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Risk assessment of quercetin dihydrate and rutin in food supplements – "Other substances"

Scientific Opinion of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy of the Norwegian Scientific Committee for Food and Environment

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Norwegian Scientific Committee for Food and Environment (VKM)
Postboks 222 Skøyen
0213 Oslo
Norway

Phone: +47 21 62 28 00
Email: vkm@vkm.no

vkm.no

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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. The project group consisted of four VKM members and one VKM staff. The Committee, by the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy, appointed specifically for the assignment, assessed and approved the final opinion.

Authors of the opinion

The authors have contributed to the opinion in a way that fulfils the authorship principles of VKM (VKM, 2019). The principles reflect the collaborative nature of the work and the authors have contributed as members of the project group and/or the VKM Panel on Nutrition, Dietetic Products, Novel Food, and Allergy, appointed specifically for the assignment.

Members of the project group (in alphabetical order after chair of the project group):

Inger-Lise Steffensen – Chair of the project group. Affiliation: 1) VKM, Chair of the Scientific Steering Committee; 2) Norwegian Institute of Public Health.

Johanna Bodin – Member of the Panel on Genetically Modified Organisms in VKM. Affiliation: 1) VKM; 2) Norwegian Institute of Public Health.

Knut Tomas Dalen – Member of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) University of Oslo.

Bente Mangschou – Project leader, VKM staff. Affiliation: VKM.

Inger Aakre – Member of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) Institute of Marine Research.

Members of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy (in alphabetical order before chair of the Panel:

Lene Frost Andersen – Vice Chair of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) University of Oslo.

Knut Tomas Dalen – Member of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) University of Oslo.

Lars T Fadnes – Member of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) University of Bergen, Haukeland University Hospital.

Ingrid Kvestad – Member of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) NORCE; 3) Innlandet Hospital Trust.

Lise Madsen – Member of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) University of Bergen.

Anine C Medin – Member of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) University of Agder.

Gry I G Schultz – Member of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) Nes municipality.

Vibeke Telle-Hansen – Member of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) OsloMet.

Inger Aakre – Member of the Panel on Nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) Institute of Marine Research.

Tor A Strand – Chair of the Panel on nutrition, Dietetic Products, Novel Food, and Allergy in VKM. Affiliation: 1) VKM; 2) Innlandet Hospital Trust, 3) University of Bergen.

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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Innhold

Summary

Background

"Other substances" are substances that have a nutritional and/or physiological effect and are not vitamins or minerals. Excessive intake of certain "other substances" may be associated with health risks. The Norwegian Food Safety Authority asked the Norwegian Scientific Committee for Food and Environment (VKM) to assess whether rutin (CAS number 153-18-4) and quercetin dihydrate (CAS number 6151-25-3) from *Sophora Japonica* (bud/flower) could pose a health risk for the Norwegian population when taken daily as oral supplements as 5 mg rutin for children from 4 years of age, 25 mg rutin for adults from 18 years of age and 500 mg quercetin dihydrate for adults from 18 years of age.

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is the prototypical representative of the flavonol subclass of flavonoids. Plants contain quercetin as an aglycone or as various conjugated forms such as glycosides, including isoquercitrin (quercetin 3-*O*-β-D-glucopyranoside; IQ) and rutin (quercetin 3-*O*-β-D-rutinoside). The composition of the diverse quercetin glycosides varies between different food plants, whereas quercetin is often present in food supplements as aglycones. Rutin is composed of quercetin and rutinose, a disaccharide of rhamnose and glucose. The low oral bioavailability of quercetin and rutin, caused by their low hydrophilic solubility, can be increased by glucosyl conjugation of these molecules, i.e. by addition of various moieties such as lecithin (a mixture of various glycerophospholipids) or sugars. Quercetin Phytosome® is formulated with sunflower lecithin in a 1:1 weight ratio.

Enzymatically modified quercetin glycoside (EMIQ) is an α-glycosyl isoquercitrin (α-oligoglucosyl quercetin 3-*O*-glucoside). EMIQ is produced through enzymatic conversion of rutin into a mixture of isoquercitrin and its α-glucosyl derivatives with 1–10 α-glucose moieties connected. A hydroxyethylation reaction has been used to add a hydroxyethyl chain on the hydroxyl groups of rutin to form *O*-(β-hydroxyethyl)-rutosides (HER). As there are four hydroxyl groups available, mono-, di-, tri- and tetra-HER in various isomeric forms are present in commercial products. In the included studies, all of these chemical forms were studied, since they all are converted to quercetin aglycone in the body.

Methods

A broad search for review studies was conducted to identify human studies and/or animal toxicity studies on adverse health outcomes related to quercetin or rutin. However, because most reviews did not present much detail on the safety assessment in the human studies, a second systematic search for randomized controlled trials was performed in five databases. From these searches, 2526 records were obtained, which were screened at the level of title/abstract. From these, 140 randomised controlled trials were obtained in full-text and screened against the eligibility criteria. Among these, 45 studies were found to meet the inclusion criteria. Publications that in any way mentioned 'safety' or 'adverse events/effects' or 'side-effects' or had analysed blood or urine or any biological measure with an expressed intent to evaluate safety were considered to meet the inclusion criteria, otherwise they were excluded.

No specific adverse health outcomes related to quercetin or rutin had been identified before the literature searches. Therefore, the included papers were sorted according to how adverse effects were obtained or measured. Fifteen publications with data based on objectively measured outcomes, such as results of analyses of blood or urine, were defined as Category 1. Eight publications with only data on adverse health effects obtained or registered by subjective methods, such as self-reported adverse effects/adverse events/side-effects by participants, were defined as Category 2. Additionally, 14 publications in Category 1 included also subjectively reported adverse effects/events, i.e. altogether 23 publications reported some data on adverse effects/events or lack of such. Twenty-two publications that only mentioned briefly "no adverse effects/events/side-effects reported", without any information on how such data were recorded or any details on the results, were defined as Category 3. The publications in Category 1 and 2 were evaluated for risk of bias using the OHAT tool and used in the risk assessment. These OHAT evaluated publications were classified as Tiers 1, 2 or 3, which represent low, moderate and high risk of bias, respectively.

Results from the systematic review of adverse effects

Among the Category 1 publications, twelve reported that parameters in blood or urine were analysed with the objective to investigate safety of the treatment without reporting any adverse effects. In three studies assessing treatments with 240 mg quercetin per day for 3 months, 150 mg quercetin dihydrate per day for 8 weeks and 150 mg quercetin dihydrate per day for 6 weeks, each reported a single sporadic effect in the direction of adversity but of mild severity (decrease in high density lipoprotein (HDL)-cholesterol, increased levels of tumor necrosis factor (TNF)-α and decreased glutathione (GSH) level, respectively). The first two effects were not supported by other endpoints measured in the same study and the third effect could be interpreted as not adverse. In addition, these effects had not been reported in the other included studies, which indicated that they may be chance findings. Therefore, no serious hazards were identified among these Category 1 results.

Among the Category 2 publications, five studies stated that no adverse effects were reported by the participants or observed. Eight publications reported adverse event/effects with at least some detail about the observations, however, the reported effects/events were all of minor severity and were either considered not to be study drug-related, the type and numbers of reported effects/events were similar between the treatment and control groups, they occurred in only one person, were self-resolving or did not show a dose-response. Therefore, no serious hazards were identified among these Category 2 results.

In addition to the systematic approach used to identify and characterize adverse effects observed in the human randomized controlled trials, additional information on absorption, distribution, metabolism and excretion (ADME), and toxic effects of the studied substances - mostly from animal studies, was included from various sources, not obtained in a systematic way.

Toxicokinetics

Quite a lot of data on toxicokinetics/ADME were available for the included substances, both from human pharmacokinetic studies and animal studies. The sugar moieties of the quercetin glycosides may modulate the quercetin bioavailability. These substances

are converted to quercetin aglycone, which is easier absorbed than substances such as rutin, probably by passive diffusion over the intestinal epithelium or directly via an intestinal transporter molecule. Quercetin may also be subsequently degraded by the colonic microbiota, mainly into different phenolic acids. After absorption, quercetin is extensively metabolised in enterocytes and liver, and it may be glucuronidated, sulfated and/or methylated. In the blood, primarily these quercetin conjugates are found, with only very low levels of the aglycone form. Quercetin is found in some tissues mostly as aglycone, while in other tissues, the unconjugated quercetin is present in smaller proportions. Ingested quercetin is rapidly excreted via urine and feces, and may also be metabolised and excreted via the lungs as CO_2. There is interindividual variation in the quantitative ratio of the various metabolites formed and in the rate of absorption and excretion of quercetin, depending on genetic variation, individual antioxidative status and co-administration of other dietary components such as fiber or fat.

Toxicological data

Based on the available literature, mutagenic and genotoxic effects have been reported in some assays *in vitro*, but quercetin, rutin and the related substances EMIQ and IQ were not found to be genotoxic *in vivo* for the doses evaluated in this risk assessment. The discrepancy between *in vitro* mutagenicity and genotoxicity, and lack of genotoxic or carcinogenic effects *in vivo,* may be related to the transient nature and the instability of the quercetin quinone methide adducts, as well as various other mechanisms.

In a 2-year feeding study by the US National Toxicology Program (1992), there was some evidence of carcinogenic activity of quercetin in male rats receiving up to 1900 mg/kg body weight per day of quercetin based on an increased incidence of renal tubule cell adenomas, but there was no evidence of carcinogenic activity of quercetin in female rats in the same doses. The renal tumor development may be associated with or may be a consequence of the chronic progressive nephropathy occurring only in male rats, with probably no or only little relevance for extrapolation to humans. Other long-term rat studies, two on quercetin and two on EMIQ, did not report any carcinogenic effects. The International Agency for Research on Cancer (IARC) concluded that "quercetin is not classifiable as to its carcinogenicity to humans" (Group 3).

Quercetin can most likely cross the placenta since effects on the fetus have been observed after maternal exposure in mice and it is shown for several other flavonoids. Rutin may be able to bind to the estrogen receptor and exert estrogen-like effects.

The available studies in mice, rats and rabbits did not find reprotoxic effects of quercetin after exposure during gestation. However, in one experiment with female mice exposed for 9 months during reproductive age, 60% reduction in number of litters was observed after exposure to 5 mg/kg body weight of quercetin for 9 months.

In a case-control study, *O*-(β-hydroxyethyl)-rutoside (HER) treatment with oral doses of 900-1000 mg HER per day for 3-5 weeks during the second and/or third month of pregnancy was found to be associated with a higher risk of certain congenital abnormalities. Similarly, malformation of the limbs the offspring (syndactyly) was found

in mice after exposure to approximately 67 mg/kg body weight of quercetin for about two weeks during gestation.

The only available information on allergenicity, sensitization and irritation was that EMIQ was not a skin sensitizer or irritant in mice.

Uncertainty

Among the publications in Category 1, ten were evaluated as having low risk of bias and five with moderate risk of bias, and among the publications in Category 2, six were evaluated as having low risk of bias and two with high risk of bias. The main objective in most of these randomized controlled trials was not to examine adverse effects, but beneficial effects. Heterogeneity or mechanisms of action could not be evaluated for most publications due to the lack of reported adverse effects. Furthermore, the publications included were heterogeneous both in relation to the outcomes examined and study duration.

In addition to the administered dose(s), the actual exposure to quercetin or rutin is determined by their purity and stability. Information on purity or stability was rarely stated in the available publications, which contribute to the uncertainty of the doses actually causing the reported effects or the lack of affects.

To be able to use the included studies in the risk assessment, the given doses of quercetin- and rutin-related substances were recalculated to the corresponding dose of the common substance quercetin aglycone, into which all the related substances are metabolized. However, mostly only one pharmacokinetic study was available per modified substance and, therefore, there is some uncertainty regarding the general applicability of this information, affecting our calculations of quercetin and rutin exposure.

Information from human studies with other designs than randomized controlled trials was not systematically included in this risk assessment.

Conclusions

Based on a systematic review of randomized controlled trials examining effects of quercetin or rutin, which resulted in the inclusion and evaluation of 23 publications with adult participants, VKM considers that exposure to the three requested doses (500 mg quercetin dihydrate, 5 and 25 mg rutin) taken daily for at least up to 3 months in adults does not pose a health risk. Two of the included publications found no adverse effects after administration for up to 6-10 months. No acute toxicity of a single or short-term (5-7 days) exposure was indicated by the results.

No specific treatment-related and dose-dependent adverse effects could be identified from the included studies which reported a few outcomes in a potentially adverse direction among the parameters measured in blood or urine, and adverse effects/events/side-effects reported by the participants. By expert judgement, the weight of evidence for absence of adverse effects related to quercetin or rutin in the 23 included randomized controlled trials is judged to be "moderate".

VKM was also requested to consider if 5 mg rutin per day could pose a health risk for children from 4 years of age. None of the included studies investigated exposure

specifically in children. None of the included studies compared susceptibility to adverse effects in adults and children. Based on the results for adults and supporting evidence from one excluded study with higher daily doses (approximately 40-70 mg rutin plus 100-150 mg quercetin) for 6.5 months, VKM concludes that 5 mg rutin per day up to 6.5 months will not cause adverse effects in children other than possibly transient irritability.

Some data indicated that *O*-(β-hydroxyethyl)-rutoside (HER) and quercetin may induce teratogenic effects in offspring, shown in humans and mice, respectively. Regarding these teratogenic effects, they were observed at similar doses in humans, but at a higher dose in mice, compared with the dose of quercetin dihydrate (recalculated to quercetin aglycone) VKM was requested to evaluate.

Because of the lack of sufficient data on pregnant women and their fetuses, and the lack of data on breast-feeding women and their infants, as well as on children in general, it is not known whether these groups may potentially be more susceptible to these substances than adults.

Some data indicate that persons with chronic nephropathy or estrogen-dependent cancer may be vulnerable to adverse effects of quercetin.

Data gaps

There were few publications having evaluation of adverse effects of quercetin and rutin as the main objective. Furthermore, many of the included studies were small and of short duration, even some with single dose administration.

Very little data were found on effects of quercetin and rutin on children and pregnant women, and no data on adolescents and breastfeeding women.

Key words: VKM, food supplements, Norwegian Scientific Committee for Food and Environment, 'other substances', quercetin, risk assessment, rutin.

Sammendrag på norsk

Bakgrunn

«Andre stoffer» er stoffer som har en ernæringsmessig og/eller fysiologisk effekt og som ikke er vitaminer eller mineraler. Overdrevent inntak av visse "andre stoffer" kan være forbundet med en helserisiko. Mattilsynet ba Vitenskapskomiteen for mat og miljø (VKM) vurdere om rutin (CAS-nummer 153-18-4) og quercetin dihydrat (CAS-nummer 6151-25-3) fra *Sophora Japonic*a (knopp/blomst) kan utgjøre en helserisiko for den norske befolkningen når det tas daglig som oralt kosttilskudd som 5 mg rutin for barn fra 4 år og eldre, 25 mg rutin for voksne fra 18 år og eldre, og 500 mg quercetin dihydrat for voksne fra 18 år og eldre.

Quercetin (3,3',4',5,7-pentahydroksyflavon) er prototypen for flavonol-underklassen av flavonoider. Planter inneholder quercetin som et aglykon eller som forskjellige konjugerte former som glykosider, inkludert isoquercitrin (quercetin 3-*O*-β-D-glukopyranosid; IQ) og rutin (quercetin 3-*O*-β-D-rutinosid). Sammensetningen av de forskjellige quercetin-glykosidene varierer mellom ulike matplanter, mens quercetin i kosttilskudd ofte er som aglykoner. Rutin er sammensatt av quercetin og rutinose, et disakkarid av rhamnose og glukose. Den lave orale biotilgjengeligheten av quercetin og rutin p.g.a. deres lave løselighet i vann kan økes ved glukosyl-konjugering av disse molekylene, dvs. ved tilsetning av forskjellige molekyler som lecitin (en blanding av forskjellige glyserofosfolipider) eller sukkermolekyler. Quercetin Phytosome® er en ny formulering av quercetin blandet med lecitin fra solsikke i et vektforhold på 1:1.

Enzymatisk modifisert quercetin-glykosid (EMIQ) er et α-glykosyl-isoquercitrin (α-oligoglukosyl quercetin 3-*O*-glukosid) produsert gjennom enzymatisk omdannelse av rutin til en blanding av isoquercitrin og dets α-glukosylderivater koblet til 1–10 glucosemolekyler. En hydroksyetyleringsreaksjon legger til en hydroksyetylkjede på hydroksylgruppene til rutin for å danne *O*-(β-hydroksyetyl)-rutosider (HER). Siden det er fire tilgjengelige hydroksylgrupper finnes mono-, di-, tri- og tetra-HER i forskjellige isomere former i kommersielle produkter. I de inkluderte studiene ble alle disse kjemiske stoffene studert, som alle omdannes til quercetin aglycon i kroppen.

Metoder

Et omfattende søk etter oversiktsartikler ble utført for å identifisere studier på mennesker og/eller toksisitetsstudier på dyr om skadelige helseutfall relatert til quercetin eller rutin. Men fordi de fleste oversiktsartiklene ikke presenterte særlig mye detaljer om risikovurderingen i studiene på mennesker, ble et nytt søk etter randomiserte kontrollerte studier utført i fem databaser, etter en systematisk prosedyre. Fra disse søkene ble det 2526 treff, som ble gjennomgått på tittel/abstrakt-nivå. Fra disse ble 140 publikasjoner innhentet i fulltekst og vurdert mot inklusjonskriteriene. Blant disse oppfylte 45 randomiserte kontrollerte studier inklusjonskriteriene. Publikasjoner som nevnte «trygghet» eller «skadelige effekter/hendelser» eller «bivirkninger», eller hadde analysert blod eller urin eller et hvilket som helst biologisk endepunkt med uttrykt intensjon om å evaluere tryggheten ble inkludert, ellers ble de ekskludert.

Ingen spesifikke uønskede helseutfall relatert til quercetin eller rutin kunne identifiseres i forkant av litteratursøkene. Derfor ble de inkluderte publikasjonene kategorisert i henhold til hvordan disse effektene ble observert eller målt. Femten publikasjoner med data basert på objektivt målte utfall, som resultater av analyser av blod eller urin, ble definert som Kategori 1. Åtte publikasjoner med kun data om skadelige helseeffekter observert eller registrert ved subjektive metoder, slik som skadelige effekter/skadelige hendelser/bivirkninger rapportert av deltakerne, ble definert som Kategori 2. I tillegg inkluderte 14 publikasjoner i Kategori 1 også subjektivt rapportert uønskede effekter/hendelser, dvs. totalt 23 publikasjoner rapporterte data om skadelige effekter/skadelige hendelser/bivirkninger eller mangel på slike. Tjueto publikasjoner som bare kort omtalte "ingen skadelige effekter/skadelige hendelser/bivirkninger rapportert", uten noen informasjon om hvordan slike data ble registrert eller innhentet, eller noen detaljer om resultatene, ble definert som Kategori 3. Publikasjonene i Kategori 1 og 2 ble vurderte for risiko for systematisk skjevhet i gjennomføringen av studiene ved hjelp av OHAT-verktøyet og brukt i risikovurderingen. De ble klassifisert som nivå 1, 2 eller 3, som representerer henholdsvis lav, moderat og høy risiko for slik skjevhet.

Resultater fra den systematiske gjennomgangen av uønskede effekter/bivirkninger

Blant Kategori 1-publikasjonene rapporterte tolv at parameterne i blod eller urin ble analysert med det formål å undersøke tryggheten ved behandlingen uten å rapportere noen bivirkninger. I tre studier som vurderte behandlinger med 240 mg quercetin per dag i 3 måneder, 150 mg quercetin dihydrat per dag i 8 uker og 150 mg quercetin dihydrat per dag i 6 uker ble det observert en enkelt sporadisk effekt i potensielt skadelig retning i hver publikasjon, men de var av mild alvorlighetsgrad (henholdsvis reduksjon i HDL-kolesterol, økt nivå av tumornekrosefaktor (TNF)-α og redusert glutation (GSH)-nivå. De to første effektene ble ikke støttet av andre endepunkter målt i de samme studiene og den tredje effekten kunne tolkes som ikke skadelig. I tillegg var disse effektene ikke rapporterte i de andre inkluderte studiene, noe som indikerte at de kan være tilfeldige funn. Konklusjonen ble dermed at det ikke ble identifisert noen alvorlige helsefarlige effekter blant disse Kategori 1-resultatene.

Blant Kategori 2-publikasjonene oppga fem studier at ingen bivirkninger ble observert eller rapportert av deltakerne. Åtte publikasjoner rapporterte skadelige effekter/skadelige hendelser med i det minste noen detaljer om observasjonene, men de rapporterte effektene/hendelsene var alle av mindre alvorlighetsgrad og ble enten ansett for ikke å være relaterte til stoffet som ble studert, typen og antall rapporterte effekter/hendelser var lik mellom behandlings- og kontrollgruppene, de forekom hos bare én person, de forsvant igjen av seg selv eller viste ingen dose-respons. Dermed ble det ikke identifisert noen alvorlige helsefarlige effekter blant disse Kategori 2-resultatene.

I tillegg til den systematiske tilnærmingen som ble brukt for å identifisere og karakterisere bivirkninger observert i humane randomiserte kontrollerte studier, ble tilleggsinformasjon om absorpsjon, distribusjon, metabolisme og utskillelse (ADME) og toksiske effekter av de studerte stoffene - hovedsakelig fra dyrestudier, inkludert fra ulike kilder, innhentet på en ikke-systematisk måte.

Toksikokinetikk

Ganske mye data om absorpsjon, distribusjon, metabolisme og utskillelse (ADME) var tilgjengelig for de inkluderte stoffene, både fra humane farmakokinetiske studier og dyrestudier. Typen sukkermolekyler i quercetin-glykosider kan modulere biotilgjengeligheten av quercetin. Disse stoffene omdannes til quercetin aglycon, som absorberes lettere enn stoffer som rutin, sannsynligvis ved passiv diffusjon over tarmepitelet eller direkte via et transportmolekyl i tarmen. Quercetin kan også senere brytes ned av mikroorganismer i tykktarmen, hovedsakelig til forskjellige fenolsyrer. Etter absorpsjon metaboliseres quercetin i stor grad i tarmceller og i lever, og det kan bli glukuronidert, sulfatert og/eller metylert. I blodet finnes først og fremst disse quercetin-konjugatene, med bare svært lave nivåer av aglykon-formen. Quercetin finnes i noen vev hovedsakelig som aglykon, mens i andre vev er det ukonjugerte quercetinet til stede i mindre mengder. Inntatt quercetin skilles raskt ut via urin og avføring, og kan også metaboliseres og skilles ut via lungene som CO_2. Det er variasjon blant individer i det kvantitative forholdet mellom de forskjellige metabolittene som dannes og i hastigheten for absorpsjon og utskillelse av quercetin. Den høye interindividuelle variasjonen avhenger av genetisk variasjon, individuell antioksidant-status og samtidig administrering av andre komponenter i maten, som fiber eller fett.

Toksikologiske data

Basert på den tilgjengelige litteraturen, selv om mutagene og gentoksiske effekter er rapportert i noen *in vitro*-tester, ble quercetin, rutin og de relaterte stoffene enzymatisk modifisert quercetin-glykosid (EMIQ) og isoquercitrin (IQ) ikke funnet å være gentoksiske *in vivo* i de dosene som ble evaluert i denne risikovurderingen. Forskjellen mellom *in vitro* mutagenitet og gentoksisitet, og mangelen på gentoksiske eller kreftfremkallende effekter *in vivo,* kan skyldes at oksidative nedbrytningsprodukter av quercetin (quercetin-kinon-metid-adduktene) er ustabile og kortlivede, så vel som forskjellige andre mekanismer.

I en 2-årig fôringsstudie utført av National Toxicology Program i USA (1992), var det noe evidens for kreftfremkallende aktivitet hos hannrotter som fikk opptil 1900 mg quercetin per kg kroppsvekt per dag basert på økt forekomst av adenomer i nyretubuliceller, men det var ingen evidens for kreftfremkallende aktivitet hos hunnrotter som fikk samme doser.

Utviklingen av nyre-svulster kan være assosiert med eller kan være en konsekvens av den kroniske progressive nyreskaden som bare forekommer hos hannrotter, med sannsynligvis ingen eller bare liten relevans for mennesker.

Andre langtidsstudier på rotter, to på quercetin og to på enzymatisk modifisert quercetin-glykosid (EMIQ), rapporterte ingen kreftfremkallende effekter. International Agency for Research on Cancer (IARC) konkluderte med at "quercetin ikke kan klassifiseres med hensyn til dets kreftfremkallende egenskaper for mennesker" (Gruppe 3).

Quercetin kan mest sannsynlig krysse placenta siden effekter på fosteret har blitt observert etter mors eksponering hos mus og det er vist for flere andre flavonoider. Rutin kan være i stand til å binde seg til østrogenreseptoren og utøve østrogenlignende effekter.

De tilgjengelige studiene på mus, rotter og kaniner fant ikke reproduksjonstoksiske effekter av quercetin etter eksponering under svangerskapet. I en studie med hunnmus eksponert i 9 måneder i reproduksjonsdyktig alder ble det imidlertid observert 60% reduksjon i antall kull etter eksponering med 5 mg quercetin/kg kroppsvekt i 9 måneder.

I en kasus-kontrollstudie på mennesker ble behandling med orale doser på 900-1000 mg *O*-(β-hydroksyetyl)-rutosider (HER) per dag i 3-5 uker i løpet av den andre og/eller tredje måneden av svangerskapet funnet å være assosiert med en høyere risiko for visse medfødte misdannelser. Tilsvarende ble misdannelse av lemmer hos avkommet (syndaktyli) funnet hos mus etter eksponering for ca. 67 mg quercetin/kg kroppsvekt i ca. to uker under svangerskapet.

Den eneste tilgjengelige informasjonen om allergenisitet, sensibilisering og irritasjon var at enzymatisk modifisert quercetin-glykosid (EMIQ) ikke var hudsensibiliserende eller irriterende hos mus.

Usikkerhet

Blant publikasjonene i Kategori 1 ble ti evaluert å ha lav risiko for systematiske skjevheter og fem med moderat risiko, og blant publikasjonene i Kategori 2 ble seks evaluert til å ha lav risiko for skjevhet og to med høy risiko for skjevhet. Hovedmålet i de fleste av disse randomiserte kontrollerte studiene var ikke å undersøke skadelige helseeffekter, men gunstige helseeffekter. Heterogenitet eller virkningsmekanismer kunne ikke evalueres for de fleste publikasjoner på grunn av mangelen på rapporterte bivirkninger. Videre var publikasjonene som ble inkludert heterogene både i forhold til de undersøkte resultatene og studienes varighet.

I tillegg til administrert dose, bestemmes den faktiske eksponeringen for quercetin eller rutin av produktenes renhet og stabilitet. Informasjon om renhet eller stabilitet ble sjelden oppgitt i de tilgjengelige publikasjonene, noe som bidrar til usikkerheten om dosene som faktisk forårsaker de rapporterte effektene eller mangelen på rapporterte effekter.

Ekspertvurderinger ble brukt til å kategorisere de inkluderte publikasjonene i Kategori 1, 2 og 3, og skåring av intern skjevhet ved bruk av OHAT-verktøyet for Kategori 1- og Kategori 2-studiene. Dette påvirket i liten grad den samlede risikovurderingen og konklusjonene.

