of participants	Participants were active duty military members and retired military members and their families.
Results	Parameters measured, methods used, and measurement time points	- Body weight and blood pressure at time zero, 4 weeks, 8 weeks, 12 weeks, and 16 weeks. - Blood tests at the screening visit/time zero, 4 weeks, 8 weeks, 12 weeks, and 16 weeks. - No description of methods used.
	Reported outcome (including measures of variance)	No significant effects on systolic or diastolic blood pressure, body weight, white blood cell count, hemoglobin, platelets, glucose, creatinine, total bilirubin, alanine aminotransferase, or aspartate aminotransferase.
Statistical analysis	Power analysis	Power analysis was performed.
	Statistical test	rANOVA
Comments		

Study characteristics	Title	Efficacy of methylsulfonylmethane (MSM) in osteoarthritis pain of the knee: a pilot clinical trial
	Author(s)	L. S. Kim, L.J. Axelrod, P. Howard, N. Buratovich
	Year of publication	2006
	Country	USA
	Funding	Financial support from Southwest College of Naturopathic Medicine & Health Sciences and grant sponsorship and products provided by Cardinal Nutrition.
	Reported conflict of interest	The authors stated that there was no conflict of interests in the preparation of the manuscript.
Methods/ intervention	Study design	Randomised, double-blind, placebo-controlled trial.
	Blinding	Double-blind
	Randomisation	Computer-generated random numbers.

	Exposure (including duration of the study)	A stepwise approach to the full dose was applied: - 2 g/day in two divided doses for 3 days. - 4 g/day in two divided doses for 4 days. - 6 g/day in two divided doses for 11 weeks. Total study duration was 12 weeks.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	50 patients enrolled, 40 completed the study: 21 (84%) in the MSM group and 19 (76%) in the placebo group.
	Inclusion/exclusion criteria for participants	Inclusion criteria: - Men and women>40 years diagnosed with knee OA according to modified criteria of the American College of Rheumatology (ACR). - ACR functional class I, II or III. - Radiographic confirmed Kellgrene-Lawrence grades 2-3 (mild to moderate osteophytes and joint space narrowing, previous 3 years). - Regular arthritis pain (arthritis pain in most days) for 3 months or more - >40 mm arthritis pain rating of target knee (100 mm visual analogue scale (VAS)) - >2 rating on patient global assessment (GA) of overall arthritis disease status (five-point Likert scale) Exclusion criteria: - Any other type of arthritis. - Rheumatoid or inflammatory arthritis. - Fibromyalgia or other chronic pain syndrome. - Arthroscopy or intra-articular corticosteroids/hyaluronic acid injections in the previous 3 months.

		- Concurrent anti-coagulant/anti-platelet drugs, corticosteroids or narcotic pain killers use. - History of epilepsy or bleeding disorders. - Gastric ulcers. - Renal or hepatic disease. - Uncontrolled hypertension. - Body mass index (BMI)>45 kg/m^2.
	Gender	Male and female; numbers per sex not reported.
	Age	40-76 years.
	Confounders and other variables as reported	Not reported
	Health and socioeconomic status of participants	Not reported
Results	Parameters measured, methods used, and measurement time points	Laboratory tests including hematology (complete blood counts and differential white blood cells), clinical chemistry (renal and hepatic functions), fasting lipid profile, urinalysis, and stool occult blood test, questionnaires, blood pressure, weight, body mass index, and other vitals were collected at baseline and 12 weeks. In addition, weekly and biweekly phone calls to patients were made.
	Reported outcome (including measures of variance)	No major adverse events reported. Hematology, clinical chemistry and urinalysis did not have any abnormal changes from baseline to 12 weeks. There were no major changes in the complete blood counts, differential white blood cell counts, hepatic and renal functions, lipid profiles, body mass index, vitals, stool occult test, swelling or tenderness of the target knee joints. Three patients did have positive hemoccult tests at 12 weeks, two in the placebo group and one in the MSM group. The hemoccult was repeated 2 weeks later; the results were negative. Changes in homocysteine (P = 0.004) and urine MDA (P =0.010) were significantly different at 12 weeks between the MSM and placebo groups.

		Side effects reported included bloating, constipation, indigestion, fatigue, concentration issues, insomnia, and headache. Patients in the MSM and placebo groups reported the symptoms in comparable frequency.
Statistical analysis	Power analysis	An intent-to-treat analysis was performed.
	Statistical test	SPSS software
Comments		

Study characteristics	Title	Associations of Herbal and Specialty Supplements with Lung and Colorectal Cancer Risk in the VITamins And Lifestyle Study
	Author(s)	J.A. **Satia**, A. Littman, C.G. Slatore, J.A. Galanko, E. White
	Year of publication	2009
	Country	USA
	Funding	National Cancer Institute grants.
	Reported conflict of interest	The authors declared no potential conflicts of interest.
Methods/ intervention	Study design	Cohort study (the VITamins And Lifestyle cohort).
	Blinding	Not reported
	Randomisation	No randomization.
	Exposure (including duration of the study)	A closed-ended format was used to inquire about current versus past use of supplements, duration of use (1-2, 3-5, 6+ years), and frequency (1-2, 3-5, 6+ days per week) over the previous 10 years. Questions on dose were not included because of lack of accurate information on potency.
Participants	Number of participants and completion rate (invited, accepted, drop out, included in follow-up if applicable)	Invited: 364,418 questionnaires were mailed; 79,300 accepted (returned questionnaire); 77,719 eligible at baseline. Participants that met inclusion criteria and were followed: n = 77,125 for lung cancer and n = 77,512 for colorectal cancer.