For å kunne bruke de inkluderte studiene i risikovurderingen ble gitte doser av quercetin- og rutin-relaterte stoffer omregnet til tilsvarende dose av stoffet quercetin aglycon, som alle de relaterte stoffene metaboliseres til i kroppen. Imidlertid var stort sett bare én farmakokinetisk studie tilgjengelig per modifisert stoff og dermed er det en viss usikkerhet angående den generelle anvendeligheten av denne informasjonen, noe som kunne påvirket våre beregninger av eksponering for quercetin og rutin.

Informasjon fra humane studier med andre design enn randomiserte kontrollerte studier ble ikke inkludert i denne risikovurderingen.

Konklusjoner

Basert på en systematisk gjennomgang av randomiserte kontrollerte studier som undersøkte effekten av quercetin eller rutin, som resulterte i inkludering og evaluering av 23 publikasjoner med voksne deltakere, vurderer VKM at eksponering for de tre forespurte dosene (500 mg quercetin dihydrat, 5 og 25 mg rutin) tatt daglig i opptil 3 måneder av voksne ikke utgjør en helserisiko. To av de inkluderte publikasjonene fant ingen bivirkninger etter administrering i opptil 6-10 måneder. Ingen akutt toksisitet av en enkelt eksponering eller kortvarig (5-7 dager) eksponering ble påvist i disse studiene.

Ingen spesifikke behandlingsrelaterte og dose-avhengige bivirkninger kunne identifiseres fra de inkluderte studiene som rapporterte noen få utfall i potensielt skadelig retning blant parameterne målt i blod eller urin, og skadelige effekter/skadelige hendelser/bivirkninger rapportert av deltakerne. Etter ekspertvurderinger ansees den samlede evidensen for fravær av skadelige effekter relatert til quercetin eller rutin i de 23 inkluderte randomiserte kontrollerte studiene å være moderat.

VKM ble også bedt om å vurdere om 5 mg rutin per dag kunne utgjøre en helserisiko for barn fra 4 år. Ingen av de inkluderte studiene hadde undersøkt eksponering spesifikt i barn. Ingen av de inkluderte studiene sammenlignet følsomhet for skadelige effekter i voksne og barn. Basert på resultatene for voksne og støttende evidens fra en ekskludert studie med barn 4-10 år med høyere daglige doser (ca. 40-70 mg rutin pluss 100-150 mg quercetin) i 6,5 måneder, konkluderer VKM med at 5 mg rutin per dag i opptil 6,5 måneder ikke antas å forårsake andre bivirkninger hos barn utover mulig forbigående irritabilitet.

Noen data indikerte imidlertid at *O*-(β-hydroksyetyl)-rutosid (HER) og quercetin kan indusere teratogene effekter hos avkom, vist hos henholdsvis mennesker og mus. Disse teratogene effektene ble observert ved omtrent like doser i mennesker, men ved en høyere dose i mus, sammenlignet med den dosen av quercetin dihydrat (omregnet til quercetin aglycon) som VKM ble bedt om a vurdere.

På grunn av mangel på tilstrekkelige data om gravide kvinner og deres fostre, og mangel på data om ammende kvinner og deres spedbarn, samt om barn generelt, er det ikke kjent om disse gruppene potensielt kan være mer sårbare for disse stoffene enn voksne.

Noen data indikerer at personer med kronisk nyreskade eller østrogenavhengig kreft kan være sårbare for uønskede effekter av quercetin.

Kunnskapshull

Det var få publikasjoner som hadde vurdering av skadelige effekter av quercetin og rutin som hovedformål. Mange av de inkluderte studiene var små og av kort varighet, til og med hadde noen administrering av kun én enkelt dose.

Svært lite data ble funnet om effekter av quercetin og rutin på barn og gravide kvinner, og ingen data om effekter på ungdom og ammende kvinner.

Abbreviations and/or glossary

Abbreviations

ADME - absorption, distribution, metabolism and excretion

BMI - body mass index

bw - body weight

CAS - unique identification number, assigned by the Chemical Abstracts Service (CAS) in USA to every chemical substance described in the open scientific literature

DNA - deoxyribonucleic acid

EFSA - European Food Safety Authority

EMIQ - enzymatically modified isoquercitrin

GSH - glutathione

HDL - high density lipoprotein

HER - *O*-(β-hydroxyethyl)-rutoside

HQ - hydroxyethylquercetin

IARC - International Agency for Research on Cancer

IQ - isoquercitrin

NFSA - Norwegian Food Safety Authority

NOAEL - no observed adverse effect level

NTP - National Toxicology Program, USA

OHAT - Office of Health Assessment and Translation

PRISMA - Preferred Reporting Items for Systematic reviews and Meta-Analyses

Q - quercetin

QD - quercetin dihydrate

QP - Quercetin Phytosome®

RCT - randomized controlled trial

RoB - risk of bias

TNF - tumor necrosis factor

VKM - Norwegian Scientific Committee for Food and Environment

Glossary

Definitions of 'adverse effects':

Changes in the morphology, physiology, growth, development, reproduction or lifespan 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/IPCS, 2009).

Change in the morphology, physiology, growth, reproduction, development or lifespan of an organism that results in impairment of functional capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences (EFSA SC, 2019).

In this risk assessment, various terms such as adverse effects, adverse events or side-effects have been used in the included publications without clear distinction and definition. We have used 'adverse effects/events' to mean all subjectively obtained effects, often self-reported by the participants, without implying clear causality.

Definitions of 'glycoside', glycone' and 'aglycone':

A glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. Glycosides are defined as any compound that contains a carbohydrate molecule that is convertible by hydrolytic cleavage into a sugar (glycone) and a non-sugar component (aglycone).

Background as provided by the Norwegian Food Safety Authority (NFSA)

"Other substances" are substances that have a nutritional or physiological effect and 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. These provisions are included in the Norwegian regulation "Forskrift om tilsetning av vitaminer, mineraler og visse andre stoffer til næringsmidler", which also implements Regulation (EC) No 1925/2006 in Norwegian law.

The intention of the national supplementary provisions is to reduce health risks that can occur when consuming certain "other substances" in foods, including food supplements.

A so-called "positive list" for the addition of certain "other substances" was introduced as Annex 3 to the regulation. It is only permitted to add "other substances" that are listed in the "positive list" in Annex 3 to foods, including food supplements. The addition must be in accordance with the terms and conditions set in the "positive list", including the threshold values that are set for the different substances.

The national supplementary provisions only apply (Section 6, second paragraph) 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 (Section 6, third paragraph) 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.

If a food business operator wants to add a higher quantity of a substance or add a substance that is included in the "positive list" to a new category of food products, the food business operator must notify NFSA (see Section 9). If a food business operator wants to add new substances, not currently included in the "positive list", the food business operator must apply for authorization to NFSA (see Section 10). The notification or application shall contain the information and scientific documentation required in Appendix 4 in the regulation.

For NFSA to process an application or notification, NSFA may request that the Norwegian Scientific Committee for Food and Environment (VKM) performs a risk assessment of higher amounts of substances listed in the "positive list", or new substances with applications for authorization.

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority asks the Norwegian Scientific Committee for Food and Environment (VKM) to assess whether rutin (CAS number 153-18-4) and quercetin dihydrate (CAS number 6151-25-3) from *Sophora Japonica* (bud/flower) in the quantities and the age groups specified below, may pose a health risk for the Norwegian population.

The Norwegian Food Safety Authority asks VKM to consider daily intake of

- 5 mg rutin (CAS number 153-18-4) per recommended daily dose in food supplements intended for children 4 years of age and older,
- 25 mg rutin (CAS number 153-18-4) per recommended daily dose in food supplements intended for adults from 18 years of age, and
- 500 mg quercetin dihydrate (CAS number 6151-25-3) per recommended daily dose in food supplements intended for adults from 18 years of age.

This includes:

- Identify and characterise adverse health effects.
    - Identify harmful health effects and describe at what doses these occur.
    - Describe uncertainty related to knowledge about adverse health effects and dose and in case of possible extrapolation from animals to humans.
- Evaluate the exposure.
    - Evaluate exposure for the dose(s) and age groups given above.
    - Describe uncertainty related to the exposure evaluations.
- Characterise health risks associated with exposure to rutin or quercetin dihydrate and describe uncertainty that may have an impact on the conclusions.
- Identify and describe knowledge gaps that may have an impact on the conclusions.

Assessment

1 Introduction

"Other substances" are substances that have a nutritional and/or physiological effect and are not vitamins or minerals. Excessive intake of certain "other substances" may be associated with health risks. On request from the Norwegian Food Safety Authority, VKM has conducted a series of risk assessment of "other substances" in food supplements and energy drinks.

In this risk assessment, we describe the relationship between quercetin and rutin and also other related substances and conduct a risk assessment of specific doses of these related substances based on previous reviews, animal studies and a broad systematic literature review of human RCTs examining adverse health effects related to exposure to quercetin dihydrate or rutin.

There are several substances structurally related to quercetin and rutin used in the included publications, and therefore, an overview of the various substances is described in Chapter 2.1 and 2.2 Quercetin/rutin and related substances.

Chapter 4 contains the hazard identification and characterization of quercetin and rutin. As the bioavailability may vary between the substances, absorption, distribution, metabolism and excretion (ADME) is described for these substances in Chapter 4.1. Chapter 4 also includes toxicological data, mostly from animal toxicological studies (Chapter 4.2-4.4) and a systematic review of adverse effects reported in human RCTs (Chapter 4.5). A summary of the Hazard identification and characterization is found in Chapter 4.6, including a summary of the included RCT studies from the systematic review (4.6.1) ADME (4.6.2) and toxicity (4.6.3).

In Chapter 5 Risk characterization, the different forms of quercetin and rutin are calculated into comparable doses as quercetin aglycone and a summary of the Risk characterization is found in Chapter 5.3.

Delimitations of the present risk assessment

In this risk assessment, the following delimitations have been made:

-The risk assessment is performed for oral intake of quercetin and rutin as food supplements and only for the doses and age groups stated in the terms of reference from the Norwegian Food Safety Authority.

-Other sources of exposure, such as intake of quercetin and rutin from foods, are not included in the risk characterization.

-Interactions between quercetin or rutin, and other components, have not been addressed.

-Data on beneficial effects of quercetin and rutin have not been evaluated.

2 Substance specifications

Naturally occurring quercetin in foods is present primarily as quercetin glycosides, whereas food supplements contain mainly the aglycone form of quercetin (Andres et al., 2018). In addition, various modifications of quercetin and rutin are available as food supplements.

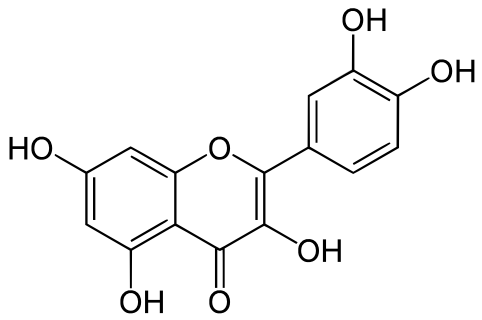
The randomised controlled trials (RCTs) obtained in the literature search used to perform the risk assessment of quercetin dihydrate and rutin as requested in the terms of reference were performed with these two specific substances or with several other variants. Therefore, the other variants (quercetin aglycone, Quercetin Phytosome®, HER, EMIQ) used in the included publications are described in the following and the differences in their molecular weight and bioavailability (see Chapter 4.1) are taken into consideration in the risk characterisation.

2.1 Quercetin and related substances

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) (Figure 2.1-1A) is the best-known, prototypical representative of the flavonol subclass, which is among the most-widespread and most-studied types of flavonoids (Owczarek-Januszkiewicz et al., 2022). Plants contain quercetin as an aglycone, or as various conjugated forms such as glycosides, among which isoquercitrin (quercetin 3-*O*-β-D-glucopyranoside; IQ) and rutin (quercetin 3-*O*-β-D-rutinoside) are the most ubiquitous (Brodowska, 2017; Santos et al., 2017) (see below). The composition of the diverse quercetin glycosides varies between different food plants, whereas quercetin is often present in food supplements as aglycones (without linked sugars) (Andres et al., 2018). The molecular formula of quercetin aglycone is $C_{15}H_{10}O_7$ and the molecular weight is 302.23 g/mol (PubChem, 2024). The CAS number of quercetin (aglycone) is 117-39-5.

Quercetin dihydrate has the synonyms 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one dihydrate and 3,3′,4′,5,7-pentahydroxyflavone dihydrate (Figure 2.1-1B). The molecular formula of quercetin dihydrate is $C_{15}H_{10}O_7 \cdot 2H_2O$ ($C_{15}H_{14}O_9$) and the molecular weight is 338.27 g/mol (PubChem, 2024). The CAS number is 6151-25-3.

A)



B)

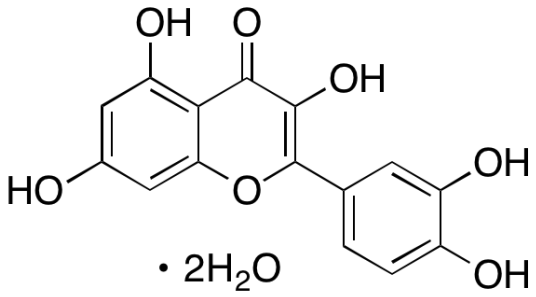


Figure 2.1-1. Chemical structure of quercetin (aglycone) (A) and quercetin dihydrate (B) (PubChem, 2024).

Quercetin Phytosome® is a new food-grade lecithin-based formulation of quercetin made to overcome the poor solubility and low oral absorption of quercetin because of its high lipophilicity. It contains quercetin formulated with sunflower lecithin (a mixture of various glycerophospholipids) in a 1:1 weight ratio together with about 1/5 part of food-grade excipients that are added to improve the physical state of the product and to standardize it to a HPLC-measured total quercetin content of about 40% (Riva et al., 2019).

2.2 Rutin and related substances

The flavonol **rutin** (quercetin-3-*O*-β-D-rutinoside) usually occurs in dietary plants such as fruits and vegetables as glycosides (with linked sugars) (Andres et al., 2018; Chua, 2013). Rutin is composed of quercetin (3,3',4',5,7-pentahydroxyflavone) and rutinose, a disaccharide of rhamnose and glucose, see Figure 2.2-1. The molecular formula of rutin is $C_{27}H_{30}O_{16}$ and the molecular weight is 610.5 g/mol (PubChem, 2024). The CAS number is 153-18-4.

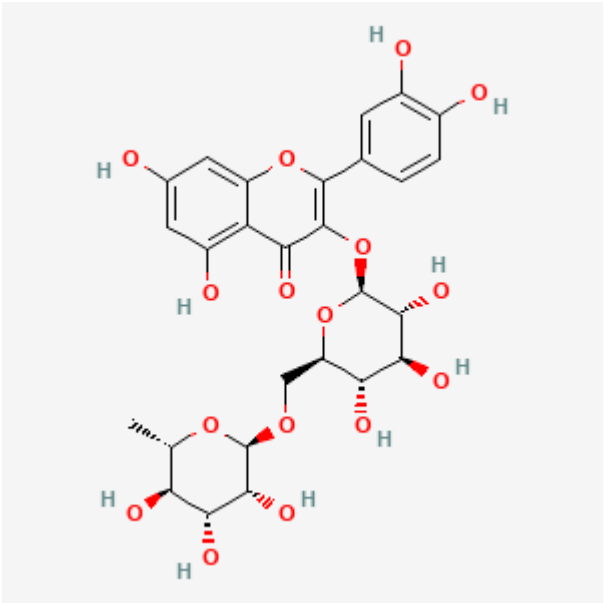


Figure 2.2-1. Structural formula of rutin (PubChem, 2024).

Enzymatically modified quercetin glycoside (EMIQ) is a mixture containing isoquercitrin and several α-oligoglycosides (Murota et al., 2010). More specific, EMIQ is an α-glycosyl isoquercitrin (α-oligoglucosyl quercetin 3-*O*-glucoside), produced through enzymatic conversion of rutin into a mixture of isoquercitrin (quercetin 3-*O*-β-D-glucopyranoside, IQ) and its α-glucosyl derivatives with 1–10 α-glucose moieties connected linearly via 1→4 linkage (IQG_1-IQG_{10}) by adding dextrin (a complex carbohydrate that is made up of many glucose molecules linked together) (Figure 2.2-2). The mean content of isoquercitrin-IQG_7 in the mixture is over 94%; free quercetin is present at a concentration less than 1%. The average molecular weight of EMIQ is about 800 Daltons.

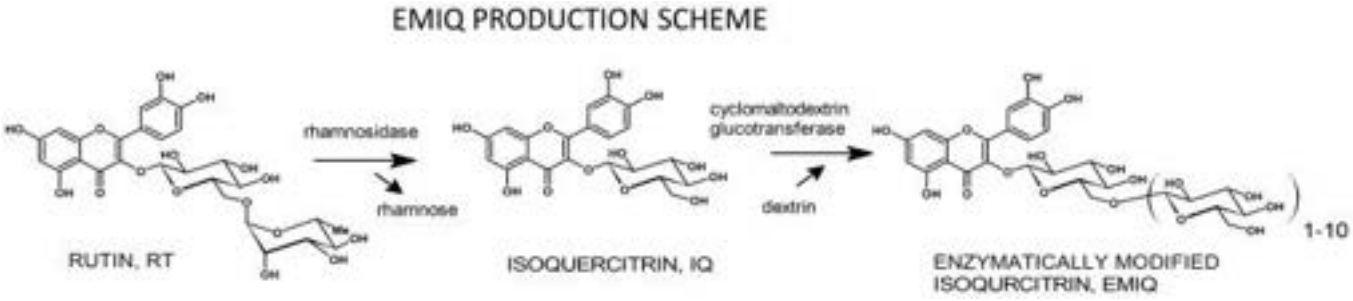


Figure 2.2-2. A simplified scheme for EMIQ production. Rutin obtained from natural sources is transformed using bacterial enzymes first into isoquercitrin (IQ) and then into isoquercitrin oligoglucosides with 1 to 10 α-glucosyl moieties (EMIQ). The figure is from Owczarek-Januszkiewicz et al. (2022), Open Source, Creative Commons Attribution License (CC BY).

***O*-(β-hydroxyethyl)-rutoside (HER, Venoruton®)** is a standardised mixture of hydroxyethylrutosides (HER), which are synthetic derivatives of the flavonoid rutin, which is highly hydrophobic (Kienzler et al., 2002). To obtain a compound with less hydrophobic properties, a hydroxyethylation reaction is used to add a hydroxyethyl chain on the hydroxyl groups of rutin to form *O*-(β-hydroxyethyl)-rutosides (HER). As there are four hydroxyl groups available, mono-, di-, tri- and tetra-HER in various isomeric forms are present in products such as Venoruton®.

3 Exposure

3.1 Doses as specified by the Norwegian Food Safety Authority

In this risk assessment, VKM has evaluated the daily intake of food supplement doses of 500 mg quercetin dihydrate for adults ≥18 years, 25 mg rutin for adults ≥18 years and 5 mg rutin for children ≥4 years. Exposure from other sources, such as in food, of quercetin dihydrate or rutin is not included in the exposure estimated by VKM.

Default body weights (bw) determined by EFSA (2012) for the EU adult population were used to estimate the daily intake of quercetin dihydrate and rutin in the unit mg/kg bw. Intake was estimated for the 5th percentile (P5) and the 50th percentile/median (P50) of bw for male and female combined (Tables 3.1-1 and 3.1-2).

Table 3.1-1 Daily dose of quercetin and default body weights used in the risk characterization.

		Body weight (bw)	
Age group	**Daily supplement dose**	P5	P50
Adults ≥18 years	500 mg	52 kg	72 kg

P5 = 5th percentile, P50 = 50th percentile (median).

Table 3.1-2 Daily doses of rutin and default body weights used in the risk characterization.

		Body weight (bw)	
Age group	**Daily supplement dose**	P5	P50
Adults ≥18 years	25 mg	52 kg	72 kg
	5 mg	52 kg	72 kg
Children 4-10 years	5 mg	14.0 kg	21.7 kg
Adolescents 10-14 years	5 mg	29.4 kg	42.0 kg
Adolescents 14-18 years	5 mg	45.0 kg	60.0 kg

P5 = 5th percentile, P50 = 50th percentile (median).

To be able to compare the doses of the various substances used in the RCTs in the included publications (describing hazard) with the doses of quercetin dihydrate or rutin requested to be evaluated by the Norwegian Food Safety Authority (the exposure), the doses have been recalculated into the doses corresponding to common substance quercetin aglycone (see Chapter 5, Table 5-1). In these recalculations of doses, we have assumed that the bioavailability of the substances will affect their potential toxicological (adverse) effects and that these relationships are linear. The information on bioavailability of Quercetin Phytosome® versus quercetin in humans is from Riva et al. (2019). The data on bioavailability of isoquercitrin and EMIQ versus rutin is from experiments in male rats (Makino et al., 2009).

3.2 Dietary sources of exposure

Fruits and vegetables are the primary dietary sources of quercetin, particularly onion, kale, citrus fruits, capers, cloves, apples, tea and red wine (Frutos et al., 2019; Phenol-Explorer 3.6). Olive oil, grapes, dark cherries and dark berries such as blueberries, blackberries, bilberries, elderberries and cranberries, are also high in quercetin. Rutin occurs in foods such as buckwheat, asparagus, unpeeled apples, figs, apricots, cherries, grapes, grapefruit, plums and oranges, as well as in black tea, green tea and elderflower tea (Frutos et al., 2019; Phenol-Explorer 3.6).

The estimated average daily intake of quercetin by an individual in the United States was 25 mg (NTP, 1992). Average daily intake of quercetin appeared to range from approximately 2 to 53 mg per day (Manach et al., 1997). Harwood et al. (2007) reported that the estimated intake of quercetin from the diet by consumers having a high fruit and vegetable intake was 200-500 mg per day, whereas Andres et al. (2018) stated that it had been estimated that quercetin intake of "high-end consumers" of fruits and vegetables was 250 mg per day.

Dietary intake of quercetin or rutin has not been addressed in this VKM risk assessment.

4 Hazard identification and characterisation

Literature searches were conducted to identify any adverse effects from oral intake of quercetin or rutin in food supplements, see Chapter 4.5.1. No specific literature search was conducted to retrieve publications on absorption, distribution, metabolism and excretion (ADME) of quercetin, rutin and related substances. However, information relevant for ADME in humans or animals was found in the publications that were identified in the literature searches for adverse effects, both in RCTs and in some reviews. In addition, further information was obtained from the reference lists of these publications. Some animal toxicity studies were obtained from the review search, including a chronic two-year rat study by NTP (1992), others were obtained from the reference lists. The data on vulnerable groups and drug interactions were obtained from similar sources as ADME and toxicity, i.e. using a non-systematic approach.

4.1 Absorption, distribution, metabolism and excretion (ADME)

4.1.1 Rutin and quercetin

Rutin is not well absorbed in the small intestine of humans. After a single oral dose of 500 mg rutin, persons show variability in kinetics of uptake, with two persons having a maximal plasma concentration of quercetin at 7 hours and the third person achieving a maximal plasma concentration at 4 hours (Boyle et al., 2000). There was also interindividual variation in the extent of absorption, with an increase in plasma concentration of 40 ± 220 ng quercetin/ml).

Rutin is further transported from the small intestine into the colon and metabolised by the gut microbiota into isoquercetin (quercetin-3-glucoside) and then quercetin, or directly into quercetin. First, one sugar moiety (rhamnose) can be hydrolysed by the enzyme α-rhamnosidase to the intermediate molecule isoquercetin and thereafter the second sugar molecule can be hydrolysed by the enzyme β-glucosidase to quercetin (Chua, 2013; Riva et al., 2020) (Figure 4.1.1-1). Alternatively, the enzyme β-rutinosidase removes both sugar moieties forming quercetin. There are considerable interindividual differences in the activity of the β-glucosidase enzyme in the small intestine, contributing to individual variations in quercetin absorption (Németh et al., 2003). The aglycone is easier absorbed, probably by passive diffusion over the intestinal epithelium. Absorption of quercetin glycoside ranges from 3 to 17% after a 100-mg dose in healthy subjects (Simioni et al., 2018). Quercetin glycosides may also be absorbed directly via intestinal sodium-dependent glucose transporter (SGLT-1) (Andres et al., 2018). Quercetin may be subsequently degraded mainly into different phenolic acids. Smaller molecules are formed by the colonic microbiota, such as 3,4-dihydroxyphenylacetic acid (3,4-DHPAA), 3,4-dihydroxytoluene (3,4-DHT), 3-hydroxyphenylacetic acid (3-HPAA) and homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid, HVA) (Chua, 2013). An overview of quercetin metabolism is shown in Figure 4.1.1-2.

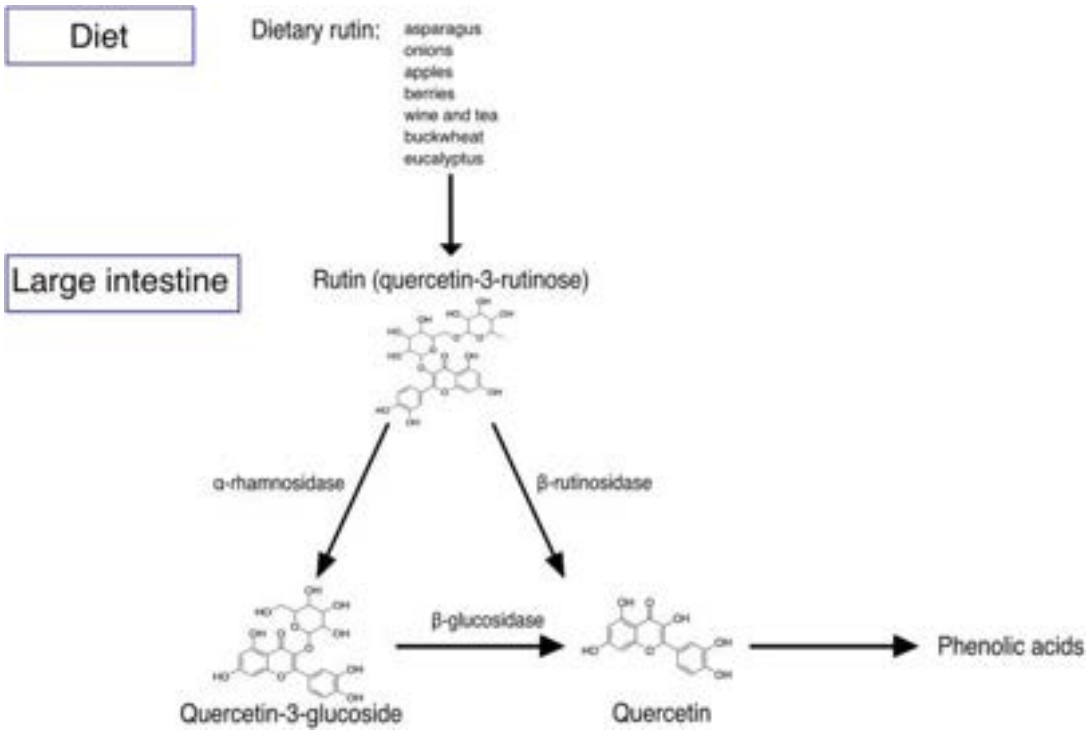


Figure 4.1.1-1. Schematic representation of rutin metabolism. Rutin is not well absorbed in the small intestine of humans and, thus, is transported into the colon and metabolized by the gut microbiota into quercetin-3-glucoside and then quercetin, or directly into quercetin. Quercetin may be subsequently degraded mainly into different phenolic acids. Reused from Riva et al. (2020), Open Source, Creative Commons Attribution License (CC BY).

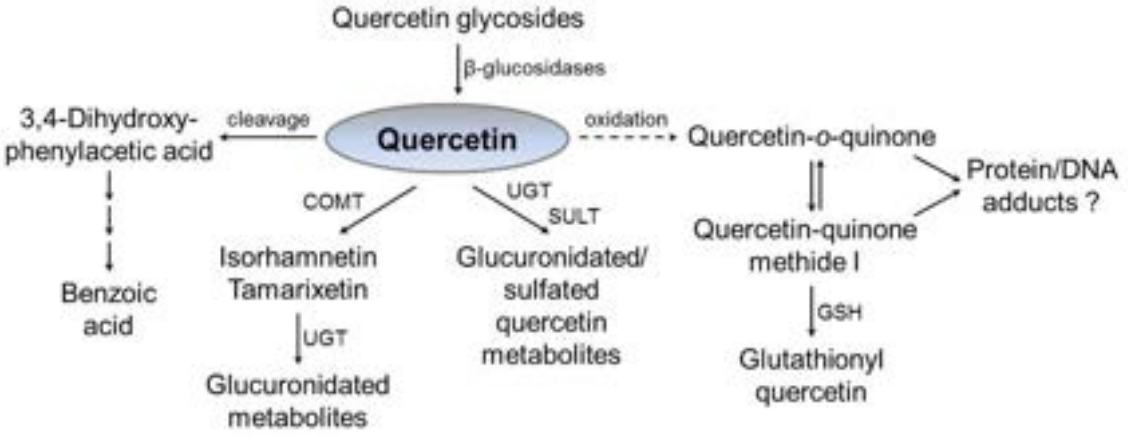


Figure 4.1.1-2. Overview of the metabolism of quercetin. Reused with permission from Andres et al. (2018, supporting Figure S1) and modified. Abbreviations: COMT: catechol-O-methyltransferase; GSH: glutathione; SULT: sulfotransferase; UGT: UDP-glucuronosyltransferase.

After absorption, quercetin is extensively metabolised in the enterocytes and in the liver, and it may be glucuronidated, sulfated and/or methylated (Andres et al., 2018).

The phase II metabolites of quercetin are secreted into the portal and lymph circulation. In addition, it may be oxidized *in vivo*, forming quercetin-quinone and quercetin-quinone methides, which may form DNA-adducts (see Chapter 4.2 Genotoxic potential and carcinogenicity). In the blood, primarily quercetin conjugates are found, with only very low levels of the aglycone form. The blood contains mostly glucuronidated and/or sulfated quercetin conjugates, without the corresponding conjugates of the methylated quercetin forms. It has been demonstrated that the predominant quercetin conjugates in analysed human plasma samples are quercetin 3-O-β-D-glucuronide (Q3GA) and quercetin-3′-sulfate (D'Andrea, 2015). The composition of various of quercetin conjugates (sulfates or glucuronides) may also modulate the biological action(s) of quercetin *in vivo*. The pharmacokinetics of quercetin can show high interindividual variability, depending on, for example, genetic variations, individual antioxidative status, food co-administration of other dietary components such as fiber or fat. With respect to quercetin glycosides as the major quercetin source in foods, the sugar moieties of the quercetin glycosides may also modulate the quercetin bioavailability (Andres et al., 2018). Apparently, there is less interindividual variation in metabolites which are derived from absorption in the small intestine compared to catabolites derived from the action of microbiota in the colon (Almeida et al., 2018).

A pharmacokinetic human trial in healthy volunteers (n = 12) compared absorption of quercetin aglycone (8, 20 and 50 mg) and rutin (16, 40 and 100 mg). The respective doses of both substances contained equimolar amounts of quercetin aglycone. It was demonstrated that quercetin and rutin were present in plasma as glucuronides and/or sulfates of quercetin and as unconjugated quercetin aglycone, but no rutin was detected (Erlund et al., 2000). The time to reach maximum plasma concentration was significantly shorter with quercetin aglycone treatment compared to rutin treatment. Thus, the aglycone is likely to have a greater biological activity than the glycoside (Kienzler et al., 2002). The absorption of quercetin aglycone was much more predicable than that of quercetin derived from rutin. The absorption of quercetin from quercetin aglycone showed small inter-individual variation and was not affected by gender or use of oral contraceptives. After rutin administration, inter-individual variations in plasma levels were considerable. The absorption was higher in females than in males and was further increased by the use of female oral contraceptives (Erlund et al., 2000).

Regarding the tissue distribution of quercetin, data are available for rats and pigs, whereby pigs seem to better reflect the metabolism seen in humans (Andres et al., 2018). In rats, the highest quercetin levels were found in lung, testis and kidney (with lower levels than in plasma) and the lowest levels in brain, spleen and white fat tissue. In pigs, some tissues, such as colon, mesentery, diaphragm, liver, lung, jejunum and brain, seem to contain quercetin either exclusively or at higher proportions as aglycone (approximately 90%), while in other tissues, such as the kidney or lymph nodes, the unconjugated quercetin was present in smaller proportions (30–60%). However, there are uncertainties in the real proportion of the quercetin aglycone in tissues because there are indications that postmortem deconjugation of flavone conjugates during the extraction procedure may occur to varying degrees in different organs (Andres et al., 2018).

Ingested quercetin is rapidly excreted via urine and feces, and may also be metabolised and excreted via the lungs as CO_2 (D'Andrea, 2015). Unchanged rutin,

quercetin or conjugated quercetin metabolites in the form of glucuronide or sulphate were not detected in the urine after oral dosing of rats, indicating that urinary excretion is not a major route (Choudhury et al., 1999). The fecal recovery of quercetin was in the range of 1.6-4.6% of the oral dose (D'Andrea, 2015). After oral exposure, rutin appeared to be excreted unmodified in the feces in germ-free rats, but as aglycones in conventional rats with normal microflora (Griffiths & Barrow, 1972).

After a single oral dose of 500 mg rutin, there was interindividual variation in the rate of quercetin clearance, which was not complete after 24 hours in one of three persons (Boyle et al., 2000). Hence, there is a possibility that significant accumulation of quercetin may occur in the blood of some individuals after repeated daily supplementation.

4.1.2 Quercetin Phytosome®

In a human clinical study in healthy volunteers (n = 12) by Riva et al. (2019), absorption of Quercetin Phytosome® was compared with absorption of quercetin after a single oral dose. In the group exposed to 500 mg quercetin, the quercetin plasma concentration was always <10 ng/ml, whereas the plasma concentration of quercetin was about 100 ng/ml and 170 ng/ml after intake of 250 mg and 500 mg Quercetin Phytosome®, respectively. Thus, it was found up to about 12 and 20 times higher plasma levels of Quercetin Phytosome® in comparison to that of quercetin with the 250 mg and 500 mg dose, respectively. The half-lives in plasma of the two doses of Quercetin Phytosome® in comparison to that of quercetin were about 60% and 54% lower with the 500 mg and 250 mg dose, respectively. These data demonstrate a dose-dependency in the pharmacokinetics of Quercetin Phytosome®.

The interaction between Quercetin Phytosome® and the human microbiota showed that the Quercetin Phytosome® formulation was more stable than unformulated quercetin after interaction with the intestinal microbiota (Di Pede et al., 2020). Quercetin Phytosome® could slow down the intestinal microbial degradation of quercetin, allowing for more time and the better dispersion of the single molecule to be absorbed, thus overcoming one of the possible reasons for quercetin's poor oral bioavailability.

4.1.3 Enzymatically modified isoquercitrin (EMIQ) and isoquercitrin (IQ)

The low oral bioavailability of quercetin and rutin caused by their insolubility in water, can be increased by glucosyl conjugation of these molecules, i.e. addition of various sugar moieties, such as in EMIQ. See Figure 2.2-2 for production of EMIQ. In addition, the chemical structure of the glucose moiety of a particular glycoside affects the small intestinal absorption of the glycosides, such as the position of linkage between glucose molecules. The following formation on ADME of EMIQ is mostly based on Owczarek-Januszkiewicz et al. (2022), Makino et al. (2009) and Murota et al. (2010). Salivary α-amylase cleaves parts of the α-(1→4) linkages of EMIQ and de-oligomerize the EMIQ components into simpler ones directly after oral intake. The conversion is faster the longer the α-glucosyl side chain is. No substantial digestion takes place in the stomach, since EMIQ is being resistant to the low pH there. In the small intestine, EMIQ is further degraded by pancreatic α-amylase, being converted into isoquercitrin and α-glucosyl derivatives with 1 or 2 α-glucose moieties. Further, the remaining α-glucosyl

derivatives are degraded to isoquercitrin and then to quercetin by the enzyme lactase-phlorizin hydrolase (LPH), acting extracellularly at the intestinal epithelial cells. The glucose released during the reaction is actively transported into the enterocytes by SGLT-1, while the aglycone is now lipophilic enough to be absorbed via passive diffusion.

Various metabolites derived from EMIQ degradation are detected in plasma as early as 15 min after oral administration of EMIQ. This indicates that the small intestine is the primary absorption site, although some polyphenols may reach the large intestine and there undergo transformation by gut microbiota. These early EMIQ metabolites are mainly quercetin glucuronide and sulfate conjugates, being transformed in the enterocytes by UDP-glucuronosultransferase and phenol sulfotransferase, respectively. Some methylated derivatives may also be formed later, by the activity of *O*-methyltransferase in the liver.

Experiments in rats showed that the bioavailability (*F* value) of EMIQ (calculated from the concentrations of total plasma quercetin levels from 0 to 12 hours after oral administration) was 35%, thus being approximately 17, 3 and 44 times higher than after oral administration of quercetin (2%), isoquercitrin (12%) and rutin (0.8%), respectively (Makino et al., 2009). In one human study, the main metabolites of EMIQ in plasma were quercetin 3-glucuronide, quercetin 3'-sulfate and isorhamnetin 3-glucuronide (Owczarek-Januszkiewicz et al., 2022). However, the quantitative ratio of these various metabolites varied substantially among individuals. Maximum plasma concentration of these metabolites was reached 1.5-2 hours after oral administration, and similar to the metabolisation in animals, reached about three times higher circulating levels than that obtained for isoquercitrin.

Metabolites of EMIQ are at least partially eliminated through urine and elevated levels of quercetin conjugates in the urine samples of laboratory animals were detected even 28 days after EMIQ exposure, suggesting that the EMIQ-derived metabolites may to some extent accumulate in the body. The quantifiable amounts of quercetin 3-glucuronide, isorhamnetin, quercetin and kaempferol were detected in the bones, cerebrum and fat of Sprague-Dawley rats after 14 days of oral administration of EMIQ (1.5, 3 and 5% in the diet). The accumulation was dose-dependent and caused a characteristic yellow discoloration of the femur. The data suggest that the effect is reversible and not connected with any histopathological changes.

4.1.4 O-(β-hydroxyethyl)-rutoside (HER, Venoruton®)

It is assumed that *O*-(β-hydroxyethyl)-rutosides (HER) are poorly absorbed from the gastrointestinal (GI) tract because of their high molecular weight and low lipophilicity and liposolubility (Kienzler et al., 2002). However, the presence of HER-glycosides and their conjugates in urine of humans is evidence of absorption from the gastrointestinal tract, although the low plasma levels suggest either a slow absorption from gastrointestinal or efficient hepatic extraction. The position and nature of the sugar residues may affect the uptake of the compound in the small intestine. It is assumed that HER are not absorbed as glycosides and are present in plasma as aglycones conjugated to glucuronic acid and/or sulfate conjugates. Apparently, HER can be considered to have a similar bioavailability as rutin and quercetin.

Most likely, β-glucosidase activity is involved in absorption of HER in humans in the distal small intestine or colon. Mono-3'-HER and mono-4'-HER are the most available among the circulating metabolites of HER, which are hydrolysed prior to absorption (Kienzler et al., 2002). Thus, it is likely that these molecules are present in plasma as aglycones (quercetin) conjugated to glucuronic acid and/or sulfate groups. The overall pharmacokinetic behaviour of mono-3'-hydroxyethylquercetin (HQ) and mono-4'-HQ was similar, with a t_{max} of about 8 hours and 7 hours, respectively, independent of the administered doses (Kienzler et al., 2002). The amount of mono-3'-HQ and mono-4'-HQ measured in the blood was negligible up to 4 hours, but occurred rapidly after this and plasma concentrations close to t_{max} were reached after 7 hours and 6 hours, respectively, showing similar absorption behaviour as rutin.

The elimination half-life of both molecules was similar with three higher doses (1000 mg, 2000 mg and 4000 mg Venorutin powder), but shorter with 500 mg (Kienzler et al., 2002). For mono-3'-HQ, terminal half-life averaged approximately 17 hours at the 500 mg dose and 26-34 hours at the three higher doses. For mono-4'-HQ, terminal half-life averaged 6 hours at the 500 mg dose and 12 hours at three higher doses (1000 mg, 2000 mg and 400 mg). The data for the lowest dose was less certain because of fewer data points above the limit of quantification.

4.2 Genotoxic potential and carcinogenicity

4.2.1 Genotoxicity, general toxicity and carcinogenicity of quercetin and rutin

4.2.1.1 *In vitro* genotoxicity

Quercetin induced gene mutations in *Salmonella typhimurium* strains TA100 and TA98 with and without exogenous metabolic activation (S9-mix, from the 9000 g supernatant of a liver homogenate containing metabolic enzymes). Positive results were also obtained in tests with and without S9 for induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells (NTP, 1992). Andres et al. (2018) also summarized *in vitro* studies of quercetin, finding positive effects on mutations, chromosomal aberrations, DNA single strand breaks and induction of micronuclei. Thus, quercetin is genotoxic *in vitro*.

4.2.1.2 Chronic toxicity, *in vivo* genotoxicity and carcinogenicity

Carcinogenicity of quercetin and rutin was examined in male and female inbred ACI strain of rats given a diet containing 1% or 5% quercetin or 5% rutin for 540 days (18 months), or 10% quercetin and 10% rutin for 850 days (about 28 months) (Hirono et al., 1981). Rats in the control groups were fed a normal basal diet. Most tumors found in treatment groups were also found in the corresponding control groups. Furthermore, there was no significant difference between the incidence of tumors in the treatment and control groups ($P > 0.05$). Thus, quercetin and rutin tested were not carcinogenic in ACI rats.

F344 rats of both sexes were exposed to a basal diet or a basal diet with 0.1% or 0.2% of purified quercetin *ad libitum* for 64 weeks (about 16 months) (Stoewsand et

al., 1984). The few lesions observed in the tissues were related to normal aging in this rat strain, thus, quercetin did not appear to be carcinogenic.

NTP (1992) performed toxicological and carcinogenesis feeding studies of quercetin (CAS No. 117-39-5) in F344/N rats (NTP TR 409). The average amounts of quercetin (>95% pure) consumed per day by the 1000, 10000 and 40000 ppm (mg/kg feed) dose groups were 40, 400 and 1900 mg/kg of body weight (bw), when calculated after week 52. It was given to groups of 50 male and female rats for 104 weeks. Ten additional animals per dose group were evaluated at 6 and 15 months.

Body weights of exposed male and female rats given 40 and 400 mg/kg bw were within 5% of controls throughout the studies. Reduced bw gain in male and female rats receiving 1900 mg/kg bw per day was observed by week 15 and the final mean bw were 87% of controls at week 104. Survival and feed consumption were similar among exposed and control groups throughout the studies.

In male rats, the principal toxic effects associated with the dietary administration of quercetin for 2 years were observed in the kidney. There were dose-related increases in the severity of chronic nephropathy (control, 2.7; low-dose, 2.7; mid-dose, 3.0; high-dose, 3.2) and a slight increased incidence in focal hyperplasia of the renal tubule epithelium (1/50, 2/50, 3/50, 4/50). Parathyroid hyperplasia, indicative of renal secondary hyperparathyroidism, also increased incidence in dosed male rats (1/43, 6/45, 6/43, 17/43).

The evaluation of single sections from the left and right kidneys revealed renal tubule adenomas in three of 50 male rats (6%) and adenocarcinoma in one other male rat (2%) receiving 1900 mg/kg bw per day of quercetin; none were seen in the controls or in the two lowest quercetin doses. Examination of additional step sections of the male rat kidney identified additional hyperplasia and adenomas in controls and all dose groups (hyperplasia:2/50 (4%), 2/50 (4%), 6/50 (12%), 8/50 (16%), respectively; adenoma: 1/50 (2%), 2/50 (4%), 7/50 (14%), 6/50 (12%), respectively. The overall incidence of renal tubule adenoma or adenocarcinoma detected in single or step sections combined in male rats with increasing doses (1/50 (2%), 2/50 (4%), 7/50 (14%), 9/50 (18%)) showed a statistically significant trend in the tumor incidences with Cochran-Armitage test ($P = 0.005$) and for pairwise comparisons with control only for the two highest doses ($P = 0.030$ and $P = 0.008$, respectively) with Fischer exact test. For untreated male F344/N historical control rats, there was an incidence for both renal tubule adenomas, and renal tubule adenomas or adenocarcinomas, of 4/499 (0.8%). There was no apparent effect of quercetin on the kidney of female rats. A single renal tubule adenoma was seen in one female of 50 (2%) receiving 400 mg/kg per day; this neoplasm was not considered biologically significant.

There was a statistically significant, dose-related decrease in the incidence of mammary gland fibroadenomas, including multiple fibroadenomas, in exposed female rats (29/50 (58%), 27/50 (54%), 16/50 (32%), 9/50 (18%), respectively) (Cochran-Armitage test for trend; $P < 0.001$, Fischer exact test for comparisons with control; $P = 0.008$ and $P = < 0.001$, for the two highest quercetin doses, respectively), which may in part be attributed to lower bw gains. There was a treatment-related accumulation of yellow-brown granular pigment adsorbed to or absorbed by the epithelial cells of the glandular stomach, ileum, jejunum and, to a lesser extent, the duodenum and colon. The severity of the pigmentation in these tissues increased with increased length of

exposure. There were no other lesions considered to be related to quercetin administration.

In conclusion, under the conditions of these 2-year feeding studies there was some evidence of carcinogenic activity of quercetin in male F344/N rats based on an increased incidence of renal tubule cell adenomas. There was no evidence of carcinogenic activity of quercetin in female F344/N rats receiving 40, 400 and 1900 mg/kg bw per day of quercetin. The incidence of renal tubule hyperplasia and the severity of nephropathy were increased in exposed male rats.

From the study of quercetin in rats by NTP (1992), the renal histopathology was re-evaluated by Hard et al. (2007) confirming the exacerbation of the chronic progressive nephropathy, the induction of renal hyperplasia and the increase of renal tumors in the mid- and high-dose groups of male rats (400 and 1900 mg/kg bw, respectively). The nephropathy was already enhanced by the high quercetin dose in interim investigations of 6 and 15 months. The authors suggested that renal tumor development may be associated with or may be a consequence of the chronic progressive nephropathy occurring only in male rats, with probably no or only little relevance for extrapolation to humans. However, taking another cautious interpretation into consideration, quercetin may have the ability to exacerbate adverse effects in pre-damaged kidneys and, thus, patients with a kidney dysfunction may be a potential risk group for long-term quercetin supplementation at high doses. However, according to Andres et al. (2018), this could not be confirmed or refuted based on the limited human data.

Andres et al. (2018) also summarized other *in vivo* studies of oral administration quercetin in mice and rats, where quercetin caused no induction of DNA strand breaks, DNA damage, micronuclei formation or chromosomal aberrations in bone marrow cells. Quercetin also did not induce unscheduled DNA synthesis in hepatocytes or genotoxic related pathways in liver and the small intestine, as demonstrated by transcriptome analyses.

A metabolic pathway for activation of quercetin to DNA-reactive species may include enzymatic and/or chemical oxidation of quercetin to quercetin-ortho-quinone, followed by isomerisation of the ortho-quinone to quinone methides (Rietjens et al., 2005). The quinone methides are suggested to be the active alkylating DNA-reactive intermediates. The formation of quercetin DNA adducts is demonstrated in exposed cells *in vitro*. However, these genotoxic characteristics of quercetin are not reflected by carcinogenicity, which may be related to i.a. the transient nature of the quercetin quinone methide adducts.

Further, covalent binding of quercetin to glutathione, protein and DNA, as well as the stability of quercetin DNA adducts in time was studied by van der Woude et al. (2005). Quercetin DNA adducts were of transient nature, independent of the presence of nucleotide excision repair (NER), suggesting chemical instability of the adducts. Together, the data indicated that formation of covalent quercetin adducts can be expected in all cells, independent of their oxidative enzyme levels, but the chemical instability provides a possible explanation for the apparent lack of *in vivo* carcinogenicity of this *in vitro* mutagen.

The discrepancy between *in vitro* mutagenicity and genotoxicity, and the lack of genotoxic or carcinogenic effects *in vivo,* have also been thoroughly discussed by

Harwood et al. (2007). Alternative possible explanations may be the transient nature of the quercetin-DNA adducts, binding of quercetin to serum proteins preventing distribution into cells, specifically the binding of the reactive quercetin ortho-quinone/quinone methide metabolites to albumin and/or to glutathione. In addition, the microbial degradation of quercetin in the intestine and the extensive metabolism (*O*-methylation, glucuronidation or sulfation) limit the *in vivo* bioavailability of the quercetin aglycone and ensure rapid excretion.

Other potential mechanisms suggested for the lack of carcinogenic effects *in vivo* are induction of cell death or cell cycle arrest, inhibition of topoisomerases and tyrosine kinases, down-regulation of oncogenes and up-regulation of tumor suppressor genes leading to the elimination of cancer cells (Andres et al., 2018).

Due to the potential tumor promoting effects of quercetin primarily in estrogen-dependent cancer found in animal (rat) and *in vitro* studies, the supplementation of quercetin at high doses was considered potentially harmful for patients with a currently diagnosed estrogen-dependent cancer (Andres et al., 2018).

The International Agency for Research on Cancer (IARC) examined the potential carcinogenic risk of quercetin to humans and, based on data available at that time, came to the overall conclusion that "quercetin is not classifiable as to its carcinogenicity to humans" (Group 3) (IARC, 1999).

Genotoxic effects of rutin were evaluated in the bone marrow of outbred Swiss–Webster mice of both sexes by Comet assay and the micronucleus test by da Silva et al. (2002). The micronucleus test showed that rutin caused no damage to the DNA of the mice bone marrow cells and the Comet assay demonstrated an increase of damage only with two injections of 1250 mg/kg bw given intraperitoneally 24 hours apart. No information on genotoxicity of rutin was found on PubChem.

4.2.2 Genotoxicity and general toxicity of EMIQ and IQ

4.2.2.1 *In vitro* and *in vivo* genotoxicity of EMIQ and IQ

The genotoxic potential of enzymatically modified isoquercitrin (EMIQ) and isoquercitrin (IQ) has been evaluated by EFSA (European Food Safety Authority), OECD (Organisation for Economic Co-operation and Development) and US Food and Drug Administration (FDA) guidance on genotoxicity and toxicity testing (referred by (Owczarek-Januszkiewicz et al., 2022).

In vitro, both substances (in doses up to 5000 µg/plate) tested positive in *Salmonella typhimurium* strains (TA98, TA100, TA1537) reverse mutation assays and the exposure to IQ induced moderate chromosomal aberrations in Chinese hamster ovary cells. However, all other *in vitro* micronuclei and chromosomal aberration assays in mammalian cells gave negative results.

In vivo, no genotoxicity was demonstrated in a micronuclei/Comet assay in rats and a MutaTM mouse mutation assay, thus, supporting the conclusion that EMIQ and IQ are not considered genotoxic *in vivo* (Hobbs et al., 2018; Owczarek-Januszkiewicz et al., 2022).

4.2.2.2 Subchronic and chronic toxicity and carcinogenicity of EMIQ

Two animal toxicity studies on EMIQ were performed by US FDA in 2007 and described in Madden et al. (2022). In a 13-week subchronic toxicity study, F344/DuCrj rats administered EMIQ in the diet at concentrations of 0.3% (188 mg/kg bw in males, 190 mg/kg bw in females), 0.625% (392 mg/kg bw in males, 396 mg/kg bw in females), 1.25% (784 mg/kg bw in males, 792 mg/kg bw in females) or 2.5% (1568 mg/kg bw in males and 1584 mg/kg bw in females) had bone discoloration at 0.625% EMIQ or higher, yellow or yellow-brown discoloration of urine for all dose levels and decreased bw gains in the 2.5% dose group. Because there were no related histopathological effects in the bone, the oral no observed adverse effect level (NOAEL) was established at 1.25% EMIQ in the diet, equivalent to an intake level of approximately 800 mg/kg bw per day of EMIQ in rats. The human equivalent dose was 9081 mg per day of EMIQ for a 70 kg individual based on body surface area comparison.

In a 104-weeks chronic animal toxicity study, no treatment-related toxicity or neoplasms were observed in F344/DuCrj rats administered 0.5% or 1.5% of EMIQ in the diet. The dietary NOAEL was established at 1.5% of EMIQ, which is equivalent to 489 mg/kg bw per day for males and 598 mg/kg bw per day for females.

4.3 Reproductive toxicity

It has been shown that several flavonoids can cross the placenta to accumulate in human fetal tissue (Todaka et al., 2005), and other experiments indicate that this is also the case for quercetin since effects on the fetus have been observed in 129/SvJ:C57BL/6J mice after maternal exposure (Vanhees et al., 2011).

Rutin is a phytoestrogen, which can bind to the estrogen receptor and exert estrogen-like effects because of structurally similarity to endogenous estrogen (Chua, 2013).

4.3.1 Reproductive toxicity and embryotoxicity

The effects of quercetin exposure on fetal development and fetal erythropoiesis were studied in female 129/SvJ:C57BL/6J mice exposed daily to quercetin (302 mg/kg feed, about 60 mg/kg bw) from 3 days before conception until day 14.5 of gestation, when the dams were sacrificed and the fetuses were isolated (Vanhees et al., 2011). No differences in litter size, fetal weight or placental weight could be observed between control and quercetin-exposed mice.

Birth outcomes and ovarian morphology were assessed in 4-week-old offspring of C57BL/6 mice receiving quercetin (calculated to be 5 mg/kg bw per day) via drinking water for 9 months during two breeding periods: from 2 to 6 months (prime reproductive age) and 8 to 11 months of age (Beazley & Nurminskaya, 2016). Quercetin did not affect maternal body weight, male fertility, birth weight or the growth of offspring. However, quercetin increased birth spacing, leading to a 60% reduction in the number of litters, but enhanced folliculogenesis in ovaries of female offspring. The litter size was increased in young females and decreased in older females.

F344 rats of both sexes were exposed to a basal diet or a basal diet with 0.1% or 0.2% of purified quercetin *ad libitum* for 64 weeks (about 16 months) (Stoewsand et

al., 1984). There were no significant weight changes due to quercetin intake. Quercetin did not have any deleterious effects on reproductive performance based on parturition index, mean viable litter size, live birth index, 3-day survival, lactation index, birth weight or day-21 weight.