	Inclusion/exclusion criteria for participants	Participants with self-reported history of lung cancer and colorectal cancer at baseline (or who did not complete the baseline medical history section) were excluded.
	Gender	Female: 40,073; Male: 37,052.
	Age	50 to 76 years.
	Confounders and other variables as reported	• Age, gender, and smoking. • Possible confounders included in the lung cancer analyses: education, physical activity, body mass index, fruit and vegetable consumption, previous history of cancer, COPD/emphysema/asthma, and first-degree family history of lung cancer. • Possible confounders included in the colorectal cancer analyses: education, physical activity, smoking status, body mass index, fruit and vegetable consumption, use of non fiber laxatives, NSAID use, sigmoidoscopy use in the past 10 years, current multivitamin use, previous history of cancer, and first-degree family history of colorectal cancer.
	Health and socioeconomic status of participants	% with high education (college/advanced degree): - Among lung cancer cases: 23%. - Among controls: 42%. - Among colorectal cancer cases: 31%, controls: 42%.
Results	Parameters measured, methods used, and measurement time points	Participants were followed for lung and colorectal cancers occurring from baseline through December 31, 2006, by linking the cohort to the Seattle-Puget Sound SEER registry.
	Reported outcome (including measures of variance)	No increase in cancer risk were reported. Adjusted hazard ratios (HR) were for lung cancer 1.00 (95% CI 0.68-1.47) and for colorectal cancer 0.46 (95% CI 0.23-0.96) for any MSM use during the previous 10 years (vs. no use).
Statistical analysis	Power analysis	-
	Statistical test	Data analyses were done using SAS (version 9.1)
Comments		

12.4.2 Animal studies

Ezaki et al. (2013) assessed the safety and efficacy of MSM on bone and knee joints in rats and mice at doses ranging from similar to recommended doses in humans up to doses 100 times recommended doses in humans (0.06, 0.6 and 6 g/kg bw/day). They reported that MSM induced adverse effects such as reduction of body and tissue weight in rats at the doses 0.6 and 6 g/kg bw/day and in mice at the dose 0.6 g/kg bw/day. Six-week-old growing male Wistar rats were used to examine the effects of MSM on bone. Rats were randomly assigned to four diet groups, of seven animals each, to receive a control diet or MSM-containing diets (0.06, 0.6, and 6 g/kg bw/day). After four weeks, all rats were killed by collecting whole blood under anesthetization. Body and tissue weights were measured, and the right femur and tibia were removed for measurement of bone mineral density. The left legs were removed for measurement of cartilage thickness. The kidney and liver were subjected to histomorphological analysis. The initial body weight of the rats did not differ among the four groups, and the food intake was not significantly different among the groups throughout the experiment. The body weight of the 6 g/kg bw/day group was significantly lower than the control group four weeks after the experiment. Liver weight in the 0.6 and 6 g/kg bw/day groups was significantly lower than that in the control group, and the spleen and kidney weights in the 6 g/kg bw/day group were significantly lower and higher compared to the control group, respectively. Serum calcium and bone mineral density of the whole body in the 6 g/kg bw/day group was significantly lower than that in the control group. Ten-week-old male mice were used to examine the efficacy of MSM on bone and cartilage of in an osteoarthritis mouse model (STR/OrtCrlj). Mice were randomly assigned to three diet groups, of six animals each, to receive a control diet or MSM-containing diets (0.06 and 0.6 mg/kg bw/day). Body weight and total food intake did not differ among the three groups. The spleen weight in the 0.6 group was significantly lower than the control group. MSM intake decreased total liver score including fat vacuole score, glycogen area, and focal necrosis score in a dose-dependent manner.

The acute and subchronic toxicity of MSM in rats at a dose five to seven times the maximum recommended dose in humans was evaluated by Horvath et al. (2002). For both studies, animals were randomly assigned to treatment groups, and equal numbers of males and females were assigned to each treatment group. Twenty Sprague–Dawley rats (10 males and 10 females, 6 weeks old) were used in the acute toxicity study. Rats in the MSM group received a single oral dose by gavage of 2 g/kg, and rats in the control group received a single oral dose of the vehicle by gavage. All treatments were administered at a volume of 10 ml/kg based on the individual animal body weights obtained on the day of dosing. Animals were checked for clinical signs and mortality twice a day. Each rat was weighed on days 1, 5, 8, 12 and 15. The organs and tissues examined were adrenals, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes (mesenteric, mandibular), mammary glands, ovaries, pancreas, parathyroid, prostate, rectum, salivary glands, skeletal

muscle, skin, spleen, stomach (fundic area), testes, thyroid, tongue, trachea, urinary bladder, uterus and vagina. No mortality was observed, no adverse effects or clinical signs of toxicity were observed, no discernible differences in weight gain were noted between treatment groups, and no gross lesions were noted on necropsy.