Female Wistar rats were exposed to quercetin (10 mg/kg per day) orally once breeding had been confirmed until parturition (Johnson et al., 2009). Dams given quercetin during pregnancy had significantly increased weight gain during pregnancy, but there were no effects on gestation length, pregnancy success rate, live birth index, litter size, birth weight, total litter weight, sex ratio, survival to postnatal day 4 or survival to weaning.

EMIQ was evaluated for embryo/fetal survival, developmental toxicity and maternal side-effects in female New Zealand White rabbits by Maronpot et al. (2020). No maternal toxicity was observed after oral gavage of EMIQ in doses up to 1000 mg/kg bw per day during gestation days 6–28. Reproductive parameters, such as gravid uterine weight, number of implantations, implantation loss, live young, the ratio of males-to-females, placental, litter and fetal weights, and litter size, were not affected. In fetal development, sporadic cases of kidney and ureter absence were recorded, however, they were considered unrelated to the treatment due to the lack of reproducibility. Based on the study results, the NOAEL for maternal toxicity and embryo/fetal development for EMIQ was specified as 1000 mg/kg bw per day (Maronpot et al., 2020).

4.3.2 Teratogenicity

Quercetin administered daily to female C57BL/6 mice (333 mg/kg feed, corresponding to approximately 67 mg/kg bw) for about two weeks during gestation resulted in numerically higher incidence of fetal forelimb syndactyly and hindlimb interdigital webbing, an effect not observed with 66 mg/kg feed (about 13 mg/kg bw) (Prater et al., 2008). The cause of these effects on the limbs may be due to dose-related effects on apoptosis required for digital sculpting or prooxidant effects of quercetin that caused a maturational delay.

A single oral dose of 0, 2, 20, 200 or 2000 mg/kg bw of quercetin was administered to pregnant Sprague-Dawley CD rats on day 9 of gestation (Willhite, 1982). Other groups of pregnant rats received similar oral doses of quercetin daily on days 6-15 of gestation. No toxic effects were observed in the dams. The mean number of living fetuses per litter and the resorption rate of fetuses were not different from the controls. The two highest dose groups (200 or 2000 mg/kg bw) exposed on day 9 of gestation showed a significant decrease in the average weight of day-20 foetuses compared with the weight in the control group. The mean bw of fetuses from dams exposed on days 6-15 of gestation with 2 and 20 mg/kg bw was significantly lower than in the controls. However, studies of the foetuses recovered on day 20 of gestation failed to reveal any reproducible dose-related teratogenic effects attributable to quercetin treatment.

In a review publication on effects of i.a. flavonols by Barenys et al. (2017), a relevant exposure threshold for developmental effects of quercetin of 50 mg/kg bw per day during the whole gestation for *in vivo* rodent studies and of 5 µM for *in vitro* studies, were estimated.

One population-based Hungarian case-control study evaluated the teratogenic potential of oral HER treatment (Pósfai et al., 2014). A standardized mixture of the semisynthetic flavonoids, 7-mono-*O*-(β-hydroxyethyl)-rutoside and structurally related di-, tri- and tetra-hydroxyethylrutosides (HER) (Venoruton®) was given in oral doses of 3 x 300 mg per day or 2 x 500 mg per day. The doses of HER treatment were daily 900–1000 mg in most pregnant women, while the mean duration of treatment was 4.2 and 4.8 months in the mothers of cases and control, respectively. Comparative analysis of HER exposure during pregnancy in the mothers of cases with congenital abnormalities and matched control newborns without any defects was performed. Of the 22843 cases with all types of congenital abnormalities, 567 (2.5%) had mothers with HER treatment, while of 38151 matched controls without congenital abnormalities, 1143 (3.0%) were born to mothers with HER treatment (OR with 95% CI: 0.8, 0.7–0.9), thus, for congenital defects in general, the difference with or without HER treatment was not statistically significant. However, an association of HER treatment during the second and/or third month of pregnancy was found with the higher risk of unilateral ocular coloboma (OR with 95% CI: 5.4, 2.2–12.9) and a new congenital abnormality syndrome including anotia/microtia, poly-/syndactyly and caudal (genital and anal) defects (OR with 95% CI: 3.0, 1.3–27.4). The authors recommended that the use of oral HER should be avoided in the second and third month of pregnancy.

4.4 Allergenicity and irritation

EMIQ exhibited no skin irritant properties when applied topically to the ears of female CBA/J mice in concentrations of 10%, 25% and 50% for three days. The compound did not induce lymphadenopathy, ear swelling, erythema, irritation and other skin lesions. The skin sensitization potential of EMIQ was assessed by the local lymph node assay *ex vivo*, and according to the assay protocol, EMIQ was classified as a non-sensitizer at all tested concentrations (Vij et al., 2022).

4.5 Adverse effects from literature searches

4.5.1 Adverse health effect identifications

4.5.1.1 Introduction to the literature searches

A two-step literature search for review studies (step one) and primary research (step two) was conducted by VKM to identify previous research on the safety and potential adverse health effects of quercetin dihydrate and rutin in humans and animals (in reviews). A research librarian was involved in the planning of and was conducting the searches. Literature searches were conducted separately for quercetin and rutin.

A broad search for review studies was conducted to identify human studies and/or animal toxicity studies on adverse health outcomes related to quercetin or rutin. However, because most reviews did not present much detail on the safety assessment in the human studies, a second search for randomized controlled trials (RCTs) in humans was performed and will be described in more detail in the following. The search on review studies will not be described further. The search terms and strategies are given in Chapter 10 Appendix I.

As no specific adverse health outcomes were identified related to quercetin or rutin exposure in previous reviews or animal studies, a broad approach was chosen for the literature search for RCTs in order to obtain all types of adverse effects.

The publication selection was performed by pairs of reviewers. The screening of title and abstract was done in Rayyan (Ouzzani et al., 2016). To ensure between-reviewer calibration, all reviewers first screened a sample of the retrieved titles and abstracts, and the results were discussed in the working group to ensure consistent application of the inclusion criteria. Publications that passed the abstract screening were evaluated in full text. A similar calibration process was performed before pairs of reviewers independently evaluated the full-text publications based on the eligibility criteria. To ensure that the eligible publications retrieved from the literature searches were of sufficient quality, a risk of bias (RoB) analysis was performed.

4.5.1.2 Search strategy for RCTs

To identify available RCTs, the five databases Ovid MEDLINE, Ovid Embase, Web of Science, Epistemonikos and Cochrane Central Register of Controlled Trials (CENTRAL) were searched from inception to September 14, 2023, for RCTs of oral intake of quercetin or rutin. There were no restrictions on outcomes. Searches were conducted separately for quercetin and rutin but were later handled together. The search terms and strategies are given in Chapter 10 Appendix I.

4.5.1.3 Eligibility criteria for RCTs

A search for primary studies was conducted to identify RCTs that addressed safety of oral intake of quercetin or rutin. The inclusion criteria, including the PICO elements (Population, Intervention, Comparator, Outcome), are listed in Table 4.5.1.3-1.

Table 4.5.1.3-1 Overview of inclusion criteria for human RCTs.

Criterion	Specification human studies
Study design	Randomized controlled trials (RCTs).
Population	Adults for both quercetin and rutin, children ≥4 years only for rutin, both sexes in all age groups.
Intervention	Oral intake of quercetin or rutin in all dose ranges. Duration of intake in prioritized order (from highest to lowest): • Chronic or repeated • Subchronic • Acute.
Comparator	Placebo (or cross-over design).
Outcome	All outcomes (self-reported, clinical examination or laboratory tests/biomarkers) described in relation to safety/ tolerance/ side-effects or potential adverse or negative health effects.
Language	No restrictions. Machine translations was performed if safety was mentioned in English title or abstract.
Date	Inception to search date.
Type of literature	Primary studies in peer-reviewed journals.

The following exclusion criteria were applied:

- Outcomes not related to safety or adverse effects, such as beneficial effects.
- Oral exposure from sources other than dietary supplements (e.g. mouth wash or oral gels).
- Human trials without randomization or a control group.
- Results for quercetin or rutin could not be separated from other bioactive components, e.g. onion extracts, tea or buckwheat cookies that contained other bioactive compounds from the food (e.g. vitamins or minerals).
- Studies without abstract in English or Scandinavian.

4.5.1.4 Study selection of RCTs

From the literature searches for quercetin and rutin, 2526 records were obtained, which were screened at the level of title/abstract. From these, 140 RCTs were obtained in full-text and screened against the eligibility criteria. Among these, 45 RCTs were found to meet the inclusion criteria.

The included publications reported RCTs with a control group, either using placebo as comparator to the intervention or with a cross-over design in which the participants were evaluated before and after the intervention.

The terms of reference requested to identify and characterise adverse health effects in general. No specific adverse health outcomes related to quercetin or rutin could be identified *a priori* to our literature search.

Publications that in any way mentioned 'safety' or 'adverse events/adverse effects/side-effects' or had analysed blood or urine or any biological measure with expressed intention to evaluate safety were included.

Publications that did not mention 'safety', 'adverse events/adverse effects/side-effects', or did not express that blood or urine or any biological measure was analysed with intention to evaluate safety, were excluded. Excluded publications are listed in Chapter 11 Appendix II, Table 11-1 with reasons for exclusion.

When more than one publication appeared to report results from the same study (identified as the same study based on the author names and other information about the RCTs), only the publication giving the most information on adverse effects was included.

Three human RCT studies in Japanese, that had safety assessment as objective according to the English title or abstract, were included. Figure 4.5.1.4-1 gives an overview of the study selection. The selection of the RCTs is reported using PRISMA flow diagrams (Haddaway et al., 2022).

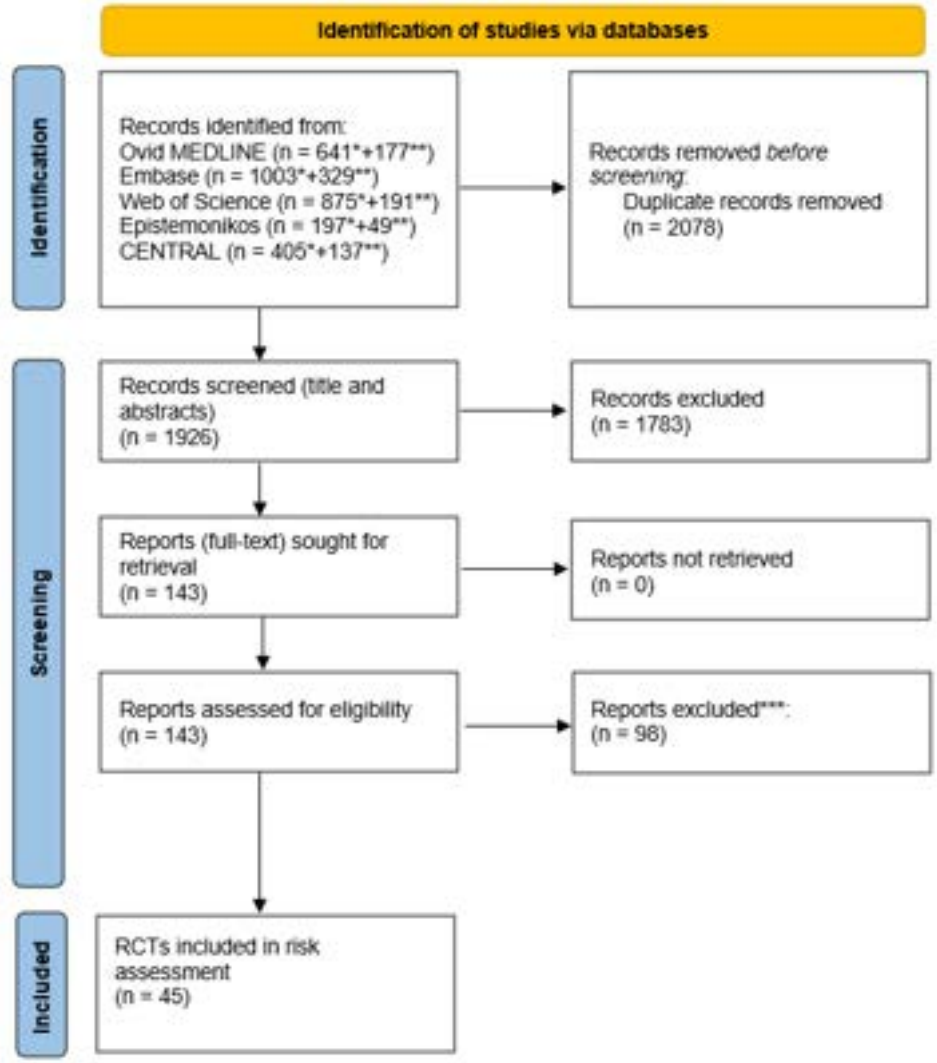


Figure 4.5.1.4-1 Flow diagram for the selection of randomized controlled trials (RCTs).

4.5.1.5 Categorization of adverse health effects

Publications addressing adverse health effects were categorized according to the reliability of how these effects were obtained or measured:

Category 1: Fifteen publications with data based on objectively measured outcomes, such as results of analyses of blood or urine, were defined as Category 1. Among these publications, thirteen reported both subjectively measured (Category 2) and objectively measured (Category 1) adverse effects and were included in Category 1 as 1(2) in Table 4.5.1.5-1.

Category 2: Eight publications with only data on adverse health effects obtained or registered by subjective methods, such as self-reported adverse effects/adverse events/side-effects by participants, were defined as Category 2. Additionally, 14 publications in Category 1 included also subjectively reported adverse effects/events, i.e. altogether 23 publications reported some data on adverse effects/events.

Category 3: Twenty-two publications that only mentioned briefly "no adverse effects/events/side-effects reported", without any information on how such data were recorded or any details on the results, were defined as Category 3. They have not been evaluated for risk of bias using OHAT and are not included in the main results in this chapter, but are mentioned in Chapter 5.4.1 Supporting evidence and listed in Chapter 11 Appendix II, Table 11-2).

Publications in Category1 and Category 2 were further evaluated for internal validity (risk of bias (RoB) analysis) using OHAT.

Table 4.5.1.5-1 Overview of publications that report Category 1 and 2 results.

Category 1 and 2	Only category 2
Annoni et al., 1986 Boyle et al., 2000* Capelli et al., 1987 Egert et al., 2009 Erlund et al., 2000 Han et al., 2020 Kienzler et al., 2002 Nakamura et al., 2022 Pfeuffer et al., 2013 Riva et al., 2019 Shatylo et al., 2021 Yamada et al., 2022 Yasutake et al., 2015 Yoshimura et al., 2008 Yoshimura et al., 2012	Bazyar et al., 2023 Bergqvist et al., 1981 Di Pierro et al., 2021 Ganio et al., 2010 Egert et al., 2008 Hirano et al., 2009 Shi & Willamson, 2016 Shoskes et al., 1999

*Only Category 1 results.

4.5.1.6 Evaluation of internal validity of RCTs

Risk of bias was evaluated using the OHAT (Office of Health Assessment and Translation) tool (OHAT, 2015; 2019) for adverse health effects in publications in Category 1 and 2. This evaluation tool offers a method to evaluate risk of bias in human and animal studies. Eight questions (Q1, Q2, Q6-Q11) addressing selection bias, performance bias, selective reporting bias, attrition/exclusion bias, detection bias and other sources of bias were used to evaluate the risk of bias in the RCTs. The questions addressing performance bias (Q6: blinding of personnel and participants) and detection bias (Q8: confidence in the exposure characterisation and Q9; confidence in the outcome assessment) were defined as key questions. The other questions were defined as non-key questions (Q1, Q2, Q7, Q10 and Q11).

The rating of key and non-key questions was integrated to classify the RCTs into tiers to characterise the overall risk of bias for the Categories 1 and 2 of adverse health effects reported in the studies, as shown in Tables 4.5.1.6-1. Tiers 1, 2 and 3 represent low, moderate and high risk of bias, respectively.

Table 4.5.1.6-1. Classification of studies into tiers according to overall risk of bias for adverse effect per study.

Tier	1 (low RoB)	2 (moderate RoB)	3 (high RoB)
Criteria for classification	All key questions are scored + or ++ AND No more than two non-key question are scored - or --	All combinations not falling under Tier 1 or Tier 3	All key questions are - or -- AND/OR Three of more non-key questions are scored - or --

Due to missing information, risk of bias for the reporting of adverse effects could not be evaluated in publications in Category 3. The risk of bias for adverse effects in these publications is therefore generally considered as potentially high. The RoB rating and tier classification for each study in Categories 1 and 2 are presented in Tables 4.5.1.6-2 and 4.5.1.6-3, respectively.

Table 4.5.1.6-2. Risk of bias rating and classification into tiers for adverse effects in Category 1.

Authors, year	Q6*	Q8*	Q9*	Q1	Q2	Q7	Q10	Q11	Tier
Annoni et al., 1986	++	--	+	+	NR	NR	+	+	2
Boyle et al., 2000	+	+	+	+	NR	+	+	+	1
Cappelli et al., 1987	++	--	+	++	++	++	+	+	2
Egert et al., 2009	++	+	+	++	NR	++	-	+	1
Erlund et al., 2000	++	+	+	+	NR	++	+	+	1
Han et al., 2020	++	+	+	++	+	+	+	-	1
Kienzler et al., 2002	--	+	++	+	NR	+	+	-	2
Nakamura et al., 2022	++	+	+	++	++	++	+	-	1
Pfeuffer et al., 2013	++	+	++	+	NR	++	++	+	1
Riva et al., 2019	+	++	+	+	NR	+	+	-	1
Shatylo et al., 2021	++	--	+	+	NR	++	+	+	2
Yamada et al., 2022	++	-	++	++	++	++	+	-	2
Yasutake et al., 2015	++	+	++	++	++	++	++	-	1
Yoshimura et al., 2008	++	+	++	+	NR	++	++	-	1
Yoshimura et al., 2012	++	+	++	+	NR	+	++	-	1

*Key questions. Definitely low risk of bias (++); probably low risk of bias (+); probably high risk of bias (-); definitely high risk of bias (--); not reported (NR) is considered as 'probably high risk of bias'.
Q1: Was administered dose or exposure level adequately randomized?
Q2: Was allocation to study groups adequately concealed?
Q6: Were the research personnel and human subjects blinded to the study group during the study?
Q7: Were outcome data complete without attrition or exclusion from analysis?
Q8: Can we be confident in the exposure characterisation?
Q9: Can we be confident in the outcome assessment?
Q10: Were all measured outcomes reported?
Q11: Were there no other potential threats to internal validity?

Among the publications in Category 1, ten were evaluated as Tier 1 and five as Tier 2.

Table 4.5.1.6-3. Risk of bias rating and classification into tiers for adverse effects in Category 2.

Authors, year	Q6*	Q8*	Q9*	Q1	Q2	Q7	Q10	Q11	Tier
Bazyar et al., 2023	++	+	+	++	++	++	+	+	1
Bergqvist et al., 1981	++	+	+	+	++	++	++	+	1
Di Pierro et al., 2021	--	+	+	+	NR	NR	NR	-	3
Egert et al., 2008	++	+	+	+	NR	++	+	+	1
Ganio et al., 2010	++	+	+	+	++	+	+	+	1
Hirano et al., 2009	++	+	+	+	NR	++	+	-	1
Shi & Williamson., 2016	++	+	+	++	++	+	+	+	1
Shoskes et al., 1999	++	--	+	+	NR	+	-	-	3

*Key questions. Definitely low risk of bias (++); probably low risk of bias (+); probably high risk of bias (-); definitely high risk of bias (--); not reported (NR) is considered as 'probably high risk of bias'.
Q1: Was administered dose or exposure level adequately randomized?
Q2: Was allocation to study groups adequately concealed?
Q6: Were the research personnel and human subjects blinded to the study group during the study?
Q7: Were outcome data complete without attrition or exclusion from analysis?
Q8: Can we be confident in the exposure characterisation?
Q9: Can we be confident in the outcome assessment?
Q10: Were all measured outcomes reported?
Q11: Were there no other potential threats to internal validity?

Among the publications in Category 2, six were evaluated as Tier 1 and two as Tier 3.

All forms for the OHAT-evaluations are presented in detail in Chapter 13 Appendix IV.

4.5.1.7 Data extraction from RCTs

To evaluate adverse effects, VKM extracted data from the 23 RCTs. The data extracted included the study ID (first author, publication year), study design, objective of the study, substance, the dose, duration of the experiment, number of participants, population/patient groups, the adverse outcome measures/ side-effects measured, and the results. Also, results in which the observed effects were reported as adverse among endpoints studied with beneficial effects as the objective were extracted.

4.5.2 Adverse health effect characterisation

4.5.2.1 Characteristics of the human RCTs

All 23 studies were performed in adults, age ranging from 18-80 years. None of the included studies reported exposure of the substances under evaluation (quercetin dihydrate, quercetin aglycone, Quercetin Phytosome®, rutin, EMIQ or HER) to children or adolescents, to pregnant and or breast-feeding women (Table 4.5.2.1-1). One publication in which quercetin was administered together with rutin and luteolin to children was obtained in the literature search but was excluded because of the mixed exposure. However, this study will be presented as supporting evidence (Chapter 5.4), since this is the only relevant study reporting intake of quercetin and rutin in children. Most studies included both men and women, only five publications reported results on

only one sex. Thus, in total the participants in the included publications can be regarded as representative of the general population, except for the lack of data for the young age groups, pregnant and breast-feeding women.

A few publications studied persons with overweight and obesity. Several studies included healthy volunteers, but a large number of these studies comprised participants with diagnoses of varying severity, such as type 2 diabetes mellitus, metabolic syndrome, chronic obstructive pulmonary disease (COPD), chronic venous insufficiency, prostate related pelvic pain, haemorrhoids, COVID infection or allergic symptoms of pollinosis (Table 4.5.2.1-1). There is no available data suggesting that these patient groups have ADME different from healthy persons.

The number of participants was quite low in approximately two-thirds of the RCTs, varying from 2-35 participants per treatment or dose group, whereas about one third of the studies included 49-100 participants per treatment or dose group (Table 4.5.2.1-1).

The doses of the substances under evaluation used in the RCTs varied from 8 mg per day to 4000 mg (4 g) per day. The quercetin intake from dietary supplements in the included studies (Table 4.5.2.1-1) was in general considerably higher than reported average background dietary intake levels. Several studied also exceeded the estimated intake levels from high consumers of fruit, berries and other food sources of quercetin (see Chapter 3.2).

Three RCTs only administered a single dose of the substances under evaluation (to obtain pharmacokinetic data), three studies used 1-2 weeks of exposure, eight studies had a duration of 3-4 weeks/1 month, 7 studies had a duration of 6-12 weeks (1.5-3 months), one study lasted 24 weeks (~3.5 months) and the study with the longest duration lasted 40 weeks (~6 months) (Table 4.5.2.1-1).

In the publications of RCTs with an objective to evaluate safety of the substances under evaluation, adverse 'effects' (sometimes called events, sometimes effects) were recorded, and included complaints on headache, vomiting, diarrhea etc. In the publications of RCTs with an objective to evaluate beneficial effects of the substances under evaluation, sometimes also adverse effects were observed and reported, as well as measured parameters going in an adverse direction, among a long list of varying endpoints, depending on the purpose of the study, the patient group involved etc. The details on how adverse effects were recorded and which beneficial effects were looked for, is given in Table 12.-1 in Chapter 12.1 Appendix III. Notably, detection of adverse effects was not the main objective in most of the studies.

Table 4.5.2.1-1 Study characteristics in RCTs included (n = 23) from the systematic literature review sorted by duration, and then in alphabetic order.

Authors, year	Study design	Primary and secondary aim of the study	Substance	Oral dose, mg per day	Study duration	Study (n) in treatment(s) and control groups	Participants' characteristics
Nakamura et al., 2022	Randomized, double-blind, placebo controlled, parallel	Investigate beneficial effects on cognitive function and cerebral blood flow (CBF). Several blood and urine analyses relevant for safety were included	EMIQ[1]	110	40 weeks	80 (40M+F/group), 33 (10M, 23F), 35 (13M, 22F)	Healthy volunteers, 60-75 years
Yoshimura et al., 2012	Randomized, double-blind, placebo controlled, parallel	Investigate beneficial effects from long-term use on body fat reduction. Several blood and urine analyses relevant for safety were included	EMIQ[1]	110	24 weeks	57 (28M, 29F), 59 (30M, 29F)	Overweight and obese subjects with BMI ≥ 25 - < 30 kg/m^2, 20-66 years
Bazyar et al., 2023	Randomized, double-blind, placebo controlled, parallel	Investigate beneficial effects on blood pressure markers, some serum antioxidant enzymes and quality of life in patients with type 2 diabetes mellitus	Rutin	500	12 weeks	25 (12M, 13F), 25 (11M, 14F)	Patients with type 2 diabetes mellitus, 18-60 years
Shatylo et al., 2021	Randomized, double-blind, placebo controlled, parallel	Investigate beneficial effects on cardio-metabolic endpoints in metabolic syndrome	Q	240	12 weeks	55 (16M, 39F), 55 (10M, 45F)	Patients with metabolic syndrome, 65-69 years
Yoshimura et al., 2008, study 2	Randomized, double-blind, placebo controlled, parallel	Investigate beneficial effect from long-term use on body fat reduction. Several blood and urine analyses relevant for safety were included	EMIQ[1]	275	12 weeks	100 (51M, 49F), 100 (51M, 49F)	Overweight and obese subjects with BMI 24-31 kg/m^2, 20-65 years

Authors, year	Study design	Primary and secondary aim of the study	Substance	Oral dose, mg per day	Study duration	Study (n) in treatment(s) and control groups	Participants' characteristics
Hirano et al., 2009	Randomized, double-blind, placebo controlled, parallel	Investigate beneficial effect on symptoms in patients with Japanese cedar pollinosis	EMIQ[2]	100	8 weeks	12 (9M, 3F), 12 (10M, 2F)	Subjects with Japanese cedar pollinosis, 37.6 ± 9.0 years
Pfeuffer et al., 2013	Randomized, double-blind, placebo controlled, cross-over	Investigate beneficial effects on risk factors of atherosclerosis, biomarkers of inflammation and oxidative stress, depending on the apolipoprotein E (APOE) genotype	QD	150	8 weeks	49M (19APOE3/3, 30APOE4)	Healthy subjects with varying apolipoprotein E (APOE) genotypes, 48-68 years
Boyle et al., 2000	Randomized, single-blind, placebo controlled, parallel	Investigate potential antioxidant effects, and pharmacokinetic parameters. Some blood analyses relevant for safety were included	Rutin	500	6 weeks	16F (8F, 8F)	Healthy non-obese normo-cholesterolaemic volunteers, 18-48 years
Egert et al., 2009	Randomized, double-blind, placebo controlled, cross-over	Investigate beneficial effects on cardiovascular risk factors and biomarkers such as blood pressure, body composition, oxidative stress, inflammation and blood lipids in overweight and obese subjects with a high-CVD risk phenotype. Several blood analyses relevant for safety were included	QD	150	6 weeks	93 (42M, 51F)	Overweight subjects with a high-cardiovascular disease risk phenotype (metabolic syndrome), 26-65 years
Annoni et al., 1986	Randomized, double-blind, placebo controlled, parallel	Investigate beneficial effects on acute symptoms of haemorrhoids. Several blood and urine analyses relevant for safety were included	HER	4000	4 weeks	20 (12M, 8F), 20 (11M, 9F)	Patients with haemorrhoids, 42.6±12.67 years

Authors, year	Study design	Primary and secondary aim of the study	Substance	Oral dose, mg per day	Study duration	Study (n) in treatment(s) and control groups	Participants' characteristics
Bergqvist et al., 1981	Randomized, double-blind, placebo controlled, parallel	Investigate beneficial effects in plethysmographic parameters and subjective symptoms in patients with chronic venous insufficiency	HER	1500+1000 i.v.	4 weeks	71 (15M, 56F), 72 (12M, 60F)	Patients with chronic venous insufficiency, intervention group 52.4 ± 11.9 years and placebo group 54.7 ± 14.2 years
Cappelli et al., 1987	Randomized, double-blind, placebo controlled, parallel	Investigate if HER might counteract the unwanted effect of oral contraceptives on venous plethysmographic parameters. Some blood and urine analyses relevant for safety were included	HER	3000	4 weeks	20F (10, 10)	Women taking oral contraceptives and suffering from venous insufficiency of the lower limbs, 19-42 years
Di Pierro et al., 2021	Prospective, randomized, controlled, open-label, parallel, no placebo	Investigate beneficial effects on early symptoms and prevention of severe outcomes of COVID-19	Q	400	4 weeks	76 (42M, 34F), 76 (46M, 30F)	COVID-19 outpatients, 18-80 years
Shi & Williamson, 2016	Randomized, double-blind, placebo controlled, cross-over	To test the hypothesis that quercetin supplementation might result in a reduction in plasma uric acid levels in male subjects with non-optimal plasma uric acid levels	QD	544	4 weeks	22M (9, 13)	Subjects with non-optimal, but still healthy, blood uric acid levels, 19-65 years
Shoshkes et al., 1999	Preliminary prospective, randomized, double-blind, placebo controlled, parallel	Investigate beneficial effects on symptoms of category III chronic prostatitis (pelvic pain syndrome)	Q	1000	4 weeks	28M (15, 13)	Men with prostate-related pelvic pain, 26-72 years

Authors, year	Study design	Primary and secondary aim of the study	Substance	Oral dose, mg per day	Study duration	Study (n) in treatment(s) and control groups	Participants' characteristics
Yamada et al., 2022	Randomized, double-blind, placebo controlled, parallel	Investigate beneficial effects on allergen-induced reactions and subjective nose/eye symptoms of pollinosis. Several blood and urine analyses relevant for safety were included	QP	200	4 weeks	32 (13M, 19F), 32 (17M, 15F)	Subjects with allergic symptoms of pollinosis, 22-78 years
Yasutake et al., 2015	Randomized, double-blind, placebo controlled, parallel	Evaluate safety of excessive intake of tea containing quercetin glucosides in healthy adults including obese subjects. Several blood and urine analyses relevant for safety were included	EMIQ[1]	330	4 weeks	24 (12M, 12F), 24 (12M, 12F)	Healthy subjects with BMI ≥ 18.5 and < 30.0 kg/m^2, 20-64 years
Egert et al., 2008	Randomized, double-blind parallel design, wash out periods, no placebo	Investigate pharmacokinetics of various doses of QD and its potential effects on parameters of the oxidant/antioxidant status, inflammation and metabolism	QD	50, 100, 150	2 weeks	35 (18M, 17F)	Healthy volunteers, 26.2 ± 3.7 years
Ganio et al., 2010	Randomized, double-blind, cross-over, wash out periods, no placebo	Investigate beneficial effects on maximal oxygen uptake	Q	1000	1 week	11 (5M, 6F)	Sedentary and untrained volunteers, 18-34 years
Han et al., 2020	Randomized, double-blind, placebo controlled, parallel	Investigate safety in patients with COPD by measures of lung function, blood profile and COPD assessment	Q	50, 1000, 2000	1 week	6 (4M, 2F), 3 (1M, 2F), 2/dose	Patients with chronic obstructive pulmonary disease (COPD), 58-78 years
Erlund et al., 2000	Randomized, double-blind,	Compare absorption and pharmacokinetics of quercetin from quercetin aglycone or	Q (total) or rutin (total)	8, 20, 50 (78) or 16,	single dose	12 (7M, 5F)	Healthy volunteers, 18-33 years

Authors, year	Study design	Primary and secondary aim of the study	Substance	Oral dose, mg per day	Study duration	Study (n) in treatment(s) and control groups	Participants' characteristics
	cross-over, no placebo	rutin. Secondary, to investigate which forms of quercetin are present in plasma. Several blood and urine analyses relevant for safety were included		40, 100 (156)			
Kienzler et al., 2002	Randomized, open, cross-over, no placebo	Evaluate pharmacokinetic parameters at various doses. A secondary objective was to evaluate the general safety of the different dosages	HER	500, 1000, 2000, 4000	single dose	16 (M, F)	Healthy volunteers, 19-48 years
Riva et al., 2019	Randomized, single-blind, cross-over, no placebo	Compare a new food-grade lecithin-based formulation, Quercetin Phytosome®, to unformulated quercetin in terms of solubility in simulated gastrointestinal fluids and oral absorption. Some blood and urine analyses relevant for safety were included	Q or QP	500Q, 250QP, 500QP	single dose	12 (M, F)	Healthy volunteers, 18-50 years

[1]EMIQ as isoquercitrin. Isoquercitrin: n = 1 glucose.
[2]EMIQ, n = 1-8 glucose.
Q: quercetin.
QD: quercetin dihydrate.
QP: Quercetin Phytosome®.
HER: *O*-(β-hydroxyethyl)-rutoside (Venoruton®).
EMIQ: enzymatically modified quercetin glycoside(s) with varying numbers of glucose molecules.
i.v.: intravenous injection.

4.5.2.2 Results of the human RCTs

Of the 23 included publications (Table 4.5.2.1-1), only two publications reported safety parameters as the primary objective (Han et al., 2020; Yashutake et al. 2015). Eleven publications reported safety parameters as secondary outcomes or had included several analyses of blood and urine that were stated as included for evaluation of safety in the publication (Annoni al., 1986; Boyle et al., 2000; Cappelli et al., 1987; Egert et al., 2009; Erlund et al., 2000; Kienzler et al., 2002; Nakamura et al., 2022; Riva et al., 2019; Yamada et al., 2022; Yoshimura et al., 2008; 2012). Three publications reported outcomes in a potentially adverse direction among outcomes measured for study of beneficial effects (Pfeuffer et al., 2013; Shatylo et al., 2021; Egert et al., 2009). The remaining eight publications measured subjective, primarily self-reported adverse effects/events (Bazyar et al., 2023; Bergqvist et al., 1981; Di Pierro et al., 2021; Egert et al., 2008; Ganio et al., 2010; Hirano et al., 2009; Shi & Williamson, 2016; Shoskes et al., 1999).

Only a very low number of the included publications which reported that they had looked for adverse effects, either as safety related biological parameters in blood or urine, or as self-reported adverse events (side-effects) in addition to their primary outcomes measured, actually observed any adverse effects.

In the sections below, in text and tables, we present the results related to adverse effects reported in the included publications. For details on all included outcomes measured, see Chapter 12.1 Appendix III, Table 12-1. The results are presented separately for publications in Category 1 and Category 2. All publications in Category 1 include more objectively measured parameters such as blood or urine analyses that have been judged as potentially relevant for safety, whereas the publications in Category 2 report more subjectively measured, often self-reported, outcomes. However, there is some overlap between the publications in Category 1 and 2. For each category, we summarize the risk of bias with reference to the OHAT Tiers for each publication presented in Chapter 4.5.1.6.

Category 1 results

Twelve studies reported that parameters in blood or urine were analysed with the objective to investigate safety of the treatment (Table 4.5.2.2-1). No adverse effects were reported from these analyses in the following publications (Annoni al., 1986; Boyle et al., 2000; Cappelli et al., 1987; Erlund et al., 2000; Han et al., 2020; Kienzler et al., 2002; Nakamura et al., 2022; Riva et al., 2019; Yamada et al., 2022; Yashutake et al. 2015; Yoshimura et al., 2008; 2012). In several of these publications, the data from these analyses were not shown, but merely reported as no adverse findings in the text.

Three publications reported one single outcome each in a potentially adverse direction among parameters analysed in blood or urine; decreased level of glutathione (GSH), possibly indicating changes in oxidative stress (Shatylo et al., 2021), increased tumor necrosis factor-α (TNF-α), indicating a slight pro-inflammatory effect (Pfeuffer et al., 2013) and a small, but significant, decrease in high density lipoprotein (HDL) cholesterol (Egert et al., 2009) (Table 4.5.2.2-1). These three studies are described in more detail in the following.

In the study by Shatylo et al. (2021), 55 elderly patients with metabolic syndrome (16 men and 39 women) were treated with 240 mg quercetin per day for 3 months. The placebo group also had 55 participants (10 men and 45 women). Among all the parameters measured, the plasma GSH level was found to be significantly decreased in the quercetin-treated group. This effect may be interpreted as a decrease in oxidative stress levels, which in turn resulted in a decreased need for GSH synthesis, or alternatively, a lower level of GSH could be regarded as a negative effect, since GSH is important in protecting the body against oxidative stress of toxic chemicals.

In the cross-over study by Pfeuffer et al. (2013), 49 healthy men with varying apolipoprotein E (APOE) genotypes (19 with APOE3/3 and 30 with APOE4) were given 150 mg quercetin dihydrate per day for 8 weeks. It was found moderately increased levels of TNFα with quercetin in men with both APOE3/3 and APOE4 genotypes, indicating a potential pro-inflammatory effect. However, other inflammatory parameters (s-E-selectin, soluble adhesion molecules s-VCAM and s-ICAM, oxidized low-density lipoprotein (oxLDL), high-sensitivity C-reactive protein (hs-CRP)) and the urinary isoprostane 8-iso-prostaglandin $F_{2\alpha}$ were not affected by the quercetin dihydrate treatment in this study.

In a cross-over study by Egert et al. (2009), 93 overweight subjects (42 men and 51 women) with a high-cardiovascular disease risk phenotype (metabolic syndrome) were treated with 150 mg quercetin dihydrate per day for 6 weeks. Among all the parameters measured, a small, but significant, decrease in HDL-cholesterol during quercetin treatment was found, but it was not associated with an increase in the low density lipoprotein to heigh density lipoprotein-cholesterol or triacylglycerol to high density lipoprotein-cholesterol ratios. Thus, the small decrease in HDL-cholesterol concentration during the quercetin supplementation was considered to be of only limited, if any, physiological or clinical relevance.

In summary, in three studies assessing treatments with 240 mg quercetin per day for 3 months (Shatylo et al., 2021), 150 mg quercetin dihydrate per day for 8 weeks (Pfeuffer et al., 2013) and 150 mg quercetin dihydrate per day for 6 weeks (Egert et al., 2009), a single sporadic effect in the direction of adversity but of mild severity (decrease in HDL-cholesterol, increased levels of TNFα and decreased GSH level, respectively) was observed in each publication. The first two effects were not supported by other endpoints measured in the same study and the third effect could be interpreted as not adverse. In addition, these effects have not been reported in the other included studies, which indicate that they may be chance findings. Therefore, no serious hazards were identified among these Category 1 results.

The publications in Category 1 (Table 4.5.2.2-1) all had either a low or moderately risk of bias, i.e. 10 publications were evaluated as Tier 1 (low risk of bias) (Boyle et al., 2000; Egert et al., 2009; Erlund et al., 2000; Han et al., 2020; Nakamura et al., 2022; Pfeuffer et al., 2013; Riva et al., 2019; Yashutake et al. 2015; Yoshimura et al., 2008; 2012), and five publications were evaluated as Tier 2 (moderate risk of bias) (Annoni et al., 1986; Cappelli et al., 1987; Kienzler et al., 2002; Shatylo et al., 2021; Yamada et al., 2022).

The adverse outcomes measured and the results of adverse effects in all included publications in Category 1 are presented in Table 4.5.2.2-1.

Table 4.5.2.2-1 Results in publications in Category 1 with objectively measured outcomes sorted by duration, and then in alphabetic order.

Authors, year	Dose, (mg per day), substance	N in treatment group, duration of study	Reported parameters and results from objectively measured outcomes (blood or urine analyses) related to evaluation of safety	Tier
Nakamura et al., 2022	110, EMIQ[1]	40, 40 weeks	Safety parameters were biological and hematological parameters (white blood cells, red blood cells, Hb, hematocrit, platelet count test), liver function indicators (aspartate aminotransferase, alanine transaminase, γ-glutamyl transferase, alkaline phosphatase and lactate dehydrogenase levels), renal function indicators (blood urea nitrogen and creatinine levels, urinalysis and estimated glomerular filtration rate), blood lipid indicators (total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol, apolipoprotein B, apolipoprotein AI, triglycerides), glycaemic index (fasting glucose, glycated haemoglobin, glycoalbumin and fasting insulin) and serum proteins (total protein, albumin), insulin-like growth factor-1 (IGF-1), plasma amyloid-β 40, amyloid-β 42, Tau, neurofilament light chain, tumor necrosis factor-α, interleukin-6 and interferon-γ and lateral index. All parameters were judged to be within normal range by a physician.	1
Yoshimura et al., 2012	110, EMIQ[1]	57, 24 weeks	Safety evaluation comprised effects based on the physical test, blood test and urinalysis, as well as anthropometry and blood pressure. Blood tests included haematology (white and red cell count, haemoglobin, haematocrit, platelet count), blood biochemistry (AST, ALT, γ-GTP, ALP, LDH, total bilirubin, total cholesterol, HDL and LDL cholesterol, triglycerides, CPK, total protein, albumin, urea nitrogen, creatinine, phospholipid, free fatty acids, fasting blood sugar, hemoglobin A1c (HbA1c), Na, Cl, K, Ca, Mg, Fe. Urinalysis included protein, glucose, urobilinogen and ketone. None of these parameters indicated adverse effects of the treatment. Abnormally high ALP, AST and ALT (1 each) were reported, but considered not to be related to quercetin treatment.	1
Shatylo et al., 2021	240, Q	55, 12 weeks	The treatment was safe and tolerable based on ECG and routine biochemical blood parameters (hemoglobin, erythrocytes, leukocytes, platelets, bilirubin, creatinine, alanine aminotransferase and aspartate transaminase) monitored weekly, as well as effects on body composition, lipid and blood sugar parameters and oxidative stress. **However, the glutathione (GSH) level was significantly decreased in the quercetin-treated group, which could either be suggestive of a decrease in oxidative stress, which in turn has resulted in a decreased need for GSH synthesis, or it could be a potential negative effect.**	2
Yoshimura et al., 2008, Study 2	275, EMIQ[1]	100, 12 weeks	Safety evaluation comprised effects based on the physical test, blood test and urinalysis, as well as anthropometry and blood pressure. Blood tests included haematology (white and red cell count, haemoglobin, haematocrit, platelet count), blood biochemical tests (AST, ALT, ALP, LDH, total bilirubin, γ-GTP, CPK, total cholesterol, HDL and LDL cholesterol, triglycerides, total protein, albumin, urea nitrogen, creatinine, phospholipid, free fatty acids, blood sugar,	1

Authors, year	Dose, (mg per day), substance	N in treatment group, duration of study	Reported parameters and results from objectively measured outcomes (blood or urine analyses) related to evaluation of safety	Tier
			HbA1c, Na, Cl, K, Ca, Mg and Fe. Urinalysis included protein, sugar, urobilinogen. Some significant changes were observed in some subjects in week 12 compared to the start of study, but all analyses were within normal limits and no differences were found between the treatment and placebo groups. An abnormal urine glucose value was observed in one subject but considered not to be related to quercetin.	
Pfeuffer et al., 2013	150, QD	49 (19APOE3/3, 30APOE4), 8 weeks	Atherosclerosis, endothelial function, blood pressure, anthropometry, metabolic and inflammatory parameters, soluble adhesion molecules and total glutathione were examined. **The only adverse result observed was that quercetin exerted moderately increased levels of TNFα, suggestive of a potential slightly pro-inflammatory effect, but with no difference by APOE genotype.**	1
Boyle et al., 2000	500, rutin	16, 6 weeks	No adverse effects observed on blood chemistry, indices of liver function, antioxidant levels and DNA damage.	1
Egert et al., 2009	150, QD	93, 6 weeks	Safety parameters such as biomarkers of liver and kidney function (alanine transaminase, aspartate transaminase, γ-glutamyl-transpeptidase, alkaline phosphatase, cholesteryl esterase, creatinine), haematology and serum electrolytes were all within normal ranges and no differences were observed between quercetin group and placebo. In addition, effects on blood pressure, lipid metabolism, markers of oxidative stress, inflammation and body composition were examined. **The only effect in a potentially adverse direction was a small, but significant decrease in serum HDL-cholesterol concentration during quercetin treatment, which was not associated with any increase in the LDL:HDL-cholesterol or TAG:HDL-cholesterol ratios. Thus, this decrease in HDL-cholesterol may be of only limited, if any, physiological or clinical relevance.**	1
Annoni et al., 1986	4000, HER	20, 4 weeks	Clinical examination, proctoscopy, and blood chemistry tests (blood sugar, urea nitrogen, blood count, urine test, ESR, SGOT, SGPT, bilirubin, total and direct, alkaline phosphatase, and time of prothrombin) were performed before and after the treatment and did not show any significant changes.	2
Cappelli et al., 1987	3000, HER	76, 4 weeks	To estimate the tolerability of the drug, before and after the treatment, the following parameters were checked: azotaemia, glycaemia, prothrombin activity, bilirubin, transaminases, alkaline phosphatase, haemochrome and urine analysis. The laboratory tests performed at the end of the treatment did not show changes of any parameters.	2
Yamada et al., 2022	200, QP	30, 4 weeks	Safety evaluation comprised reactions based on the physical test, blood test and urinalysis. Blood tests included white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, differential leukocyte count (percentage and count of neutrophils, lymphocytes, monocytes, eosinophils and basophils), aspartate aminotransferase, alanine transaminase,	2

Authors, year	Dose, (mg per day), substance	N in treatment group, duration of study	Reported parameters and results from objectively measured outcomes (blood or urine analyses) related to evaluation of safety	Tier
			γ-glutamyl transferase, alkaline phosphatase, lactate dehydrogenase, leukocyte alkaline phosphatase, total bilirubin, direct and indirect bilirubin, cholinesterase, total protein, urea nitrogen, creatinine, uric acid, creatine kinase (CK), calcium, serum amylase, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glycoalbumin, serum iron (Fe), sodium (Na), potassium (K), chlorine (Cl), inorganic phosphorus (P), glucose and hemoglobin A1c (HbA1c). Urinalysis covered protein, glucose, urobilinogen, bilirubin, occult blood reaction, ketone body, specific gravity, and pH. Some significant inter-group differences observed (systolic and diastolic blood pressure, and blood glucose level) were variations within the criterion range of the parameters concerned. Also, some parameters outside the criterion range were still considered not to be a medical problem.	
Yasutake et al., 2015	330, EMIQ[1]	24, 4 weeks	Safety was the primary purpose of the study. Parameters measured were anthropometry (height, weight, BMI, body fat percentage), systolic and diastolic blood pressure and pulse rate, hematology (white and red cell count, hemoglobin, hematocrit, platelet count), blood biochemical tests (AST (GOT), ALT (GTP), LDH, total bilirubin, ALP, γ-GTP, CPK, total cholesterol, HDL and LDL cholesterol, triglycerides, total protein, albumin, urea nitrogen, creatinine, uric acid, fasting blood sugar, HbAlc, Na, Cl, K, Ca and urinalysis (protein, sugar, urobilinogen, ketone bodies). None observed changes in test values were of clinical concern. There was no causal relationship between the adverse events reported during the treatment period and the treatment, and there was no difference in incidence rate between the treatment and control groups.	1
Han et al., 2020	50, 1000, 2000, Q	2, 1 week	No treatment-related effects were reported based on the safety parameters postbronchodilator FEV1 and other parameters of lung function, complete blood count and a comprehensive metabolic panel examined. Parameters of liver and kidney functions were within the normal range at run-in and posttreatment.	1
Erlund et al., 2000	8, 20, 50 (78) or 16, 40, 100 (156) Q (total) or rutin (total)	12, single dose	At screening and at the study completion, blood and urine samples were assessed for safety parameters: haematology, biochemical profile, urinalysis, electrocardiogram (ECG), blood pressure and heart rate, and no adverse effects were observed.	1
Kienzler et al., 2002	500, 1000, 2000, 4000, HER	16, single dose	Safety parameters were haematology, biochemical profile, urinalysis, ECG, blood pressure and heart rate. There was no modification of vital signs, ECG or safety laboratory results during the study.	2

Authors, year	Dose, (mg per day), substance	N in treatment group, duration of study	Reported parameters and results from objectively measured outcomes (blood or urine analyses) related to evaluation of safety	Tier
Riva et al., 2019	500Q, 250QP, 500QP	12, single dose	Clinical safety (evaluation of vital signs (blood pressure, heart and respiratory rate, temperature) and biological safety (evaluation of blood count, blood chemistry results and urine sediment) were monitored to ensure that they were within normal limits by clinical judgement of the investigator. No significant differences were observed between the three treatments in vital signs, physical examination results or ECG results.	1

[1]EMIQ as isoquercitrin. Isoquercitrin: n = 1 glucose.
Q: quercetin.
QD: quercetin dihydrate.
QP: Quercetin Phytosome®.
HER: *O*-(β-hydroxyethyl)-rutoside (Venoruton®).
EMIQ: enzymatically modified quercetin glycoside(s) with varying numbers of glucose molecules.
i.v.: intravenous injection.

Category 2 results

Twenty-two publications give some information about subjectively obtained adverse effects, mostly self-reported by the participants (all except Boyle et al. (2000)). Of these, five studies stated that no adverse effects were reported by the participants or observed (Bazyar et al., 2023; Egert et al., 2008; Ganio et al., 2010; Hirano et al., 2009; Shi & Williamson, 2016) (Table 4.5.2.2-2).

In eight publications that reported adverse effects/events (Annoni et al., 1986; Bergqvist et al., 1981; Di Pierro et al., 2021; Han et al., 2020; Pfeuffer et al., 2013; Shoskes et al., 1999; Yoshimura et al., 2008; 2012), the results on various adverse outcomes subjectively reported are described in more detail in the following (Table 4.5.2.2-2).

Annoni et al. (1986) treated 20 adult patients (12 men and 8 women) with very advanced haemorrhoids with 4000 mg per day of *O*-(β-hydroxyethyl)-rutoside (HER) or 20 patients (11 men and 9 women) with placebo for 28 days. The patients were questioned at each visit to the clinic about possible appearance of any adverse effects of the treatment. In the HER-treated group, one case each of gastric heartburn, epigastric weight and diarrhea was reported, whereas in the placebo group, one case each of epigastric weight and diarrhea was reported, thus, the adverse effects reported were quite similar after treatment with HER and placebo.

Bergqvist et al. (1981) treated 71 patients (15 men and 56 women) with chronic venous insufficiency with a single i.v. injection of 1000 mg and 1500 mg per day orally of *O*-(β-hydroxyethyl)-rutoside (HER, Venoruton®) for 4 weeks, whereas 72 participants (12 men and 60 women) received placebo. The adverse effects they observed, assessed using a standardised questionnaire, were evenly distributed between the HER and placebo group, and of minor severity: discomfort (12, 9), vomiting (1, 0), diarrhoea (3, 9), constipation (9, 6), headache (15, 14), dizziness (7, 6), tiredness (15, 9), dry mouth (18, 15), pruritus (6, 2), sleeping problems (0, 3), others (12, 20), in total (98, 93) with number of patients given HER and placebo in parentheses, respectively. The difference in the number of adverse events between the two groups was not statistically significant (*t*-test, P = 0.861). Two of the patients with chronic venous insufficiency, both on placebo, stopped the treatment because of side-effects (not reported which effects).

Di Pierro et al. (2021) treated COVID outpatients with 1000 mg per day of Quercetin Phytosome® for 30 days together with standard care or gave only standard care as control treatment. No peculiar side-effects were reported by the patients. Only a few cases of gastric pain and reflux, constipation, diarrhoea, meteorism, flatulence and sleep disorders were experienced, which self-resolved in a few days and occurred also in the control group, demonstrating they were likely not caused by quercetin.

Han et al. (2020) treated 6 patients (4 men and 2 women) with chronic obstructive pulmonary disease (COPD) with chews containing 500, 1000 or 2000 mg quercetin per day (2 persons per dose) in a dose-escalation manner for 7 days, whereas placebo was given to 3 patients (1 man and 2 women). Patient-reported adverse events were gastro-oesophageal reflux disease (GERD), stomach upset, breathlessness, chest congestion, headache and nausea. Two subjects, one in the placebo group and

another in the 500 mg quercetin group (the lowest dose tested), reported symptoms of GERD and the subject in the placebo group needed treatment to reduce the GERD symptoms. Thus, GERD was not specifically related to the treatment with quercetin.

Pfeuffer et al. (2013) gave 49 healthy men with varying apolipoprotein E (APOE) genotypes (19 with APOE3/3 and 30 with APOE4), 150 mg quercetin dihydrate per day for 8 weeks, in a cross-over study. One subject with APOE3/4 genotype dropped out of the study right at onset because of night sweat, which may be a treatment-related effect according to the authors. No further information was given.

Shoskes et al. (1999) treated 15 men with prostate-related pelvic pain with quercetin, 1000 mg per day for one month, whereas the placebo group comprised 13 men. They reported that one patient taking placebo developed a rash that resolved when he stopped taking the capsules. One patient taking quercetin developed a headache after the first few doses, which resolved, and one patient taking quercetin noted mild tingling of the extremities after each dose. However, all these adverse effects resolved after cessation of therapy.

Yoshimura et al. (2008) exposed 100 overweight and obese persons (51 men and 49 women) with BMI 24-31 kg/m^2 and age 20-65 years to enzyme-treated isoquercitrin (EMIQ), 275 mg per day for 12 weeks. The placebo group also consisted of 100 persons (51 men and 49 women). In the EMIQ group, 75 cases of adverse events were reported (cold symptoms (25), headache (13), stiff shoulders (7) menstrual pain (7), rhinitis (4), hay fever (4), gastric discomfort (4), constipation, bladder cystitis, fatigue, abdominal pain, back pain, tooth suppuration, tooth inflammation, strained back, allergic symptoms, gout, sprain (1 each)). There were 75 adverse events reported in the control group (cold symptoms (40), headache (7), menstrual pain (3), rhinitis (2), toothache (2), constipation (2), hay fever (2), diarrhoea, stiff shoulders, car sickness, stye, palpitations, broken bones, injuries, canker sores, influenza, boils, cystitis, stomach discomfort, stomach pain, skin inflammation, allergic symptoms, hangover, positive urine sugar (1 each)). However, the reported adverse events in both groups were judged not to have a causal relationship with the test drink, based on the timing and circumstances of their occurrence. (This publication was translated from the obtained pdf using Print Screen and Google translate Image function from Japanese to English.)