To examine subchronic toxicity of MSM, eighty Wistar rats, 40 males and 40 females, 5 weeks old, were used (Horvath et al., 2002). Rats in the MSM treatment group received a daily dose of 1.5 g/kg of MSM by gavage in a volume of 10 ml/kg distilled water for 90 days. Rats in the control group received 10 ml/kg distilled water for 90 days. Animals were checked twice daily for mortality and clinical signs of toxicity. Oral administration of MSM in a dose of 1.5 g/kg bw/day for 90 days did not cause any mortality. No adverse effects were observed for any animals. Body weight and estimated food consumption were not affected. All hematological data, erythrocyte, leukocyte and platelet counts, hematocrit and mean corpuscular hemoglobin, were within normal limits. All blood chemistry values were within normal physiologic ranges. Urinanalysis was normal without glucosuria, proteinuria or hematuria. No gross pathological lesions were noted and there was no difference in organ weights in comparison to the control group. The autopsy performed on day 91 after initiation of treatment did not reveal any gross pathological changes or any differences in organ weights, with the exception of the kidneys in the males, which weighed slightly greater than those of controls. The authors concluded that the slight increase in the kidney weight related to the body weight in the males was not supported by histopathological findings, and suggested that the slight statistical weight difference was probably due to low within-group deviations rather than any significant toxicological effect or event. VKM noted several deviations from the OECD guideline for repeated dose 90-day oral toxicity study in rodents (408) (REF). The most severe being lack of histopathology except for kidneys, and aggregated or individual data is not reported. In addition, serum total T4, T3 and TSH, was not measured. Due to lack of available data, a NOAEL could not be established.

The hepatoprotective effect of MSM against carbon tetrachloride-induced acute liver injury in rats was evaluated by Kamel and El Morsy (2013). Fifty female Sprague–Dawley rats were divided into 5 groups, 10 rats per group. The control group received 10 % tween 80 solution orally by gavage for 5 days, and one hour after the last dose they received an intraperitoneal injection of corn oil (0.1 ml/100 g bw) on the 5th day. The MSM group received 400 mg MSM/kg bw dissolved in 10 % tween 80 solution orally by gavage for 5 days, and one hour after the last dose they received an intraperitoneal injection of corn oil (0.1 ml/100 g bw) on the 5th day. The other groups received carbon tetrachloride, MSM and carbon tetrachloride, and silymarin and carbon tetrachloride, respectively. MSM treatment increased SOD and CAT activities (antioxidant defences) and CYP2E1 (involved in toxic substrate metabolism) level significantly compared to control. No histopathological changes were recorded in liver sections of rats treated with MSM. (Increased CYP2E1 may indicate metabolic activation)

Developmental toxicity of MSM was studied by Magnuson et al. (2007b). MSM was administered orally to pregnant Sprague–Dawley rats during the period of major organogenesis and histogenesis. A preliminary teratogenicity study was performed to determine the dose for a definitive teratogenicity study. Five groups with 8–9 timed bred primiparous dams/group were administered vehicle only, 50, 250, 500, or 1000 mg MSM/kg via oral gavage. Dosing occurred daily on gestation days 6–20. Maternal body weight, body weight gain and feed consumption were measured throughout gestation. Dams were euthanized on their respective 21st day of gestation. The uteri were weighed, opened and inspected for implantation sites; fetuses were harvested, weighed and given a gross external examination. No evidence of maternal or foetal toxicity was observed. Maternal body weight, uterus weight, body weight gain and feed consumption were not adversely affected by treatment.

The definitive teratogenicity study was conducted according to OECD guidelines for developmental toxicity (No 414). The study consisted of four groups of 24–25 timed bred primiparous dams/group. Rats were administered vehicle only, 50, 500, or 1000 mg MSM/kg via oral intubation at a constant volume of 5 ml/kg. Dosing occurred daily on gestation days 6–20. Uterine weights and body weights were similar across all groups. Dams appeared normal throughout the study and no clinical signs of toxicity were observed. No evidence of maternal toxicity by MSM was observed. No statistically significant differences were detected in the number of live or total implants, resorptions, corpora lutea, or percent pre- or post- implantation loss. One dead fetus was observed in the low dose group, which was not considered an adverse or treatment related effect. No significant differences in male, female or combined fetal weights were detected. Male-to-female fetal ratios were similar across groups. No gross external anomalies were observed; all fetuses appeared normal and none were malformed. One fetus in the low dose group had a red mark on its neck; this was related to removal of the fetus from the uterus and was not considered an adverse or treatment-related finding. No visceral malformations were observed in any of the fetuses examined. No abnormalities were seen in the control or high dose fetuses. No treatment-related skeletal anomalies were seen in the low, mid or high dose groups. No evidence of embryo or fetal toxicity, or treatment related alterations in fetal body weights or fetal examinations (gross external, visceral, cephalic, or skeletal) was observed in this study at doses up to 1000 mg/kg MSM. VKM noted that TSH, T3 and T4 were not measured. VKM identified a NOAEL of 1000 mg/kg MSM (the highest dose) for maternal and developmental toxicity.

Study characteristics	Title	Assessment of safety and efficacy of methylsulfonylmethaneon bone and knee joints in osteoarthritis animal model
	Author(s)	J. Ezaki, M. Hashimoto, Y. Hosokawa, Y. Ishimi
	Year of publication	2013

	Country	Japan
	Funding	Not reported
	Reported conflict of interest	The authors declared no conflict of interest.
Type of study	Good laboratory practice (yes/no)	No
	Guideline study (yes/no; if yes, specify)	No
	Study design (including number of groups/ number of animals per group)	Rats were randomly assigned to four diet groups, of seven animals each. Mice were randomly assigned to three diet groups, of six animals each.
Animal model	Species/(sub)strain/line	Wistar rats and STR/OrtCrlj mice.
	Disease models (e.g. allergy)	The mice were an osteoarthritis model.
Study design and exposure	Sex and age	Male rats, 6-week-old; male mice, 10-week-old.
	Feed (name, source)	Rat: control diet or MSM-containing diets, based on an AIN-93G diet. Mice: control diet (STR-cont) or MSM-containing diets, based on an AIN-93 M diet.
	Compound purity	Pure MSM, 100 %.
	Vehicle used	Not reported
	Dose regimen and frequency	Rats: MSM doses were 0.06, 0.6, and 6 g/kg bw/day. Mice: MSM doses were 0.06 and 0.6 mg/kg bw/day.
	Route of administration	Oral
	Exposure duration	Rats were exposed for 4 weeks. Mice were exposed for 13 weeks.

Results	Parameters measured, methods used, and measurement time points	Rats: After 4 weeks, all rats were sacrificed by collecting whole blood under anesthetization. Body and tissue weights (thymus, liver, spleen, kidneys) were measured, and the right femur and tibia were removed for measurement of bone mineral density. The left legs were removed for measurement of cartilage thickness. The kidney and liver were subjected to histomorphological analysis. Mice: After 13 weeks, all mice in the four groups were sacrificed by collecting whole blood under anesthetization. Body and tissue weights (thymus, liver, spleen, kidneys, and testes) were measured, and the right femur and tibia were removed for measurement of bone mineral density. The left leg was removed for measurement of histomorphologic analysis of the stifle joint. The liver was subjected to histomorphological analysis. • Bone mineral density and bone area was analysed by dual-energy X-ray absorptiometry. • Serum biochemical markers, total cholesterol, triglyceride, glutamate oxaloacetate transaminase, and glutamate pyruvate transaminase, was analysed by enzymatic colorimetric methods. • Serum calcium and phosphorus was measured by atomic absorption spectrophotometry. • Histological sections of organs were prepared and stained with hematoxylin and eosin. • Cartilage thickness of rats was measured by an image analysis system. Histomorphological grading of sections of the knee in STR mice was performed independently by two blinded observers.
	Reported outcome (including measures of variance)	Rat: 0.6 and 6 g MSM/kg bw/day: decreased liver weight, lean body mass, and bone mineral density. Serum triglyceride and serum calcium were significantly reduced. Body weight, spleen and kidney weights in the 6 g/kg bw/day group were significantly lower and higher than those in the control group, respectively. Mice: the spleen weight in the 0.6 g MSM/kg bw/day group was significantly lower than the control group. MSM intake decreased total liver score including fat vacuole score, glycogen area, and focal necrosis score in a dose-dependent manner.
Statistical analysis	Power analysis	Not reported
	Statistical test	Statistical analyses were performed by the SPSS (Version 16.0 J for Windows).
Comments		

Study characteristics	Title	Toxicity of methylsulfonylmethane in rats
	Author(s)	K. Horvath, P.E. Noker, S. Somfai-Relle , R. Glavits , I. Financsek , A.G. Schauss
	Year of publication	2002
	Country	Corresponding author from the USA.
	Funding	Not reported
	Reported conflict of interest	Not reported
Type of study	Good laboratory practice (yes/no)	Yes; animal care was in compliance with Good Laboratory Practice Regulations for Nonclinical Laboratory Studies of the United States Food and Drug Administration and Hungarian Act 1998:XXVIII, regulating animal protection.
	Guideline study (yes/no; if yes, specify)	No
	Study design (including number of groups/ number of animals per group)	Acute toxicity: 20 rats. Animals were assigned randomly to treatment groups based on body weight. An equal number of males and females were assigned to each treatment group. Subchronic toxicity: 80 rats. Animals were randomly assigned to treatment group based on body weight. An equal number of males and females were assigned to each treatment group.
Animal model	Species/(sub)strain/line	Sprague–Dawley rats.
	Disease models (e.g. allergy)	No
Study design and exposure	Sex and age	Acute toxicity: Males and females, 6 weeks old. Subchronic toxicity: Males and females, 5 weeks old.
	Feed (name, source)	Certified Rodent Diet #5002 (P.M.I. Feeds, Inc., St Louis, MO, USA).
	Compound purity	Cardinal OptiMSM™, Vancouver, WA, USA; Lot #98019.
	Vehicle used	Distilled water.
	Dose regimen and frequency	Acute toxicity: Each rat received a single oral dose by gavage of 2 g/kg of MSM or vehicle alone. Subchronic toxicity: Each rat in the MSM treatment group received a daily dose of 1.5 g/kg of MSM by gavage