In a similar study by Yoshimura et al. (2012), 57 overweight and obese persons (28 men and 29 women) with BMI ≥25 - <30 kg/m^2 and age 20-66 years were treated with enzyme-treated isoquercitrin (EMIQ), 110 mg per day for 24 weeks. The placebo group consisted of 59 persons (20 men and 29 women). In the EMIQ group, 33 persons reported 67 cases of adverse events (cold symptoms (47), headache (3), tooth ache (2), diarrhoea (2), influenza (2), stress-induced gastric ulcer (2), stomach discomfort (2), stomach pain, stiff shoulders, constipation, tendonitis, herpes, heel pain, abnormally high alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine aminotransferase (ALT) (1 each)). There were 54 adverse events in 26 persons in the control group (cold symptoms (24), headache (11), toothache (5), stomach discomfort (4), stomach pain (3), abdominal pain, lower back pain, stiff shoulders, rhinitis, diarrhoea, strained back, eczema (1 each). The adverse events in both groups were determined by the study director to have no causal relationship with the test drinks, based on timing of occurrence, circumstances etc. (This publication was

translated from the obtained pdf using Print Screen and Google translate Image function from Japanese to English.)

In summary, among the Category 2 results, eight publications reported adverse event/effects with at least some detail about the observations (Annoni et al., 1986; Bergqvist et al., 1981; Di Pierro et al., 2021; Han et al., 2020; Pfeuffer et al., 2013; Shoskes et al., 1999; Yoshimura et al., 2008; 2012) (Table 4.5.2.2-2). However, the reported effects/events were all of minor severity and were considered not to be study drug-related (Han et al., 2020; Yoshimura et al., 2008; 2012), the type and numbers of reported effects/events were similar between the treatment and control groups (Annoni et al., 1986; Bergqvist et al., 1981; Di Pierro et al., 2021; Yoshimura et al., 2008; 2012), each occurred in only one person (Pfeuffer et al., 2013; Shoskes et al., 1999), were self-resolving (Di Pierro et al., 2021; Shoskes et al., 1999) or did not show a dose-response (Han et al., 2020). Therefore, no serious hazards were identified among these Category 2 results.

Most of the publications in Category 2 had a low risk of bias, i.e. 6 publications were evaluated as Tier 1 (Bazyar et al., 2023; Bergqvist et al., 1981; Egert et al., 2008; Ganio et al., 2010; Hirano et al., 2009; Shi & Williamson, 2016). Two publications were evaluated as Tier 3 (high risk of bias) (Di Pierro et al., 2021; Shoskes et al., 1999) (Table 4.5.2.2-2.).

The results of adverse effects subjectively reported in all included publications in Category 2 are presented in Table 4.5.2.2-2.

Table 4.5.2.2-2 Results in publications in Category 2 with subjectively measured outcomes sorted by duration, and then in alphabetic order.

Authors, year	Dose, (mg per day), substance	N in treatment group, duration of study	Reported parameters and results from subjectively measured adverse effects/events/side-effects	Tier
Nakamura et al., 2022	110, EMIQ[1]	40, 40 weeks	There were no safety issues in conducting the study or the intake of the test beverage. A causal relationship between frequent urination and the study exposures was considered possible, owing to the likely association between increased water intake and the study beverage (barley tea). However, this would be the case after exposure to both EMIQ and placebo, both being administered in barley tea. No other adverse events were causally related to the study.	1
Yoshimura et al., 2012	110, EMIQ[1]	57, 24 weeks	**In the EMIQ group, 33 persons reported 67 cases of adverse events (cold symptoms (47), headache (3), tooth ache (2), diarrhoea (2), influenza (2), stress-induced gastric ulcer (2), stomach discomfort (2), stomach pain, stiff shoulders, constipation, tendonitis, herpes, heel pain, abnormally high ALP, AST and ALT (1 each)). There were 54 adverse events in 26 persons in the control group (cold symptoms (24), headache (11), toothache (5), stomach discomfort (4), stomach pain (3), abdominal pain, lower back pain, stiff shoulders, rhinitis, diarrhoea, strained back, eczema (1 each)). Adverse events in both groups were determined by the study director to have no causal relationship with the test drinks, based on timing of occurrence, circumstances etc.** (This publication was translated from the obtained pdf using Print Screen and Google translate Image function from Japanese to English.)	1
Bazyar et al., 2023	500, rutin	25, 12 weeks	Side-effects of rutin consumption were assessed during these three months. This was done via phone call or text message from the researcher apparently twice a month. The patients did not report any side-effects from rutin consumption during the study.	1
Shatylo et al., 2021	240, Q	55, 12 weeks	None of patients developed adverse events that required discontinuation of the treatment.	2
Yoshimura et al., 2008, Study 2	275, EMIQ[1]	100, 12 weeks	**In the EMIQ group, 75 cases of adverse events were reported (cold symptoms (25), headache (13), stiff shoulders (7) menstrual pain (7), rhinitis (4), hay fever (4), gastric discomfort (4), constipation, bladder cystitis, fatigue, abdominal pain, back pain, tooth suppuration, tooth inflammation, strained back, allergic symptoms, gout, sprain (1 each)). There were 75 adverse events reported in the control group (cold symptoms (40), headache (7), menstrual pain (3), rhinitis (2), toothache (2), constipation (2), hay fever (2), diarrhoea, stiff shoulders, car sickness, stye, palpitations, broken bones, injuries, canker sores, influenza, boils, cystitis, stomach discomfort, stomach pain, skin inflammation, allergic**	1

Authors, year	Dose, (mg per day), substance	N in treatment group, duration of study	Reported parameters and results from subjectively measured adverse effects/events/side-effects	Tier
			symptoms, hangover, positive urine sugar (1 each)). Adverse events in both groups were judged not to have a causal relationship with the test drink, based on the timing and circumstances of their occurrence. (This publication was translated from the obtained pdf using Print Screen and Google translate Image function from Japanese to English.)	
Hirano et al., 2009	100, EMIQ[2]	12, 8 weeks	The severity of subjective symptoms was evaluated by a scoring system and the participants were asked to record symptom scores and activities of daily living (ADL) every day during the testing period (for 10 weeks). No adverse effects, such as gastrointestinal symptoms, allergic reactions or cardiovascular symptoms, were observed during the entire period.	1
Pfeuffer et al., 2013	150, QD	19 or 30, 8 weeks	**On subject with APOE3/4 genotype dropped out of the study right at onset because of night sweat, which may be treatment-related.** No further information was given.	1
Egert et al., 2009	150, QD	93, 6 weeks	The participants were asked to document observed side-effects, deviations from their normal physical activity or any other observations considered relevant in a study diary.	1
Annoni et al., 1986	4000, HER	20, 4 weeks	Great attention was paid to the possible appearance of indirect effects, questioning patients at each visit. The side-effects were mild, so that no patient had to interrupt treatment, even temporarily. **In the treatment group, there was one case each of gastric heartburn, epigastric weight and diarrhea, and in the placebo group, there was one case each of epigastric weight and diarrhea**. Tolerability was good and similar for both treatments.	2
Bergqvist et al., 1981	1500+1000 i.v., HER	71, 4 weeks	The side-effects, assessed using a standardised questionnaire, were evenly distributed between the HER and placebo group (numbers not statistically significantly different), and of a minor severity: **discomfort (12, 9), vomiting (1, 0), diarrhoea (3, 9), constipation (9, 6), headache (15, 14), dizziness (7, 6), tiredness (15, 9), dry mouth (18, 15), pruritus (6, 2), sleeping problems (0, 3), others (12, 20)**, for number of patients given HER and placebo, respectively. Two of the patients with chronic venous insufficiency, both on placebo, stopped treatment because of side-effects (not reported which effects).	1
Cappelli et al., 1987	3000, HER	10, 4 weeks	No side-effects were observed.	2
Di Pierro et al., 2021	400, Q	76, 4 weeks	Adherence, tolerability and side-effects as a direct consequence of the quercetin supplementation to COVID-19 outpatients was assessed. Quercetin was generally well tolerated with no apparent toxicity. No peculiar side-effects were reported by the patients and **the few cases of gastric pain and reflux, constipation, diarrhoea, meteorism, flatulence and sleep disorders were self-resolving in few days and similarly occurred in the**	3

Authors, year	Dose, (mg per day), substance	N in treatment group, duration of study	Reported parameters and results from subjectively measured adverse effects/events/side-effects	Tier
			control group (given standard care), demonstrating that they likely could not be attributed to the use of quercetin.	
Shi & Williamson, 2016	544, QD	22, 4 weeks	At the end of each 4-week period, call-back questionnaires were used to record changes in physical activity and intensity and the occurrence of any side-effects. No adverse events after receiving quercetin or placebo were reported.	1
Shoshkes et al., 1999	1000, Q	15, 4 weeks	**One patient taking placebo developed a rash that resolved when he stopped taking the capsules. One patient taking quercetin developed a headache after the first few doses, which resolved, and one patient taking quercetin noted mild tingling of the extremities after each dose.** All these side-effects resolved after cessation of therapy.	3
Yamada et al., 2022	200, QP	32, 4 weeks	Adverse events developed in some of the subjects, but all events subsided following oral medication or application of an ophthalmic solution. They were confirmed to have no causal relationship to the test food.	2
Yasutake et al., 2015	330, EMIQ[1]	24, 4 weeks	There was no causal relationship between the adverse events reported during the test period and the test drink, and there was no difference in incidence rate between the test and control groups.	1
Egert et al., 2008	50, 100, 150, QD	35, 2 weeks	No adverse effects of quercetin intake were reported.	1
Ganio et al., 2010	1000, Q	11, 1 week	No participant exhibited or self-reported side-effects due to food bar consumption.	1
Han et al., 2020	500, 1000, 2000, Q	2, 1 week	Patient-reported adverse events were **gastro-oesophageal reflux disease (GERD), stomach upset, breathlessness, chest congestion, headache and nausea**. Two subjects, one in the placebo group and the other one in the 500 mg quercetin group (the lowest dose tested), reported symptoms of **GERD**, and the subject in the placebo group needed treatment to reduce the GERD symptoms. **Thus, GERD was not specifically related to the treatment with quercetin.**	1
Erlund et al., 2000	8, 20, 50 (78) or 16, 40, 100 (156) Q	12, single dose	Subjects were asked to record adverse events in their patient's diaries that were collected daily. No adverse events related to the study compounds occurred during the study.	1

Authors, year	Dose, (mg per day), substance	N in treatment group, duration of study	Reported parameters and results from subjectively measured adverse effects/events/side-effects	Tier
	(total) or rutin (total)			
Kienzler et al., 2002	500, 1000, 2000, 4000, HER	16, single dose	All adverse events were completely documented and reported. The subjects were asked after each blood sampling, in an open manner without prompting, if they experienced unusual or adverse events. Any condition that was not present at the initial examination was recorded. The authors concluded that the different doses of the study medication were safe and well tolerated.	2
Riva et al., 2019	500Q, 250QP, 500QP	12, single dose	No notable side-effects of Quercetin Phytosome® were ported and it was well tolerated.	1

[1]EMIQ as isoquercitrin. Isoquercitrin: n = 1 glucose.
[2]EMIQ, n = 1-8 glucose.
Q: quercetin.
QD: quercetin dihydrate.
QP: Quercetin Phytosome®.
HER: *O*-(β-hydroxyethyl)-rutoside (Venoruton®).
EMIQ: enzymatically modified quercetin glycoside(s) with varying numbers of glucose molecules.
i.v.: intravenous injection.

4.6 Summary of the hazard identification and characterization

4.6.1 RCTs

Among the 23 included RCTs, only eleven reported any adverse effects/events at all, three publications reported outcomes in a potentially adverse direction among the parameters measured in blood or urine (Category 1), and eight were among publications with subjectively obtained adverse effects, often self-reported (Category 2).

Among the publications in Category 1 (Table 4.5.2.2-1), in the three studies assessing treatments with 240 mg quercetin per day for 3 months (Shatylo et al., 2021), 150 mg quercetin dihydrate per day for 8 weeks (Pfeuffer et al., 2013) and 150 mg quercetin dihydrate per day for 6 weeks (Egert et al., 2009), a single sporadic effect in the direction of adversity but of mild severity (decrease in HDL-cholesterol, increased levels of TNFα and decreased GSH level, respectively) was observed in each publication. The first two effects were not supported by other endpoints measured in the same study and the third effect could be interpreted as not adverse. In addition, these effects have not been reported in the other included studies, which indicate that they may be chance findings. Therefore, no serious hazards were identified among these Category 1 results.

Among the publications in Category 2 (Table 4.5.2.2-2), eight publications reported adverse event/effects with at least some detail about the observations (Annoni et al., 1986; Bergqvist et al., 1981; Di Pierro et al., 2021; Han et al., 2020; Pfeuffer et al., 2013; Shoskes et al., 1999; Yoshimura et al., 2008; 2012). In these publications, the exposure lasted from 5 days to 24 weeks, using doses up to 2000 mg per day of quercetin or 4000 mg per day of HER. However, the reported effects/events were all of minor severity and were considered not to be study drug-related (Han et al., 2020; Yoshimura et al., 2008; 2012), the type and numbers of reported effects/events were similar between the treatment and control groups (Annoni et al., 1986; Bergqvist et al., 1981; Di Pierro et al., 2021; Yoshimura et al., 2008; 2012), each occurred in only one person (Pfeuffer et al., 2013; Shoskes et al., 1999), were self-resolving (Di Pierro et al., 2021; Shoskes et al., 1999) or did not show a dose-response (Han et al., 2020). Therefore, no serious hazards were identified among these Category 2 results.

The publications in Category 1 all had either a low or moderately risk of bias, i.e. 10 publications were evaluated as Tier 1 (low risk of bias) and five publications were evaluated as Tier 2 (moderate risk of bias). Most of the publications in Category 2 had a low risk of bias, i.e. 6 publications were evaluated as Tier 1. Two publications were evaluated as Tier 3 (high risk of bias). However, the main objective in most of these studies were not to examine adverse effects, but beneficial effects. Heterogeneity or mechanisms of action could not be evaluated since for most publications there was a lack of adverse effects. Furthermore, the publications included were heterogeneous both in relation to outcomes examined and study duration. Thus, the confidence in the overall evidence for absence of adverse effects related to quercetin or rutin in the 23 included RCTs is considered to be moderate.

In addition to the systematic approach used to identify and characterize adverse effects observed in the human RCTs, some additional information, mostly from animal studies, was included from other sources, not obtained in a systematic way.

4.6.2 ADME

Rutin and its aglycone quercetin have low oral bioavailability caused by their insolubility in water, but this can be increased by glucosyl conjugation of these molecules, i.e. addition of various sugar moieties, such as in the substances EMIQ and isoquercetin. Similarly, to overcome the poor solubility and low oral absorption of quercetin, a new food-grade lecithin-based formulation of quercetin, called Quercetin Phytosome®, has been made.

The absorption of rutin in the small intestine is limited and varies based on individual pharmacokinetics and other factors (Boyle et al., 2000). Rutin is further transported from the small intestine into the colon and metabolised by the gut microbiota into isoquercetin (quercetin-3-glucoside) and then quercetin, or directly into quercetin (Chua, 2013; Riva et al., 2020). The quercetin aglycone is easier absorbed, probably by passive diffusion over the intestinal epithelium or directly via intestinal sodium/glucose co-transporter-1 (Andres et al., 2018). Quercetin may also be subsequently degraded by the colonic microbiota, mainly into different phenolic acids (Chua, 2013). After absorption, quercetin is extensively metabolised in enterocytes and liver, and it may be glucuronidated, sulfated and/or methylated (Andres et al., 2018). In the blood, primarily these quercetin conjugates are found, with only very low levels of the aglycone form. Quercetin is found in some tissues mostly as aglycone, while in other tissues, the unconjugated quercetin is present in smaller proportions (Andres et al., 2018). Ingested quercetin is rapidly excreted via urine and feces, and may also be metabolised and excreted via the lungs as CO_2 (D'Andrea, 2015). There is also interindividual variation in the rate of excretion of quercetin (Boyle et al., 2000).

It was shown in humans that Quercetin Phytosome® had higher absorption (up to 20 times) and lower half-life in plasma than quercetin and that these effects were dose-dependent (Riva et al., 2019). Quercetin Phytosome® was more stable than quercetin after interaction with the intestinal microbiota, allowing for more time and the better dispersion of the single molecule to be absorbed, thus, overcoming one of the possible reasons for quercetin's poor oral bioavailability (Di Pede et al., 2020).

In EMIQ and isoquercetin, the chemical structure of the glucose moiety of a particular glycoside affects the small intestinal absorption of the glycosides, such as the position of linkage between glucose molecules (Makino et al., 2009). De-oligomerisation of EMIQ starts in the mouth, and in the small intestine, EMIQ is further degraded by pancreatic α-amylase, being converted into isoquercitrin and α-glucosyl derivatives with 1 or 2 α-glucose moieties (Owczarek-Januszkiewicz et al., 2022; Makino et al., 2009; Murota et al., 2010). Further, the remaining α-glucosyl derivatives are also degraded to isoquercitrin and then to quercetin by the enzyme lactase-phlorizin hydrolase (LPH), acting extracellularly at the intestinal epithelial cells. Early EMIQ metabolites are mainly quercetin glucuronide and sulfate conjugates (Makino et al., 2009). The quantitative ratio of the various metabolites varies substantially among individuals. Maximum plasma concentration of metabolites of EMIQ was reached 1.5-2 hours after oral administration and reached about 17, 3 and 44 times higher circulating levels than that

obtained for quercetin, isoquercitrin and rutin (Owczarek-Januszkiewicz et al., 2022). Metabolites of EMIQ are detected in various tissues dose-dependently and are at least partially eliminated through urine.

It is assumed that *O*-(β-hydroxyethyl)-rutosides (HER) are poorly absorbed from the gastrointestinal tract because of their high molecular weight and low lipophilicity and liposolubility (Kienzler et al., 2022). The position and nature of the sugar residues may affect the uptake of the compound in the small intestine. HER are not absorbed as glycosides and are present in plasma as aglycones conjugated to glucuronic acid and/or sulfate conjugates. Apparently, HER can be considered to have a similar bioavailability as rutin and quercetin. β-Glucosidase activity is involved in absorption of HER in humans in the distal small intestine or colon (Kienzler et al., 2022). Mono-3'-HER and mono-4'-HER are the most available among the circulating metabolites of HER, which are hydrolysed prior to absorption and are present in plasma as quercetin aglycone conjugated to glucuronic acid and/or sulfate groups. The elimination half-life of both mono-3'-HER and mono-4'-HER are dose-dependent (Kienzler et al., 2022).

4.6.3 Toxicity

Based on the available literature, although mutagenic and genotoxic effects have been reported in some assays *in vitro*, quercetin, rutin and the related substances EMIQ and IQ are not found to be genotoxic *in vivo* for the doses evaluated in this risk assessment (NTP, 1992; Andres et al., 2018; da Silva et al., 2002).

In vitro, quercetin may be activated to DNA-reactive metabolites by enzymatic and/or chemical oxidation of quercetin to quercetin ortho-quinone, followed by isomerisation of the ortho-quinone to quinone methides, which are suggested to be the active alkylating DNA-reactive intermediates. The discrepancy between *in vitro* mutagenicity and genotoxicity, and lack of genotoxic or carcinogenic effects *in vivo,* may be related to the transient nature and the instability of the quercetin quinone methide adducts, as well as various other mechanisms (Rietjens et al., 2005; Woude et al., 2005, Harwood et al., 2007).

In a 2-year feeding study by NTP (1992), there was some evidence of carcinogenic activity of quercetin in male rats based on an increased incidence of renal tubule cell adenomas, but there was no evidence of carcinogenic activity of quercetin in female rats receiving up to 1900 mg/kg bw per day of quercetin. The incidence of renal tubule hyperplasia and the severity of nephropathy were increased in exposed male rats. Thus, the renal tumor development may be associated with or may be a consequence of the chronic progressive nephropathy occurring only in male rats, with probably no or only little relevance for extrapolation to humans. Other long-term rat studies, two on quercetin (Hirono et al., 1981; Stoewsand et al., 1984) and two on EMIQ (FDA, 2007, reported in Madden et al., 2022) did not report any carcinogenic effects.

IARC concluded that "quercetin is not classifiable as to its carcinogenicity to humans" (Group 3) (IARC, 1999).

Quercetin can most likely cross the placenta, since effects on the fetus have been observed after maternal exposure in mice (Vanhees et al., 2011), and is shown for several other flavonoids (Todaka et al., 2005).

Rutin may be able to bind to the estrogen receptor and exert estrogen-like effects (Chua, 2013).

The available studies in mice, rats and rabbits did not find reprotoxic effects of quercetin after exposure during gestation (Vanhees et al., 2011; Johnson et al., 2009; Maronpot et al., 2020). When the mice dams were exposed to 5 mg/kg bw per day via drinking water for 9 months during 2 to 6 months and 8 to 11 months of age, quercetin increased birth spacing, leading to a 60% reduction in the number of litters, but enhanced folliculogenesis in ovaries of female offspring (Beazley & Nurminskaya, 2016).

In a human case-control study by Pósfai et al. (2014), HER treatment with oral doses of 900-1000 mg HER per day (corresponding to 414-460 mg quercetin aglycone per day or 837-930 mg rutin per day) for 3-5 weeks during the second and/or third month of pregnancy was found to be associated with a higher risk of unilateral ocular coloboma and a new congenital abnormality syndrome including anotia/microtia, poly-/syndactyly and caudal (genital and anal) defects. Similar effects (syndactyly) were found in mice after exposure for approximately 67 mg/kg bw of quercetin for about two weeks during gestation (Prater et al., 2008).

The only available information on allergenicity, sensitization and irritation was that EMIQ was not a skin sensitizer or irritant in mice (Vij et al., 2022).

5 Risk characterisation of the specified doses

VKM was requested by NFSA to evaluate the potential risk of 500 mg quercetin dihydrate per day in food supplements for adults from 18 years of age. Further, VKM was requested to evaluate the potential risk from intake of 5 mg rutin per day in food supplements for children 4 years of age and older, and 25 mg rutin per day in food supplements for adults from 18 years of age.

The bioavailability of quercetin is generally low due to the low water solubility, variable between subjects and depending on several factors (see Chapter 4.1). Therefore, a lot of research have been performed to modify quercetin as well as rutin into substances with higher bioavailability.

Among the 23 included publications used in this risk assessment, various substances were used as intervention in the RCTs: quercetin aglycone (assumed also when only stated quercetin), quercetin dihydrate, rutin and modified substances with increased bioavailability, such as Quercetin Phytosome® (QP), enzymatically modified quercetin glycoside (EMIQ), *O*-(β-hydroxyethyl)-rutoside (HER, Venoruton®) and isoquercitrin (see Chapter 2). All these substances are metabolized to quercetin aglycone in the body (see the information on ADME in Chapter 4.1). The doses used in the publications were therefore recalculated based on differences in molecular weight and bioavailability to this common substance (quercetin aglycone) for comparisons with the daily doses VKM was requested to evaluate by NSFA (Table 5-1).

Table 5-1. Substances assessed in the included publications used in this risk assessment recalculated as quercetin (aglycone) or rutin (age groups for use in parentheses as requested by NFSA).

Substances used in the included publications	Doses as requested by NFSA	Molecular weight (g/mol)	Differences in bioavailability or other comparisons based on chemistry	Comparable dose of substance of quercetin or rutin
Quercetin-related substances				
Quercetin aglycone		302.23[1]		
Quercetin dihydrate	**500 mg** quercetin dihydrate (from 18 years of age and older)	338.27[2]	Quercetin dihydrate will be dissociated to quercetin aglycone in the body	302.23/338.27 = 0.89. 500 mg x 0.89 = **445 mg** quercetin aglycone
Quercetin Phytosome® (QP)			20 times higher bioavailability of QP than quercetin[5]	445 mg x 20 = **8900 mg** quercetin aglycone
Rutin-related substances				
Rutin	**5 mg** rutin (from 4 years of	610.52[3]		302.23/610.52 = 0.49. 5 mg x 0.49 = **2.5 mg** quercetin aglycone

Substances used in the included publications	Doses as requested by NFSA	Molecular weight (g/mol)	Differences in bioavailability or other comparisons based on chemistry	Comparable dose of substance of quercetin or rutin
	age and older) **25 mg** rutin (from 18 years of age and older)			25 mg x 0.49 = **12.3 mg** quercetin aglycone
EMIQ (n = glucose 1-10)			17 times higher bioavailability of EMIQ than quercetin[6] 44 times higher bioavailability of EMIQ than rutin[6]	445 mg x 17 = **7565 mg** quercetin aglycone 5 mg x 44 = **220 mg** rutin 25 mg x 44 = **1100 mg** rutin
Isoquercitrin (n = glucose 1)			3 times lower bioavailability of isoquercitrin than EMIQ[6]	7565 mg/3 = **2522 mg** quercetin aglycone 220 mg/3 = **73 mg** rutin 1100 mg/3 = **367 mg** rutin
O-(β-hydroxyethyl)-rutoside (HER, Venoruton®)		654.6 g/mol[4]	HER apparently has a similar bioavailability as rutin and quercetin	302.23/654.6 = 0.46, 445 mg x 0.46 = **204.7 mg** quercetin aglycone 610.5/654.6 = 0.93, 5 mg x 0.93 = **4.7 mg** rutin 610.5/654.6 = 0.93, 25 mg x 0.93 = **23.3 mg** rutin

[1]Pubchem (2024), CAS no. 117-39-5.
[2]Pubchem (2024), CAS no. 6151-25-3.
[3]Pubchem (2024), CAS no. 153-18-4.
[4]Pubchem (2024), CAS no. 13190-92-6.
[5]Riva et al. (2019).
[6]Makino et al. (2009).

5.1 Risk characterization based on Category 1 results

In the following, the doses of quercetin, rutin or modified related substances used in the studies are recalculated based on molecular weight and bioavailability information to corresponding doses of quercetin aglycone (Table 5-1) to enable comparisons with the doses VKM was requested to evaluate by NFSA.

The three publications that reported outcomes in a potentially adverse direction among the parameters measured in blood or urine (Table 4.5.2.2-1) used doses recalculated to quercetin aglycone of 240 mg per day for 3 months (Shatylo et al., 2021), 134 mg per day for 2 months (Pfeuffer et al., 2013) and 134 mg per day for 6 weeks (Egert et

al., 2009), all of which are lower than the dose VKM was asked to evaluate (500 mg per day of quercetin hydrate, corresponding to 445 mg per day of quercetin aglycone). However, two potentially adverse effects (decrease in HDL-cholesterol and increased levels of TNFα) were not supported by other endpoints measured in the same studies and the third effect (decreased GSH level) could be interpreted as not adverse. Furthermore, these effects were not reported in other included studies, which indicate that they may be chance findings. Therefore, VKM does not consider these results to indicate a risk from intake of quercetin aglycone or quercetin dihydrate at the requested doses.

Among the included publications that did not report any adverse effects observed among the parameters measured in blood or urine, some had given only one single dose of quercetin or Quercetin Phytosome®, quercetin or rutin, and HER, corresponding to a quercetin aglycone dose of up to 10000 mg (Riva et al., 2019), 78 mg (Erlund et al., 2000) and 1840 mg (Kienzler et al., 2002), respectively.

One study used a dose of up to 2000 mg per day for only 7 days (Han et al., 2020), indicating no acute adverse effects.