		in a volume of 10 ml/kg distilled water for 90 days. Rats in the control group received 10 ml/kg distilled water for 90 days.
	Route of administration	Oral
	Exposure duration	Acute toxicity: a single dose. Subchronic toxicity: daily exposure for 90 days.
Results	Parameters measured, methods used, and measurement time points	Acute toxicity: animals were checked for clinical signs and mortality twice a day. Each rat was weighed on days 1, 5, 8, 12 and 15. Animals were sacrificed on day 15. Subchronic toxicity: Animals were checked twice daily for mortality and clinical signs of toxicity. They were weighed weekly during treatment and after overnight fasting on the day of necropsy. Laboratory tests were performed before initiation of treatment and once a week 7 from the retroorbital sinuses of five males and five female rats in each treatment group. Acute toxicity: Necropsy examinations included inspection of all external surfaces, organs and orifices. Organs and tissues examined included adrenals, aorta, brain, cecum, colon, duodenum, epididymides, esophagus, eyes, heart, ileum, jejunum, kidneys, larynx, liver, lungs, lymph nodes (mesenteric, mandibular), mammary glands, ovaries, pancreas, parathyroid, prostate, rectum, salivary glands, skeletal muscle, skin, spleen, stomach (fundic area), testes, thyroid, tongue, trachea, urinary bladder, uterus and vagina. Subchronic toxicity: Prior to necropsy, blood samples were obtained from all animals. Parameters examined included erythrocyte, leukocyte and platelet counts, hematocrit and mean corpuscular hemoglobin using a Coulter AcT8 cytometer (Coulter Diagnostics, FL, USA). Differential leukocyte count was determined manually with a light microscope. Blood coagulation was determined from blood obtained in tubes containing trisodium citrate. Prothrombin time was analysed by a Coagulometer Bank. Liver enzymes, lipid profile, serum protein, albumin, and blood chemistry with the exception of serum sodium and potassium were analysed with a FP-901 Analyser. Serum sodium and potassium were obtained with an IL 943 flame photometer. Urinalysis was carried out on five males and five females prior to treatment and on week 7. Appearance, volume, specific gravity, pH, protein, glucose and blood were checked by dipstick (Heptaphan, Lachema, Brno, Czech Republic). Necropsy was performed 91 days after initiation of treatment. Rats were fasted at least 16 h prior to autopsy. Immediately prior to necropsy, each rat was anesthetized by ether inhalation and exsanguinated. A full gross necropsy was performed. The following organs were weighed, examined then fixed in 8% buffered

		formaldehyde solution: liver, kidneys, adrenals, left testicle, spleen, brain, thymus, heart, mesenteric lymph nodes, submandibular lymph nodes, stomach, duodenum, pancreas, lungs, pituitary, trachea, esophagus, thyroids, parthyroids, left epididymis, prostate, uterus and ovaries. Right testes and epididymis were fixed in Bouin's solution. Bone marrow smears were fixed in absolute ethanol and stored at room temperature.
	Reported outcome (including measures of variance)	Acute toxicity: MSM administered in a single gavage dose of 2 g/kg resulted in no adverse events or mortality. Subchronic toxicity: MSM administered as a daily dose of 1.5 g/kg for 90 days by gavage resulted in no adverse events or mortality. Necropsy did not reveal any gross pathological lesions or changes in organ weights. Renal histology of treated animals was normal. All hematology data were within normal limits, differences between groups were attributed to low within-group variation. All blood chemistry values were within normal physiologic ranges, differences were attributed to low within-group differences. I
Statistical analysis	Power analysis	Not reported
	Statistical test	Bartlett's test of variances was used to compare variances among treatment groups. If the variances proved to be homogeneous, one-way analysis of variance (ANOVA) was performed. If ANOVA detected significant differences, a Dunnett's test for comparing treatment means with a control was used. If the values for the treatment groups failed Bartlett's homogeneity test, the Kruskal–Wallis non-parametric ANOVA was performed. If significant differences were found among the groups, distribution-free multiple comparisons were performed.
Comments		

Study characteristics	Title	Hepatoprotective effect of methylsulfonylmethane against carbon tetrachloride-induced acute liver injury in rats
	Author(s)	R. Kamel, E.M. El Morsy
	Year of publication	2013
	Country	Egypt
	Funding	Not reported
	Reported conflict of interest	Not reported