More relevant for long-term exposure, no adverse effects were observed after 4 weeks (Annoni et al., 1986; Cappelli et al., 1987; Yamada et al., 2022; Yasutake et al., 2015), 6 weeks (Boyle et al., 2000), 12 weeks (Yoshimura et al., 2008), 24 weeks (Yoshimura et al., 2012) and 40 weeks (Nakamura et al., 2022) of exposure, with doses of quercetin aglycone of approximately 1840, 1380, 4000, 1870, 245, 1558, 623 and 623 mg per day, respectively, of which seven doses are higher than all the three doses (recalculated to quercetin aglycone) that VKM was requested to evaluate by NFSA. Thus, based on these results, VKM considers that exposure to the requested doses does not pose a risk at least up to 3 months. Two of the included publications found no adverse effects after administration for up to 6-10 months.

5.2 Risk characterization based on Category 2 results

No serious hazards of the administered substances were identified among the self-reported or observed adverse effects/events. Eight publications reported a few adverse effects/events of low severity (Annoni et al., 1986; Bergqvist et al., 1981; Di Pierro et al., 2021; Han et al., 2020; Pfeuffer et al., 2013; Shoskes et al., 1999; Yoshimura et al., 2008; 2012) (Table 4.5.2.2-2).

One of these studies administered up to 2000 mg quercetin aglycone for 7 days (Han et al., 2020), without observing any adverse effects, thus, indicating no acute toxicity.

Of relevance for more long-term toxicity, four studies exposed the study participants for 4 weeks (Annoni et al., 1986; Bergqvist et al, 1981; Di Pierro et al., 2021 and Shoskes et al., 1999) with doses of 1840, 690 orally + 460 i.v., 400 and 1000 mg per day of quercetin aglycone, respectively. Pfeuffer et al (2013) administered 134 mg per day for 8 weeks, Yoshimura et al. (2008) used 1558 mg per day for 12 weeks and Yoshimura et al. (2012) used 624 mg per day for 24 weeks. Among these studies, five used doses that were higher than all the three doses (recalculated to quercetin aglycone) requested by NFSA to evaluate. Thus, based on these results, VKM considers that exposure to the requested doses does not pose a risk at least up to 3 months, or even up to 6 months.

Among the studies that did not report any subjectively obtained adverse effects, Ganio et al. (2010) did not observe any adverse effects after exposure to 1000 mg per day for 5 days, indicating no acute toxicity.

The studies with long-term exposure used doses of 245 mg per day for 3 months (Bazyar et al., 2023), 1700 mg per day for 2 months (Hirano et al., 2009) and 484 mg per day for 1 month (Shi & Williamson, 2016), without observing any adverse effects. With the same results, Egert et al. (2008) used daily doses of up to 134 mg for 2 weeks.

Thus, based on these results, VKM considers that exposure to the requested doses do not pose a risk taken daily for at least up to 2 months.

5.3 Summary of the risk characterization based on the RCTs and toxicity data

This risk characterization is based on a systematic review of 23 RCTs, which included both a few outcomes in a potentially adverse direction among the parameters measured in blood or urine, and adverse effects/events reported by the adult participants. VKM considers that exposure to the requested doses of 500 mg quercetin dihydrate or 25 mg rutin do not pose a risk taken daily for at least up to 3 months. Two of the included publications found no adverse effects after administration for up to 6-10 months. No acute toxicity of a single or short-term (5-7 days) exposure was indicated by the results.

Quercetin and rutin are not genotoxic *in vivo*, and based on weight of the available evidence in animal studies, are not found to be carcinogenic even in much higher doses than the doses VKM was requested to evaluate.

Some data in humans and mice indicated that *O*-(β-hydroxyethyl)-rutoside (HER) and quercetin, respectively, may induce teratogenic effects in the offspring. HER treatment with oral doses of 900-1000 mg HER per day (corresponding to 414-460 mg quercetin aglycone per day or 837-930 mg rutin per day (corresponding to 7-8 mg quercetin aglycone/kg bw per day or 14-16 mg rutin/kg bw per day, for a 60 kg woman) for 3-5 weeks during the second and/or third month of pregnancy was found to be associated with a higher risk of certain congenital abnormalities in a case-control study. Similarly, malformation of the limbs the offspring (syndactyly) was found in mice after exposure to approximately 67 mg/kg bw of quercetin for about two weeks during gestation.

Regarding these teratogenic effects, they were observed at similar doses in humans (approximately 7-8 mg quercetin aglycone/kg bw), but at a higher dose in mice (about 67 mg/kg bw per day), compared with the daily dose of 500 mg quercetin dihydrate (445 mg quercetin aglycone, or 7.4 mg/kg bw, for a 60 kg woman) VKM was requested to evaluate.

Because of the lack of sufficient data on pregnant women and their fetuses, and the lack of data on breast-feeding women and their infants, as well as on children in general, it is not known whether these groups may potentially be more susceptible to these substances than adults.

Table 5.3-1 Results for adverse effects in the included RCTs, sorted by Categories and Tiers.

Authors, year	Category	Tier	Substance	Oral dose (mg per day)	Dose as quercetin aglycone (Q) (mg per day, rounded values)	Study duration	Study (n) in treatment(s) and control groups, respectively	Participants' characteristics	Treatment-related adverse effects
Category 1, Tier 1									
Ganio et al., 2010	2	1	Q	1000	1000	5 days	11 (5M, 6F)	Sedentary and untrained volunteers	No
Han et al., 2020	1(2)[1]	1	Q	50, 1000, 2000	50, 1000, 2000	7 days	6 (4M, 2F), 3 (1M, 2F), 2/dose	Patients with chronic obstructive pulmonary disease (COPD)	No
Egert et al., 2009	1(2)[1]	1	QD	150	134	6 weeks	93 (42M, 51F)	Overweight with a high-cardiovascular disease risk phenotype (metabolic syndrome)	Decreased HDL-cholesterol
Pfeuffer et al., 2013	1	1	QD	150	134	8 weeks	49M (19APOE3/3, 30APOE4)	Healthy men with varying apolipoprotein E (APOE) genotypes	Increased TNF-α. Night sweat
Riva et al., 2019	1(2)[1]	1	Q or QP	500Q, 250QP, 500QP	500, 5000, 10000	single dose	12 (M, F)	Healthy volunteers	No
Boyle et al., 2000	1	1	Rutin	500	245	6 weeks	16F (8F, 8F)	Healthy non-obese normocholesterolaemic volunteers	No
Erlund et al., 2000	1(2)[1]	1	Q (total) or rutin (total)	8, 20, 50 (78) or 16, 40, 100 (156)	8, 20, 50 (78) or 8, 20, 49 (76)	single dose	12 (7M, 5F)	Healthy volunteers	No
Bergqvist et al., 1981	1(2)[1]	1	HER	1500+1000 i.v.	690+460 i.v.	4 weeks	71 (15M, 56F), 72 (12M, 60F)	Patients with chronic venous insufficiency	No
Nakamura et al., 2022	1(2)[1]	1	EMIQ (as isoquercitrin)[2]	110	623	40 weeks	ss 80 (40M+F/group), pp 33 (10M, 23F), 35 (13M, 22F)	Healthy volunteers	No

Authors, year	Category	Tier	Substance	Oral dose (mg per day)	Dose as quercetin aglycone (Q) (mg per day, rounded values)	Study duration	Study (n) in treatment(s) and control groups, respectively	Participants' characteristics	Treatment-related adverse effects
Yasutake et al., 2015	1(2)[1]	1	EMIQ (as isoquercitrin)[2]	330	1870	4 weeks	24 (12M, 12F), 24 (12M, 12F)	Healthy adults 20-64 years with BMI ≥ 18.5 and < 30.0 kg/m^2	No
Yoshimura et al., 2008	1(2)[1]	1	EMIQ (as isoquercitrin)[2]	275	1558	12 weeks	100 (51 M, 49F), 100 (51M, 49F)	Overweight and obese adults 20-65 years with BMI 24-31 kg/m^2	No
Yoshimura et al., 2012	1(2)[1]	1	EMIQ (as isoquercitrin)[2]	110	623	24 weeks	57 (28M, 29F), 59 (30 M, 29F)	Overweight and obese adults 20-66 years with BMI ≥ 25 - < 30 kg/m^2	No
Category 1, Tier 2									
Shatylo et al., 2021	1(2)[1]	2	Q	240	240	3 months	55 (16M, 39F), 55 (10M, 45F)	Elderly patients with metabolic syndrome	Decreased GSH
Yamada et al., 2022	1(2)[1]	2	QP	200	4000	4 weeks	ss 32 (13M, 19F), 32 (17M, 15F), pp 30, 30	Persons with allergic symptoms of pollinosis	No
Kienzler et al., 2002	1(2)[1]	2	HER	500, 1000, 2000, 4000	230, 460, 920, 1840	single dose	16 (M, F)	Healthy male and female volunteers	No
Annoni et al., 1986	2	2	HER	4000	1840	28 days	20 (12M, 8F), 20 (11M, 9F)	Male and female patients with haemorrhoids	No
Cappelli et al., 1987	2	2	HER	3000	1380	28 days	20F (10, 10)	Women taking oral contraceptives and suffering from venous insufficiency of the lower limbs	No
Category 2, Tier 1									
Egert et al., 2008	2	1	QD	50, 100, 150	45, 89, 134	2 weeks	35 (18M, 17F)	Male and female healthy volunteers	No

Authors, year	Category	Tier	Substance	Oral dose (mg per day)	Dose as quercetin aglycone (Q) (mg per day, rounded values)	Study duration	Study (n) in treatment(s) and control groups, respectively	Participants' characteristics	Treatment-related adverse effects
Shi & Williamson, 2016	2	1	QD	544	484	4 weeks	22M (9, 13)	Men with non-optimal but still healthy, blood uric acid levels	No
Bazyar et al., 2023	2	1	Rutin	500	245	3 months	25 (12M, 13F), 25 (11M, 14F)	Male and female patients with type 2 diabetes mellitus	No
Hirano et al., 2009	2	1	EMIQ[3]	100	1700	8 weeks	12 (9M, 3F), 12 (10M, 2F)	Males and females with Japanese cedar pollinosis	No
Category 2, Tier 3									
Shoshkes et al., 1999	2	3	Q	1000	1000	1 month	28M (15, 13)	Men with prostate related pelvic pain	No
Di Pierro et al., 2021	2	3	Q	400	400[4]	30 days	76 (42M, 34F), 76 (46M, 30F)	Male and female COVID-19 outpatients	No

[1]Means that adverse effects were obtained with both objective (Category 1) and subjective (Category 2) methods.
[2]Isoquercitrin: n = 1 glucose.
[3]EMIQ (n = 1-8 glucose).
[4]The dose of Quercetin Phytosome® used was stated to correspond to about 2.5 times less quercetin.
In **bold**: Eight publications with study of subjectively obtained results reported adverse event/effects with at least some detail about the observations. However, the reported effects/events were all of minor severity and were considered not to be study drug-related, or the type and numbers of reported effects/events were similar between the treatment and control groups, each occurred in only one person, they were self-resolving or did not show a dose-response.
In *italic*: Among the publications which reported objectively obtained results, a single sporadic effect in the direction of adversity but of mild severity (decrease in HDL-cholesterol, increased levels of TNFα and decreased GSH level, respectively) was observed in one publication each. The first two effects were not supported by other endpoints measured in the same study and the third effect could be interpreted as not adverse. In addition, these effects were not reported in the other included studies.
Q: quercetin.
QD: quercetin dihydrate.
QP: Quercetin Phytosome®.
HER: *O*-(β-hydroxyethyl)-rutoside (Venoruton®).
EMIQ: enzymatically modified quercetin glycoside(s) with varying numbers of glucose molecules.

5.4 Supporting evidence

5.4.1 Excluded RCTs because of lack of details on adverse effects (Category 3)

Twenty-two publications were initially included as full-text in our systematic literature review of adverse health effects, but only briefly mentioned "no adverse effects/events/side-effects reported", without any information on how the data on such effects were recorded or any details on the results. These were considered to contain Category 3 results and excluded from the main results in Chapter 4.5.2, see Chapter 4.5.1.5. These 22 publications include 618 subjects in intervention groups receiving doses of various substances related to quercetin or rutin in the range from 100-2000 mg per day administered either as a single dose, or daily from some days and up to 12 weeks. The population groups include adults, healthy volunteers and various patient groups of various ages. All Category 3 studies are listed in Table 11-2 in Chapter 11 Appendix II.

5.4.2 Studies in which administration also included vitamins

David C. Nieman and colleagues have performed a double-blinded randomized controlled clinical study to identify potential beneficial effects of quercetin supplementation in a large number of individuals (n = 1002). Shanely et al. (2010) was the first of a total 8 publications (7 occurred in our literature search) from this study. Participants received placebo, 500 mg quercetin per day or 1000 mg quercetin per day for 12 weeks. The study included male and female subjects varying in age (18-85 years) with body mass index ranging from 16.7–52.7 kg/m^2. The number of individuals in the groups were (total = 1002): placebo (n = 335; male = 123, female = 212), 500 mg quercetin (n = 334; male = 138, female = 196) and 1000 mg quercetin (n = 333; male = 134, female = 199). Subjects recruited to the study had to be non-institutionalized and women were excluded if pregnant or lactating. No other exclusion criteria were employed and both diseased and healthy subjects were admitted into the study, with monitoring of disease status and medication use. Thirty-seven percent of the subjects reported past or current history for one or more chronic diseases: hypertension (19%), arthritis (16%), cancer (6%), cardiovascular disease (4%) and diabetes (4%). Hence, the RCT may be considered designed to detect potential adverse effects at the population level (across age, gender and common diseases), although the objective of this publication was to evaluate beneficial effects.

All manuscripts from this study were, however, excluded from our systematic review since they failed to meet several of our inclusion criteria. Quercetin was given together with vitamin C and niacin, since the authors claimed that such co-administration increases the uptake of quercetin. Interpretation of the effects of only quercetin intake will not be possible since these ingredients were not added to the placebo group. However, subjects completed a monthly log to verify adherence to the supplementation regimen, physical activity and diet status, change in disease status and medication use, gastrointestinal (constipation, heartburn, bloating, diarrhea, nausea, vomiting), skin (rash, dryness, flushing), allergy and mental symptoms (energy, headache, stress, focus/concentration). Only a brief statement, such as "no

adverse effects were reported", was given in some of the publications, which made it difficult to evaluate the quality of the results behind this statement. It is relevant to mention a potential conflict of interest with these publications. The study received support from Coca-Cola and Quercegen, and David C. Nieman is a board member of Quercegen Pharma. Despite these limitations, VKM will briefly summarize outcomes from the studies on this cohort, because of the large number of individuals (final n = 1002). This study was not evaluated for risk of bias using OHAT. Vitamin C may also have a beneficial effect per se, as shown by Askari et al. (2012) where the anti-inflammatory effect by the combination of quercetin and vitamin C actually was due to the vitamin C and not seen for the quercetin exposure only.

Of relevance for risk assessments, of the 1023 subjects recruited into the study, 1002 completed all phases of the study. The 21 dropouts were evenly distributed (seven from the placebo group, six from the 500 mg quercetin per day group and eight from the 1000 mg quercetin per day group). Twelve participants failed to take the supplement and/or adhere to testing procedures and nine reported adverse symptoms from taking the supplement. Follow-up revealed no consistent pattern of symptoms that could be ascribed to the quercetin supplements. Plasma levels of quercetin (overnight fasted) were ~100 µg/l for all groups at the start of the intervention and increased to ~150 µg/l in the placebo group, ~400 µg/l in the group receiving 500 mg quercetin per day and ~600 µg/l in the group receiving 1000 mg quercetin per day (Shanley et al., 2010). The increase in plasma quercetin was highly variable within each quercetin supplementation group but was unrelated to age, gender, BMI, fitness levels or diet intake. The fluctuations in plasma quercetin levels pre- and post-study were considerably lower in the placebo group compared to the two quercetin supplemental groups (Jin et al., 2010).

The beneficial effects that were evaluated within this cohort in the eight publications are described in Chapter 12.2 Appendix III.

Even if the effects from quercetin could not be separated from the other bioactive compounds administered simultaneously, VKM remarks that any potential frequently occurring adverse effect of quercetin supplementation would be expected to be observed in this study, given the large number of participants of both sex of various ages and with many individuals being affected by diseases frequently occurring in the common population. The lack of any reported adverse effects, nor any alterations in a broad range of measured biological markers relevant for safety, supports the findings in the risk characterisation that the risk of quercetin supplementation at this dose level is low, at least with exposure up to 12 weeks.

5.4.3 The only available study on children

One open-label pilot study with objective of assessing the effectiveness and tolerability of a mixed supplement in children with autism spectrum disorders (ASD) was found (Taliou et al., 2013). The dietary supplement as soft gel capsules containing luteolin from chamomile (100 mg/capsule), the quercetin glycoside rutin (30 mg/capsule) and quercetin (70 mg/capsule) from *Sohora japonica* leaves, was administered to two age groups of children 4-6 years old (n = 25) and 7-10 years old (n = 25), in total 42 boys and 8 girls, for 26 weeks. The doses were one soft gel capsule per 10 kg bw per day with food. This study was excluded from the systematic screening of the obtained

literature since it contained a mixture of substances, but was included in this assessment since it was the only study found on children for either quercetin or rutin. This study was not evaluated for risk of bias using OHAT.

Both flavonoids were >95% pure. The dose administered in this study was one capsule per 10 kg body weight per day. Since study-specific body weights were not reported, the doses were calculated to be approximately in the range of 42-65 mg for rutin, 98-152 mg for quercetin and 140-217 mg for luteolin per day based on default values from EFSA (2012) for these age groups of children (Table 3.1-2).

The children were evaluated at the baseline visit, mid-trial visit at 18 weeks and final visit at 26 weeks. Parents were interviewed for any possible improvements they noticed and instructed to report any unusual adverse events. Adverse events were systematically recorded on an adverse event form by using scales indicating severity, relationship to the study procedures, action taken and any therapy required. A total of 40 children (35 boys and 5 girls) completed the study by protocol. Six children withdrew from the study due to increased irritability caused by the formulation (2 of them were good responders to the formulation; 2 others had not responded until the mid-visit); 1 showed poor compliance; parents were unable to administer the capsule or its content in 2 children; and in 1 case, the parents decided not to participate after completing the baseline assessment. The most frequent adverse effect was an increased irritability (27 of 50), of various durations, which led to study withdrawal in 6 participants. Irritability was usually transient, lasting 1 to 8 weeks in 66% of cases; in most cases, it started 2 to 7 days after the first administration (20 of 27). In one subject, however, it started 2 months after onset of treatment. In some cases, it was addressed with reduction or splitting the dosage over the day. Less frequent adverse events were noted in 12 children and included events such as disorientation, abdominal pain, increased frequency of urination, sleeping difficulties, increased appetite. The authors concluded that the only adverse effect noted was transient irritability.

Based on the much higher doses of rutin (in addition to the high dose of quercetin, into which rutin is metabolised) given in this study, VKM considers that a dose of 5 mg rutin per day up to up to 6.5 months will not cause adverse effects in children other than possibly transient irritability.

In addition to the endpoints reported in the studies discussed above, a large number of various other endpoints have been reported in the publications as part of their objective to evaluate beneficial effects, without observing any adverse effects. These endpoints were not evaluated for risk of bias using OHAT. However, this information adds to the evidence regarding the safety of the requested doses and can be found in Table 12-1 in Chapter 12.1 Appendix III.

5.5 Vulnerable groups

In a human case-control study by Pósfai et al. (2014), HER treatment with oral doses of 900-1000 mg HER per day (corresponding to 414-460 mg quercetin aglycone per day or 837-930 mg rutin per day) for 3-5 weeks during the second and/or third month of pregnancy was found to be associated with a higher risk of unilateral ocular coloboma and a new congenital abnormality syndrome including anotia/microtia, poly-

/syndactyly and caudal (genital and anal) defects. Similar effects (syndactyly) were found in mice after exposure to approximately 67 mg/kg bw of quercetin for about two weeks during gestation (Prater et al., 2008). Thus, these data indicate that the developing fetus may be vulnerable to adverse effects of quercetin.

No studies were found on the effects of these substances on young children (<4 years of age) and there were no studies available on breastfeeding women.

Because of the lack of sufficient data on pregnant and breastfeeding women, and their fetuses/infants, as well as on children in general, it is not known whether these groups may potentially be more susceptible to these substances than adults.

Although there are no human studies indicating adverse effects on the kidneys and the human relevance is not clear, some evidence in rats indicate that especially high doses (above 400 mg/kg bw in males and 1900 mg/kg bw in females) of quercetin may increase nephrotoxicity, primarily in rats with existing chronic nephropathy (NTP, 1992).

Potential tumor promoting effects of quercetin, primarily in estrogen-dependent cancers, are indicated by animal studies (Andres et al., 2018).

Thus, some data indicate that persons with chronic nephropathy or estrogen-dependent cancer may be vulnerable to adverse effects of quercetin.

5.6 Drug interactions

Andres et al. (2018) summarized information on drug interaction of quercetin. In humans, varying results were observed with single or repeated intake of 300–1500 mg quercetin per day. No significant changes of drug bioavailability were observed for nifedipine (antihypertensive drug), rosiglitazone (antidiabetic drug), saquinavir (anti-HIV drug), digoxin (heart medication), warfarin (anticoagulant) or cefprozil (antibiotic drug). Reduced bioavailability (or only a non-significant statistical trend) was reported for midazolam (sedative) and talinolol (antihypertensive drug). Increased bioavailability was observed for cyclosporine (an immunosuppressive agent), pravastatin (cholesterol-lowering drug) and fexofenadine (antihistamine drug). Also in animals, quercetin has been shown to interact with a long list of various drugs, sometimes with different results compared with humans.

Quercetin may modulate the activity of several drug-metabolising enzymes. In a human cross-over trial with male volunteers (n=12), it was shown that quercetin (500 mg per day in 13 days) inhibited cytochrome P450 (CYP) 1A2 activity, but enhanced CYP2A6, *N*-acetyltransferase (NAT2) and xanthine oxidase (XO) activities (Chen et al., 2009).

Regarding mechanisms for drug interactions with quercetin or rutin, increased absorption of drugs may be related to inhibition of the cytochrome P450 enzyme CYP3A4, which is responsible for metabolism of many drugs, and by inhibition of the multidrug membrane-bound P-glycoprotein (P-gp) efflux pump in the intestinal mucosa, as shown for the cancer-chemotherapeutic drug paclitaxel (Kumar et al., 2015; Andres et al., 2018).

6 Uncertainties

The main objective in the majority of the RCTs that were available for this risk assessment was to detect beneficial effects of the food supplements under study. In such publications, there may be a risk for less stringent inclusion of observed adverse effects. This issue is discussed in Chapter 19 on the Adverse effects in Cochrane Handbook for Systematic Reviews of Interventions (Higgins et al., 2023). Across multiple investigations of published versus unpublished studies, Golder et al. (2016) found a median of 43% of published studies reported adverse events data, compared with a median of 83% of unpublished studies. A wider range of specific adverse events was found in sources other than published journal publications. In addition, when published and unpublished reports of the same study were compared, it was shown that the unpublished version was more likely to contain adverse effects data (median 95%) compared with the published version (median 46%). Similarly, a study of an obesity drug (Orlistat or Xenical) by Schroll et al. (2016) compared study documents (protocol, clinical study report and published report) and identified important inconsistencies. For example, adverse events in published studies were coded to appear less severe, with reduced incidence, compared with events reported in the unpublished clinical study reports. Of the total number of adverse events reported by trial investigators in clinical study reports, between 3% and 33% were subsequently reported in the corresponding published journal publications. However, in this risk assessment, very few of the publications had as objective to assess adverse effects of quercetin or rutin. This is often the case for "other substances".

In addition to the administered dose(s), the actual exposure to quercetin or rutin is determined by their purity and stability. In the applications to NSFA that were the reason for the request to VKM for this risk assessment, one application specified quercetin dihydrate (on anhydrous basis) with purity of 95% and another application specified rutin with ≥98% purity, demonstrating that these substances are available with quite high purity. Shelf-life of both quercetin and rutin was stated to be two years, indicating a reasonable stability of these compounds in food supplements.

However, very few of the included studies stated the purity of the quercetin or rutin products used, neither was this information found on the producer's website, when the producers' names occasionally were stated in the publications. Information on stability was even more rarely stated in the available publications.

Quercetin may undergo several chemical changes under food processing and storage, such as oxidation (Wang et al., 2016). Chemical stability of quercetin is influenced by factors such as oxygen concentration, pH value, temperature, concentrations of other antioxidants, presence of metal ions and storage time.

The lack of information on purity and stability of the quercetin and rutin administered in the included publications contribute to the uncertainty of the doses actually causing the reported effects or the lack of reported affects.

Expert judgement was used to categorize the included publications into Category 1, 2 and 3, and scoring of internal bias using the OHAT tool for the Category 1 and 2 studies. The evaluations and grouping of studies into the three categories were done by at least two persons, or when needed, discussed in the whole project group.

However, if one or more studies have not been placed in the most appropriate Category, this should not affect the overall risk evaluation and our conclusions.

The overall weight of evidence for the absence of adverse effects based on the RCTs obtained in the systematic literature review of quercetin or rutin was found to be "moderate" by expert judgement.

The modified substances of quercetin and rutin used in the included studies varied in their molecular weight and bioavailability. To be able to use these studies in the risk assessment, the given doses of these substances were recalculated to the corresponding dose of the common substance quercetin aglycone, into which all the related substances are metabolized. This enabled comparison of the doses related to effects of these modified substances with the doses requested by NSFA, by taken into account differences in molecular weight and information from pharmacokinetic studies in the literature. However, mostly only one pharmacokinetic study was available per modified substance and, therefore, there is some uncertainty regarding the general applicability of this information, affecting our calculations of the quercetin and rutin exposure.

Shatylo et al. (2021) stated that although quercetin was shown to cause no substantial toxicity in experimental models, the risk for adverse side-effects could be non-negligible due to its quite narrow therapeutic dose window. This statement could not be examined further due to lack of relevant data. Further, they stated that, in most *in vitro* models, toxic effects of quercetin appeared at concentrations as low as 1 µM, which is only 2-5 times higher than that shown to produce the largest therapeutic effect. However, *in vitro* studies were not included in the present risk assessment.

In this risk assessment, the information from human studies was mostly from RCTs. In addition, some information about ADME and toxicity were obtained from experimental animal studies. Thus, additional information from human studies with other designs was not included in this risk assessment.

7 Conclusions (with answers to the terms of reference)

NFSA asked VKM to assess whether rutin and quercetin dihydrate in the quantities and the age groups specified below, might pose a health risk for the Norwegian population, and asked VKM specifically to consider daily intake of

- 5 mg rutin (CAS number 153-18-4) per recommended daily dose in food supplements intended for children 4 years of age and older,
- 25 mg rutin (CAS number 153-18-4) per recommended daily dose in food supplements intended for adults from 18 years of age, and
- 500 mg quercetin dihydrate (CAS number 6151-25-3) per recommended daily dose in food supplements intended for adults from 18 years of age.

Conclusions

Based on a systematic review of RCTs examining effects of quercetin or rutin, which resulted in the inclusion and evaluation of 23 publications with adult participants, VKM considers that exposure to the requested doses (500 mg quercetin dihydrate or 25 mg rutin) does not pose a risk taken daily for at least up to 3 months by adults. Two of the included publications found no adverse effects after administration for up to 6-10 months. No acute toxicity of a single or short-term (5-7 days) exposure was indicated by the results.