Type of study	Good laboratory practice (yes/no)	No
	Guideline study (yes/no; if yes, specify)	No
	Study design (including number of groups/ number of animals per group)	Fifty female Sprague–Dawley rats, divided into 5 groups, 10 per group. Group 1 was the control group, group 3 was the MSM group.
Animal model	Species/(sub)strain/line	Sprague–Dawley rats.
	Disease models (e.g. allergy)	No
Study design and exposure	Sex and age	Female, age not reported.
	Feed (name, source)	Not reported
	Compound purity	Analytical grade
	Vehicle used	10 % tween 80 solution.
	Dose regimen and frequency	Control group received 10 % tween 80 solution orally by gavage for 5 days. 1 hour after the last dose, they received an intraperitoneal injection of corn oil (0.1 ml/100 g body weight) on the 5th day. MSM alone group received 400 mg/kg dissolved in 10 % tween 80 solution orally by gavage for 5 days. 1 hour after the last dose, they received an intraperitoneal injection of corn oil (0.1 ml/100 g body weight) on the 5th day.
	Route of administration	Oral
	Exposure duration	Five days exposure, animals were sacrificed day six.
Results	Parameters measured, methods used, and measurement time points	All samples for measurement were taken day six. • Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured as indicators of hepatic injury using standard diagnostic kits (Quimica Clinica Aplicada S.A., Spain). • Malondialdehyde level (lipid peroxidation was assessed by measuring malondialdehyde) in the liver homogenates supernatants was measured using an assay depending on colorimetric determination. • Catalase and superoxide dismutase activity was determined using assays based on formation of a coloured product measured at different wavelengths. • Total protein was measured using an assay based on formation of a coloured product measured at 500 nm.

		• Cytokine levels were determined by enzyme linked immunosorbent assay kits specific for rats based on colour change measured spectrophotometrically. • Cytochrome P450 2E1 quantitative determination was carried out using sandwich enzyme immunoassay kit (Uscn Life Science & Technology Company, Missouri, USA). • Immunostaining was evaluated by examination of slides under a bright field microscope (CX21, Olympus, Japan) at a magnification of 200 and images were captured through a digital camera for measurement of intensity. Intensities of immunostained cells were estimated by densitometry using image analysis software (Image J, 1.46a, NIH, USA). Hematoxylin and eosin staining was used for histopathological examination through the light microscope.
	Reported outcome (including measures of variance)	No histopathological changes were recorded in liver sections of rats treated with MSM. No significant differences in ALT, AST, MDA, TNF-α or IL-6 in the MSM group compared to the control group, whereas catalase and superoxide dismutase activity were significantly increased. The content of CYP2E1 was significantly increased in the MSM group compared to the control group.
Statistical analysis	Power analysis	Not reported
	Statistical test	Different groups were compared using one way analysis of variance (ANOVA) followed by Tukey–Kramer test for multiple comparisons.
Comments		

USA	Title	Oral developmental toxicity study of methylsulfonylmethane in rats
	Author(s)	B.A. Magnuson, J. Appleton, B. Ryan, R.A. Matulka
	Year of publication	2007
	Country	USA
	Funding	Not reported
	Reported conflict of interest	Not reported

Type of study	Good laboratory practice (yes/no)	Yes; the studies were conducted in accordance with US Food and Drug Administration (FDA) Good Laboratory Practice (GLP) regulations.
	Guideline study (yes/no; if yes, specify)	Yes; OECD guideline teratogenicity study (414).
	Study design (including number of groups/ number of animals per group)	Approximately 145 nulliparous rats were mated to attain a minimum of 80 pregnant rats. The study consisted of four groups of 24-25 timed-bred primiparous dams/group. Dams determined as having mated were randomly assigned to the study groups, based on body weight measured on day zero.
Animal model	Species/(sub)strain/line	Sprague–Dawley rats.
	Disease models (e.g. allergy)	No
Study design and exposure	Sex and age	Male and female.
	Feed (name, source)	Harlan Teklad Certified Rodent Diet #8728C.
	Compound purity	99.9 %
	Vehicle used	Deionized water.
	Dose regimen and frequency	Vehicle only, 50, 500, and 1000 mg/kg; daily.
	Route of administration	Oral intubation.
	Exposure duration	Exposure occurred daily on gestation days 6–20.
Results	Parameters measured, methods used, and measurement time points	Body weights were recorded on gestation days 0 (sperm-positive day/randomisation), 3, 6, 9, 12, 15, 18, and 21. All dams were sacrificed day 21 of gestation. Dams: pregnancy status, number of corpora lutea, number and distribution of live fetuses and embryonic/fetal deaths, individual pup weights, and sex were recorded for each dam. The sex of the fetus was determined as part of the gross external examination. The uteri of dams that appeared to be non-gravid were stained with 10% ammonium sulfide and examined. The uterus and ovaries were weighed. Uterine weights collected from non-gravid animals were excluded from calculations. Fetuses: evaluation of the shape of the body and head, size and extension of the limbs, enumeration of all separate digits, and inspection of the skin, umbilicus region, anus and genitals, as well as inspection of the

		nares, pinna, eyes, and oral cavity was conducted on each fetus. Each uterine horn was inspected for tissue resorptions and fetal deaths. Implantation sites were counted, recorded and classified as: early resorption (placenta only); late resorption (placenta and fetal remains); early death (fetus weight less than 0.8 g); and late death (fetus weight of more than 0.8 g). Fetuses were euthanized by the induction of hypothermia. One-half of the fetuses from each litter were randomly assigned to receive either a skeletal examination or were decapitated and subjected to a visceral and cephalic examination. For fetuses designated for visceral examination, external and internal sex was determined. For skeletal examinations, fetuses were fixed in alcohol and stained with Alizarin red/potassium hydroxide solution. Cephalic examinations were performed on decapitated fetal heads fixed in Bouin's solution using a modified method of Wilson's razor blade sectioning technique. Examinations were done on the control and high dose groups, and included: palate and upper lip, nasal septum, olfactory lobes of the brain, ventricles I, II and III of the brain, optic cup, retina, lens and cornea.
	Reported outcome (including measures of variance)	All dams were euthanized using CO_2 asphyxiation on their 21st day of gestation and underwent a cesarean section. Gross necropsy consisted of examination of the brain and all organs in the thoracic and abdominal cavities of the dams. No evidence of maternal toxicity and no significant differences in litter viability, litter size, or litter body weight were detected. Fetal evaluations showed no biologically significant increase in the incidence of anomalies in the MSM treated groups, and no malformations were seen in any of the fetuses. No evidence of fetal mortality, alterations to growth, or structural alterations were observed in the fetuses of dams administered 50–1000 mg/kg bw/day. Under the conditions of this study, NOAEL for maternal and developmental toxicity was 1000 mg/kg bw/day.
Statistical analysis	Power analysis	Not reported.
	Statistical test	Dam weights, litter body weights, and viability data were analysed by a two-way analysis of variance (ANOVA). In the presence of a significant main effect, all post-hoc comparisons were performed using Dunnett's test (two-tail). Gross, visceral, cephalic, and skeletal data were analysed by Chi-Square/Fisher's Exact tests (fetal N) when incidence in the treated rats was higher (i.e., when the difference in the absolute number of foetuses affected is greater than three) than controls.
Comments		

12.5 Rating of confidence in evidence

The reasons for the upgrading/downgrading of the confidence in the body of evidence is shown in Table 12.5-1 to 12.5-9.

Table 12.5-1. Detailed evaluation of the confidence in evidence for the outcome blood pressure.

Blood pressure	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
RCT								
N=2 Crawford et al. (2019), Kim et al. (2006). Initial rating: ++++	Both RCTs tier 1. Not serious	No significant effects reported. Not serious	The studies were designed to evaluate adverse effects for time periods of 12 and 16 weeks. Not serious	Only two RCTs were included, and data were only reported in one of the studies. Serious	No effects were reported. Not large	There was no dose-response relationship across the studies. No	Similar study populations and conditions in the two RCTs. Consistency can therefore not be evaluated.	Moderate

Table 12.5-2. Detailed evaluation of the confidence in evidence for the outcome body weight and organ weight.

Body and organ weight	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
RCT								
N=2 Crawford et al. (2019), Kim et al. (2006). Initial rating ++++	Both RCTs tier 1. Not serious	No significant effects reported. Not serious	The studies were designed to evaluate adverse effects for time periods of 12 and 16 weeks. Not serious	Only two RCTs were included, and data were only reported in one of the studies. Serious	No effects were reported. Not large	There was no dose-response relationship across the studies. No	Similar study populations and conditions in the two RCTs. Consistency can therefore not be evaluated.	Moderate
Animal experimental studies								
N=4 Horvath et al. (2002), Magnuson et al. (2007b), Ezaki et al. (2013) (rat and mice). Initial rating: ++++	One study tier 1, two studies tier 2. Serious	Some results were conflicting, and not possible to explain. Serious	The studies were designed to evaluate adverse effects. Not serious	Four animal studies were included, however, data were reported for three of the studies. Not serious	Not large	There was no dose-response relationship across the studies. No	No consistency in the body of evidence across the animal studies. No	Low

Table 12.5-3. Detailed evaluation of the confidence in evidence for the outcome cancer.

Cancer	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
Cohort								
N=1 Satia et al. (2009). Initial rating: +++	The study was classified as tier 1. Not serious	Not evaluated as only one study addresses this outcome.	Dietary supplements and effects on cancer were the focus of the study. Not serious	Only one study was included. Serious	Not large	No doses were reported, exposure was only reported as use or no use. No	Not evaluated as only one study addresses this outcome.	Moderate

Table 12.5-4. Detailed evaluation of the confidence in evidence for the outcome developmental toxicity.

Developmental toxicity	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
Animal experimental studies								
N=1 Magnuson et al. (2007b). Initial rating: ++++	The study was classified as tier 1. Not serious	Not evaluated as only one study addresses this outcome.	The study addressed developmental toxicity. Not serious	Only one study was included. Serious	No effects reported. Not large	There was no dose-response relationship. No	Not evaluated as only one study addresses this outcome.	Moderate

Table 12.5-5. Detailed evaluation of the confidence in evidence for the outcome hematology and clinical biochemistry.

Hematology and clinical biochemistry	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
RCT								
N=2 Crawford, 2019, Kim, 2006. Initial rating ++++	Both RCTs tier 1. Not serious	No significant effects reported. Not serious	The studies were designed to evaluate adverse effects for time periods of 12 and 16 weeks. Not serious	Only two RCTs were included, and data were only reported in one of the studies. Serious	Not large	There was no dose-response relationship across the studies. No	Similar study populations and conditions in the two RCTs. Consistency can therefore not be evaluated.	Moderate
Animal experimental studies								
N=3 Horvath et al. (2002), Ezaki et al. **(2013), Kamel and El Morsy (2013).** Initial rating: ++++	Two studies, both tier 2. Serious	No unexplained inconsistency. Not serious	The studies were designed to evaluate adverse effects. Not serious	Three animal studies were included, data were only reported for two of the studies. Serious	Not large	There was no dose-response relationship across the studies. No	No consistency in the body of evidence across the animal studies. No	Low

Table 13.5-6. Detailed evaluation of the confidence in evidence for the outcome kidney toxicity.

Kidney toxicity	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
RCTs								
N=2 Crawford, 2019, Kim, 2006. Initial rating ++++	Both RCTs tier 1. Not serious	No significant effects reported. Not serious	The studies were designed to evaluate adverse effects for time periods of 12 and 16 weeks. Not serious	Only two RCTs were included, and data were only reported in one of the studies. Serious	No effects were reported. Not large	There was no dose-response relationship across the studies. No	Similar study populations and conditions in the two RCTs. Consistency can therefore not be evaluated.	Moderate
Animal experimental studies								
N=3 Horvath et al. (2002), Ezaki et al. (2013) (rat and mice). Initial rating: ++++	Both studies tier 2. Serious	Some unexplained inconsistencies. Serious	The studies were designed to evaluate adverse effects. Not serious	Three animal studies were included, data were reported for two studies. Serious	Some conflicting results. Not large	There was no dose-response relationship across the studies. No	No consistency in the body of evidence across the animal studies. No	Very low

Table 12.5-7. Detailed evaluation of the confidence in evidence for the outcome liver toxicity.

Liver toxicity	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
RCT								
N=2 Crawford, 2019 Kim, 2006. Initial rating ++++	Both RCTs tier 1. Not serious	No significant effects reported. Not serious	The studies were designed to evaluate adverse effects for time periods of 12 and 16 weeks. Not serious	Only two RCTs were included, and data were only reported in one of the studies. Serious	No effects were reported. Not large	There was no dose-response relationship across the studies. No	Similar study populations and conditions in the two RCTs. Consistency can therefore not be evaluated.	Moderate
Animal experimental studies								
N=e Horvath et al. (2002), Kamel and El Morsy (2013), Ezaki et al. (2013) (rat and mice). Initial rating: ++++	One study tier 1, two studies tier 2. Serious	Conflicting results reported in studies with the same species and similar doses. Serious	The studies were designed to evaluate adverse effects. Not serious	Four animal studies were included, however, data were only reported for three of the studies. Not serious	As some of the results were conflicting, and only three studies are included, we concluded that the effects was not large. Not large	There was no dose-response relationship across the studies. No	No consistency in the body of evidence across the animal studies. No	Low

Table 12.5-8. Detailed evaluation of the confidence in evidence for the outcome oxidative stress.

Oxidative stress	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
RCT								
N=1 Kim et al. (2006). Initial rating: ++++	The study was classified as tier 1. Not serious	Not evaluated as only one study addresses this outcome.	The study was designed to evaluate adverse effects. Not serious	Only one study. Serious	Not large	Only one dose used in the study. No	Not evaluated as only one study addresses this outcome.	Moderate
Animal experimental studies								
N=1 Kamel and El Morsy (2013). Initial rating: ++++	The study was classified as tier 1. Not serious	Not evaluated as only one study addresses this outcome.	The study was designed to evaluate adverse effects. Not serious	Only one study. Serious	Not large	Only one dose used in the study. No	Not evaluated as only one study addresses this outcome.	Moderate

Table 12.5-9. Detailed evaluation of the confidence in evidence for the outcome "other side effects".

"Other side effects"	Elements triggering downgrading				Elements triggering upgrading			Overall rating
	Risk of bias	Unexplained inconsistency	Indirectness	Imprecision	Large effect	Dose–response relationship	Consistency	
RCT								
N=1 Kim et al. (2006). Initial rating: ++++	The study was classified as tier 1. Not serious	Not evaluated as only one study addresses this outcome.	The study addressed the topic of this risk assessment. Not serious	Only one study, and limited events reported. Serious	Not large	There was no dose-response relationship. No	Not evaluated as only one study addresses this outcome.	Moderate