No specific treatment-related and dose-dependent adverse effects could be identified from the included studies which reported a few outcomes in a potentially adverse direction among the parameters measured in blood or urine, and adverse effects/events reported by the participants. The weight of evidence for absence of adverse effects related to quercetin or rutin based on the 23 included RCTs is considered to be moderate.

None of the included studies investigated exposure specifically in children. None of the included studies compared susceptibility to adverse effects in adults and children. Based on the results for adults and supporting evidence from one excluded study with higher daily doses (approximately 40-70 mg rutin plus 100-150 mg quercetin) for 6.5 months, VKM concludes that 5 mg rutin per day up to 6.5 months will not cause adverse effects in children other than possibly transient irritability.

Some data indicated that *O*-(β-hydroxyethyl)-rutoside (HER) and quercetin may induce teratogenic effects in offspring, shown in humans and mice, respectively. Regarding these teratogenic effects, they were observed at similar doses in humans, but at a higher dose in mice, compared with the dose of quercetin dihydrate VKM was requested to evaluate.

Because of the lack of sufficient data on pregnant women and their fetuses, and the lack of data on breast-feeding women and their infants, as well as on children in general, it is unclear whether these groups may potentially be more susceptible to these substances than adults.

Some data indicate that persons with chronic nephropathy or estrogen-dependent cancer may be vulnerable to adverse effects of quercetin.

Uncertainties and data gaps are described in Chapters 6 and 8, respectively.

8 Data gaps

There were few publications that evaluated the adverse effects of quercetin and rutin as the main objective. Furthermore, many of the included studies were small and of short duration, even some with single dose administration.

Very little data were found on the effects of quercetin and rutin on children and pregnant women, and no data on adolescents and breastfeeding women.

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10 Appendix I – Literature search strategies

10.1 Literature searches quercetin

Database: **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions <1946 to September 13, 2023>**
Date: 14.09.2023
Hits: 90 SR and 641 RCT

1	Quercetin/	12108
2	(Quercetin or quercetine or Quercetindihydrat? or Quercetinedihydrat? or dikvertin or dikvertine or flavin or flavine or hippuroflavin or hippuroflavine or meletin or meletine or meltin or meltine or quercetol or quercetole or quercitin or quercitine or quertin or quertine or sophoretin or sophoretine or "Pentahydroxyflavone dihydrat?" or 6151-25-3 or 117-39-5 or "6151253" or "117395" or cas6151253 or cas117395).tw,kf,nm.	42620
3	1 or 2	42620
4	Dietary Supplements/ or Diet/ or Food/ or Eating/	332773
5	(oral or diet* or supplement? or intake or ingestion? or eat*).tw,kf.	1735480
6	4 or 5	1830453
7	3 and 6	6047
8	Animals/ not (animals/ and humans/)	5120641
9	7 not 8	4036
10	limit 9 to "reviews (maximizes specificity)"	69
11	9 and (Meta-Analysis/ or Network Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.)	83
12	10 or 11	90
13	limit 9 to "therapy (maximizes specificity)"	186
14	9 and (("randomized controlled trial" or "controlled clinical trial").pt. or (randomized or randomised or randomly or rct or placebo or trial or groups).tw,kf,bt.)	673
15	13 or 14	673
16	15 not 12	641

Database: **Embase <1974 to 2023 September 13>**
Date: 14.09.2023
Hits: 370 SR and 1003 RCT

1	Quercetin/	43657
2	(Quercetin or quercetine or Quercetindihydrat? or Quercetinedihydrat? or dikvertin or dikvertine or flavin or flavine or hippuroflavin or hippuroflavine or meletin or meletine or meltin or meltine or quercetol or quercetole or quercitin or quercitine or quertin or quertine or sophoretin or sophoretine or "Pentahydroxyflavone dihydrat?").tw,kf.	44198
3	(6151-25-3 or 117-39-5).rn,tw,kf. or ("6151253" or "117395" or cas6151253 or cas117395).tw,kf.	41119
4	1 or 2 or 3	61879
5	dietary supplement/ or exp diet/ or exp food/ or food intake/ or eating/	1639081
6	(oral or diet* or supplement? or intake or ingestion? or eat*).tw,kf.	2276841

7	5 or 6	3259507
8	4 and 7	18282
9	limit 8 to (conference abstracts or embase or "preprints (unpublished, non-peer reviewed)")	15503
10	(animal/ or exp nonhuman/ or Animal experiment/) not ((animal/ or exp nonhuman/ or Animal experiment/) and exp human/)	6791910
11	9 not 10	8847
12	limit 11 to "reviews (maximizes specificity)"	156
13	11 and (exp Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.)	370
14	12 or 13	370
15	limit 11 to "therapy (maximizes specificity)"	291
16	limit 11 to (randomized controlled trial or controlled clinical trial)	267
17	11 and (randomized or randomised or randomly or rct or placebo or trial or groups).tw,kf,bt.	1034
18	15 or 16 or 17	1098
19	18 not 14	1003

Database: **Web of Science Core Collection: Science Citation Index Expanded (SCI-EXPANDED) --1987-present, Social Sciences Citation Index (SSCI) --1987-present, Arts & Humanities Citation Index (A&HCI) --1987-present, Emerging Sources Citation Index (ESCI) --2015-present**
Date: 14.09.2023
Hits: 91 SR and 875 RCT

1	TS=(Quercetin or quercetine or Quercetindihydrat$ or Quercetinedihydrat$ or dikvertin or dikvertine or flavin or flavine or hippuroflavin or hippuroflavine or meletin or meletine or meltin or meltine or quercetol or quercetole or quercitin or quercitine or quertin or quertine or sophoretin or sophoretine or "Pentahydroxyflavone dihydrat$" or "6151-25-3" or "117-39-5" or 6151253 or 117395 or cas6151253 or cas117395) *Exact search*	54325
2	TS=(oral or diet* or supplement$ or intake or ingestion$ or eat*) *Exact search*	2034340
3	#2 AND #1	9027
4	TS=((animal or animals or canine* or dog or dogs or feline or hamster* or lamb or lambs or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) NOT (human* or patient*)) *Exact search*	3480347
5	#3 NOT #4	6127
6	TS=((systematic* NEAR/1 review*) or (review and ((structured or database* or systematic*) NEAR/1 search*)) or "integrative review*" or (evidence NEAR/1 review*)) OR TI=(metaanal* or "meta anal*") OR AB=(metaanal* or "meta anal*") *Exact search*	582483
7	#6 AND #5	91
8	TS=(randomized or randomised or randomly or rct or placebo or trial or groups) *Exact search*	4746027
9	#8 AND #5	911

10	#9 NOT #7	875

Database: **Epistemonikos**
Date: 14.09.2023
Hits: 60 SR (3 Broad Synthesis, 0 Structured Summary, 57 Systematic Review), 197 RCT (Primary study)

Search 1 in Broad Synthesis, Structured Summary, Systematic Review
Title/Abstract: (Quercetin or quercetine or Quercetindihydrat* or Quercetinedihydrat* or dikvertin or dikvertine or flavin or flavine or hippuroflavin or hippuroflavine or meletin or meletine or meltin or meltine or quercetol or quercetole or quercitin or quercitine or quertin or quertine or sophoretin or sophoretine or "Pentahydroxyflavone dihydrat" or "Pentahydroxyflavone dihydrate" or "6151-25-3" or "117-39-5" or "6151 25 3" or "117 39 5" or 6151253 or 117395 or cas6151253 or cas117395) AND (oral or diet* or supplement or supplements or intake or ingestion or ingestions or eat*)

Search 2 in Primary Study
Title/Abstract: (Quercetin or quercetine or Quercetindihydrat* or Quercetinedihydrat* or dikvertin or dikvertine or flavin or flavine or hippuroflavin or hippuroflavine or meletin or meletine or meltin or meltine or quercetol or quercetole or quercitin or quercitine or quertin or quertine or sophoretin or sophoretine or "Pentahydroxyflavone dihydrat" or "Pentahydroxyflavone dihydrate" or "6151-25-3" or "117-39-5" or "6151 25 3" or "117 39 5" or 6151253 or 117395 or cas6151253 or cas117395) AND (oral or diet* or supplement or supplements or intake or ingestion or ingestions or eat*) AND (randomized or randomised or randomly or rct or placebo or trial or groups)

Database: **Cochrane Database of Systematic Reviews: Issue 9 of 12, September 2023, Central Register of Controlled trials: Issue 8 of 12, August 2023**
Date: 14.09.2023
Hits: 0 SR (CDSR), 405 RCT (Trials)

#1	[mh ^Quercetin]	242
#2	(Quercetin or quercetine or Quercetindihydrat? or Quercetinedihydrat? or dikvertin or dikvertine or flavin or flavine or hippuroflavin or hippuroflavine or meletin or meletine or meltin or meltine or quercetol or quercetole or quercitin or quercitine or quertin or quertine or sophoretin or sophoretine or (Pentahydroxyflavone NEXT dihydrat?) or "6151-25-3" or "117-39-5" or "6151253" or "117395" or cas6151253 or cas117395):ti,ab	612
#3	#1 or #2	655
#4	[mh ^"Dietary Supplements"]	14118
#5	[mh ^Diet]	11332
#6	[mh ^Food]	2459
#7	[mh ^Eating]	3693
#8	(oral or diet* or supplement? or intake or ingestion? or eat*):ti,ab	300879
#9	#4 or #5 or #6 or #7 or #8	307838
#10	#3 AND #9	405

10.2 Literature searches rutin

Database: **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Daily and Versions <1946 to September 13, 2023>**
Date: 14.09.2023
Hits: 11 SR and 177 RCT

1	rutin/ or hydroxyethylrutoside/	4075
2	(rutin or rutoside or rutaside or rutocide or rutabion or rutinion or rutozyd or birutan or citroflavone or eldrin? or farutin? or globulariacitrin? or melin or myrticolorin? or "neorutin 300" or osyritin? or osyritrin? or phytomelin? or sclerutin? or sophorin? or tanrutin? or violaquercitrin? or "vitamin P" or "vitamin P4" or (("rhamnosyl glucosyl" or rhamnosylglucosyl or rhamnoglucoside or rutinoside) adj2 quercetin?) or "pentahydroxyflavone 3 rhamnoglucoside" or hydroxyethylrutoside or hydroxyethylrutaside or hydroxyethylrutin? or "hydroxyethyl rutine" or "trihydroxyethyl rutine" or trihydroxyethylrutin? or trihydroxyethylrutoside or trihydroxyethylrutaside or venoruton or rutorbin? or ruhexatal or oxerutin? or Paroven or Posorutin? or Relvene or Rheoflux or Teboven or Troxerutin? or Troxeven or Vastribil or Veinamitol or "Veniten retard" or "Veno SL" or Venorutin? or "Venotrulan Trox" or "neo semhyten" or neosemhyten or raumannite or (maxitate adj2 Rauwolfia) or isonaringin? or monoxerutin? or narirutin? or benrubin? or cilkanol or troxevasin? or varemoid or 153-18-4 or 22519-99-9 or 23869-24-1 or 55965-63-4 or 14259-46-2 or 7085-55-4 or 84932-19-4 or "153184" or "22519999" or "23869241" or "55965634" or "14259462" or "7085554" or "84932194" or cas153184 or cas22519999 or cas23869241 or cas55965634 or cas14259462 or cas7085554 or cas84932194).tw,kf,nm.	10496
3	1 or 2	10496
4	Dietary Supplements/ or Diet/ or Food/ or Eating/	332773
5	(oral or diet* or supplement? or intake or ingestion? or eat*).tw,kf.	1735480
6	4 or 5	1830453
7	3 and 6	1616
8	Animals/ not (animals/ and humans/)	5120641
9	7 not 8	1023
10	limit 9 to "reviews (maximizes specificity)"	8
11	9 and (Meta-Analysis/ or Network Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.)	11
12	10 or 11	11
13	limit 9 to "therapy (maximizes specificity)"	57
14	9 and (("randomized controlled trial" or "controlled clinical trial").pt. or (randomized or randomised or randomly or rct or placebo or trial or groups).tw,kf,bt.)	185
15	13 or 14	185
16	15 not 12	177

Database: **Embase <1974 to 2023 September 08>**
Date: 11.09.2023
Hits: 82 SR and 329 RCT

1	monoxerutin/ or narirutin/ or rutoside/ or rutoside derivative/ or troxerutin/ or ascorbic acid plus mannitol hexanitrate plus reserpine plus rutoside/ or ascorbic acid plus mannitol hexanitrate plus reserpine plus rutoside plus theophylline/ or ascorbic acid plus rutoside/ or mannitol hexanitrate plus phenobarbital plus rauwolfia extract plus rutoside/ or mannitol hexanitrate plus rauwolfia extract plus rutoside/	15674
2	(rutin or rutoside or rutaside or rutocide or rutabion or rutinion or rutozyd or birutan or citroflavone or eldrin? or farutin? or globulariacitrin? or melin or myrticolorin? or "neorutin 300" or osyritin? or osyritrin? or phytomelin? or sclerutin? or sophorin? or tanrutin? or violaquercitrin? or "vitamin P" or "vitamin P4" or (("rhamnosyl glucosyl" or rhamnosylglucosyl or rhamnoglucoside or rutinoside) adj2 quercetin?) or "pentahydroxyflavone 3 rhamnoglucoside" or hydroxyethylrutoside or hydroxyethylrutaside or hydroxyethylrutin? or "hydroxyethyl rutine" or "trihydroxyethyl rutine" or trihydroxyethylrutin? or trihydroxyethylrutoside or trihydroxyethylrutaside or venoruton or rutorbin? or ruhexatal or oxerutin? or Paroven or Posorutin? or Relvene or Rheoflux or Teboven or Troxerutin? or Troxeven or Vastribil or Veinamitol or "Veniten retard" or "Veno SL" or Venorutin? or "Venotrulan Trox" or "neo semhyten" or neosemhyten or raumannite or (maxitate adj2 Rauwolfia) or isonaringin? or monoxerutin? or narirutin? or benrubin? or cilkanol or troxevasin? or varemoid).tw,kf.	11410
3	(153-18-4 or 22519-99-9 or 23869-24-1 or 55965-63-4 or 14259-46-2 or 7085-55-4 or 84932-19-4).rn,tw,kf. or ("153184" or "22519999" or "23869241" or "55965634" or "14259462" or "7085554" or "84932194" or cas153184 or cas22519999 or cas23869241 or cas55965634 or cas14259462 or cas7085554 or cas84932194).tw,kf.	14761
4	1 or 2 or 3	18245
5	dietary supplement/ or exp diet/ or exp food/ or food intake/ or eating/	1637314
6	(oral or diet* or supplement? or intake or ingestion? or eat*).tw,kf.	2274715
7	5 or 6	3256396
8	4 and 7	6397
9	limit 8 to (conference abstracts or embase or "preprints (unpublished, non-peer reviewed)")	5410
10	(animal/ or exp nonhuman/ or Animal experiment/) not ((animal/ or exp nonhuman/ or Animal experiment/) and exp human/)	6788725
11	9 not 10	2673
12	limit 11 to "reviews (maximizes specificity)"	37
13	11 and (exp Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.)	82
14	12 or 13	82
15	limit 11 to "therapy (maximizes specificity)"	112
16	limit 11 to (randomized controlled trial or controlled clinical trial)	119
17	11 and (randomized or randomised or randomly or rct or placebo or trial or groups).tw,kf,bt.	334
18	15 or 16 or 17	359
19	18 not 14	329

Database: **Web of Science Core Collection: Science Citation Index Expanded (SCI-EXPANDED) --1987-present, Social Sciences Citation Index (SSCI) --1987-present, Arts & Humanities Citation Index (A&HCI) --1987-present, Emerging Sources Citation Index (ESCI) --2015-present**

Date: 11.09.2023

Hits: 10 SR and 191 RCT

1	TS=(rutin or rutoside or rutaside or rutocide or rutabion or rutinion or rutozyd or birutan or citroflavone or eldrin$ or farutin$ or globulariacitrin$ or melin or myrticolorin$ or "neorutin 300" or osyritin$ or osyritrin$ or phytomelin$ or sclerutin$ or sophorin$ or tanrutin$ or violaquercitrin$ or "vitamin P" or "vitamin P4" or (("rhamnosyl glucosyl" or rhamnosylglucosyl or rhamnoglucoside or rutinoside) NEAR/1 quercetin$) or "pentahydroxyflavone 3 rhamnoglucoside" or hydroxyethylrutoside or hydroxyethylrutaside or hydroxyethylrutin$ or "hydroxyethyl rutine" or "trihydroxyethyl rutine" or trihydroxyethylrutin$ or trihydroxyethylrutoside or trihydroxyethylrutaside or venoruton or rutorbin$ or ruhexatal or oxerutin$ or Paroven or Posorutin$ or Relvene or Rheoflux or Teboven or Troxerutin$ or Troxeven or Vastribil or Veinamitol or "Veniten retard" or "Veno SL" or Venorutin$ or "Venotrulan Trox" or "neo semhyten" or neosemhyten or raumannite or (maxitate NEAR/1 Rauwolfia) or isonaringin$ or monoxerutin$ or narirutin$ or benrubin$ or cilkanol or troxevasin$ or varemoid or "153-18-4" or "22519-99-9" or "23869-24-1" or "55965-63-4" or "14259-46-2" or "7085-55-4" or "84932-19-4" or "153184" or "22519999" or "23869241" or "55965634" or "14259462" or "7085554" or "84932194" or cas153184 or cas22519999 or cas23869241 or cas55965634 or cas14259462 or cas7085554 or cas84932194) *Exact search*	14011
2	TS=(oral or diet* or supplement$ or intake or ingestion$ or eat*) *Exact search*	2034105
3	#2 AND #1	2224
4	TS=((animal or animals or canine* or dog or dogs or feline or hamster* or lamb or lambs or mice or monkey or monkeys or mouse or murine or pig or pigs or piglet* or porcine or primate* or rabbit* or rats or rat or rodent* or sheep* or veterinar*) NOT (human* or patient*)) *Exact search*	3480154
5	#3 NOT #4	1440
6	TS=((systematic* NEAR/1 review*) or (review and ((structured or database* or systematic*) NEAR/1 search*)) or "integrative review*" or (evidence NEAR/1 review*)) OR TI=(metaanal* or "meta anal*") OR AB=(metaanal* or "meta anal*") *Exact search*	582284
7	#6 AND #5	10
8	TS=(randomized or randomised or randomly or rct or placebo or trial or groups) *Exact search*	4745496
9	#8 AND #5	193
10	#9 NOT #7	191

Database: **Epistemonikos**
Date: 11.09.2023
Hits: 9 SR (0 Broad Synthesis, 1 Structured Summary, 8 Systematic Review), 49 RCT (Primary study)

Search 1 in Broad Synthesis, Structured Summary, Systematic Review
Title/Abstract: (rutin or rutoside or rutaside or rutocide or rutabion or rutinion or rutozyd or birutan or citroflavone or eldrin or eldrine or farutin or farutine or globulariacitrin* or melin or myrticolorin* or "neorutin 300" or osyritin* or osyritrin* or phytomelin* or sclerutin* or sophorin* or tanrutin* or violaquercitrin* or "vitamin P" or "vitamin P4" or (("rhamnosyl glucosyl" or rhamnosylglucosyl or rhamnoglucoside or rutinoside) AND quercetin*) or "pentahydroxyflavone 3 rhamnoglucoside" or hydroxyethylrutoside or hydroxyethylrutaside or hydroxyethylrutin* or "hydroxyethyl rutine" or "trihydroxyethyl rutine" or

trihydroxyethylrutin* or trihydroxyethylrutoside or trihydroxyethylrutaside or venoruton or rutorbin* or ruhexatal or oxerutin* or Paroven or Posorutin* or Relvene or Rheoflux or Teboven or Troxerutin* or Troxeven or Vastribil or Veinamitol or "Veniten retard" or "Veno SL" or Venorutin* or "Venotrulan Trox" or "neo semhyten" or neosemhyten or raumannite or (maxitate AND Rauwolfia) or isonaringin* or monoxerutin* or narirutin* or benrubin* or cilkanol or troxevasin* or varemoid or 153-18-4 or 22519-99-9 or 23869-24-1 or 55965-63-4 or 14259-46-2 or 7085-55-4 or 84932-19-4 or 153184 or 22519999 or 23869241 or 55965634 or 14259462 or 7085554 or 84932194 or cas153184 or cas22519999 or cas23869241 or cas55965634 or cas14259462 or cas7085554 or cas84932194) AND (oral or diet* or supplement or supplements or intake or ingestion or ingestions or eat*)

Search 2 in Primary Study
Title/Abstract: (rutin or rutoside or rutaside or rutocide or rutabion or rutinion or rutozyd or birutan or citroflavone or eldrin or eldrine or farutin or farutine or globulariacitrin* or melin or myrticolorin* or "neorutin 300" or osyritin* or osyritrin* or phytomelin* or sclerutin* or sophorin* or tanrutin* or violaquercitrin* or "vitamin P" or "vitamin P4" or (("rhamnosyl glucosyl" or rhamnosylglucosyl or rhamnoglucoside or rutinoside) AND quercetin*) or "pentahydroxyflavone 3 rhamnoglucoside" or hydroxyethylrutoside or hydroxyethylrutaside or hydroxyethylrutin* or "hydroxyethyl rutine" or "trihydroxyethyl rutine" or trihydroxyethylrutin* or trihydroxyethylrutoside or trihydroxyethylrutaside or venoruton or rutorbin* or ruhexatal or oxerutin* or Paroven or Posorutin* or Relvene or Rheoflux or Teboven or Troxerutin* or Troxeven or Vastribil or Veinamitol or "Veniten retard" or "Veno SL" or Venorutin* or "Venotrulan Trox" or "neo semhyten" or neosemhyten or raumannite or (maxitate AND Rauwolfia) or isonaringin* or monoxerutin* or narirutin* or benrubin* or cilkanol or troxevasin* or varemoid or 153-18-4 or 22519-99-9 or 23869-24-1 or 55965-63-4 or 14259-46-2 or 7085-55-4 or 84932-19-4 or 153184 or 22519999 or 23869241 or 55965634 or 14259462 or 7085554 or 84932194 or cas153184 or cas22519999 or cas23869241 or cas55965634 or cas14259462 or cas7085554 or cas84932194) AND (oral or diet* or supplement or supplements or intake or ingestion or ingestions or eat*) AND (randomized or randomised or randomly or rct or placebo or trial or groups)

Database: **Cochrane Database of Systematic Reviews: Issue 9 of 12, September 2023, Central Register of Controlled trials: Issue 8 of 12, August 2023**
Date: 11.09.2023
Hits: 18 SR (CDSR), 137 RCT (Trials)

#1	[mh ^rutin]	114
#2	[mh ^hydroxyethylrutoside]	102
#3	((rutin NOT routine) or rutoside or rutaside or rutocide or rutabion or rutinion or rutozyd or birutan or citroflavone or eldrin? or farutin? or globulariacitrin? or melin or myrticolorin? or "neorutin 300" or osyritin? or osyritrin? or phytomelin? or sclerutin? or sophorin? or tanrutin? or violaquercitrin? or "vitamin P" or "vitamin P4" or (("rhamnosyl glucosyl" or rhamnosylglucosyl or rhamnoglucoside or rutinoside) NEAR/2 quercetin?) or "pentahydroxyflavone 3 rhamnoglucoside" or hydroxyethylrutoside or hydroxyethylrutaside or hydroxyethylrutin? or "hydroxyethyl rutine" or "trihydroxyethyl rutine" or trihydroxyethylrutin? or trihydroxyethylrutoside or trihydroxyethylrutaside or venoruton or rutorbin? or ruhexatal or oxerutin? or Paroven or Posorutin? or Relvene or Rheoflux or Teboven or Troxerutin? or Troxeven or Vastribil or Veinamitol or "Veniten retard" or "Veno SL" or Venorutin? or "Venotrulan Trox" or "neo semhyten" or neosemhyten or raumannite or (maxitate NEAR/2 Rauwolfia) or isonaringin? or monoxerutin? or narirutin? or benrubin? or	375

	cilkanol or troxevasin? or varemoid or "153-18-4" or "22519-99-9" or "23869-24-1" or "55965-63-4" or "14259-46-2" or "7085-55-4" or "84932-19-4" or "153184" or "22519999" or "23869241" or "55965634" or "14259462" or "7085554" or "84932194" or cas153184 or cas22519999 or cas23869241 or cas55965634 or cas14259462 or cas7085554 or cas84932194):ti,ab	
#4	#1 or #2 or #3	459
#5	[mh ^"Dietary Supplements"]	14118
#6	[mh ^Diet]	11332
#7	[mh ^Food]	2459
#8	[mh ^Eating]	3693
#9	(oral or diet* or supplement? or intake or ingestion? or eat*):ti,ab	300879
#10	#5 or #6 or #7 or #8 or #9	307838
#11	#4 AND #10	155

11 Appendix II – Publications not included in the results

11.1 Excluded publications

An overview of the publications considered not to fulfil the eligibility criteria given in Table 4.5.1.3-1.

Table 11-1 Publications considered not eligible and reason for exclusion.

Authors, year	Reason for exclusion
Abbey & Rankin, 2011	No mention of adverse effects or any safety parameters measured
Akkaya & Salici, 2021	No mention of adverse effects or any safety parameters measured
Askari et al., 2012	No mention of adverse effects or any safety parameters measured
Askari, Ghiasvand, et al., 2013	No mention of adverse effects or any safety parameters measured
Askari, Hajishafiee, et al., 2013	No mention of adverse effects or any safety parameters measured
Auteri et al., 1990	No mention of adverse effects or any safety parameters measured
Baron et al., 2018	No mention of adverse effects or any safety parameters measured
Baumhackl et al., 2005	Not relevant exposure
Bazzucchi et al., 2019	No mention of adverse effects or any safety parameters measured
Belcaro & Candiani, 1991	No mention of adverse effects or any safety parameters measured
Bhavnani et al., 2001	*In vitro* study
Bigelman et al., 2010	No mention of adverse effects or any safety parameters measured
Bobe et al., 2008	No mention of adverse effects or any safety parameters measured
Bondonno et al., 2016	No mention of adverse effects or any safety parameters measured
Bondonno et al., 2017	Abstract only
Bondonno et al., 2020	No mention of adverse effects or any safety parameters measured
Boots et al., 2007	No mention of adverse effects or any safety parameters measured
Boots et al., 2009	No mention of adverse effects or any safety parameters measured
Boots et al., 2011	No mention of adverse effects or any safety parameters measured
Buonerba et al., 2018	Results for quercetin could not be separated from other components
Butov et al., 2016	Not oral exposure
Cesarone et al., 2006a	No control group
Cesarone et al., 2005	Not RCT, no control group
Cesarone et al., 2019	No placebo or control group
Conquer et al., 1998	Results for quercetin could not be separated from other components
Cruz-Correa et al., 2006	Results for quercetin could not be separated from other components
Cureton et al., 2009	No mention of adverse effects or any safety parameters measured
Dagher et al., 2021	Protocol
Daneshvar et al., 2013	No mention of adverse effects or any safety parameters measured
Darvishi et al., 2013	Abstract only

Authors, year	Reason for exclusion
De Groote et al., 2011	Abstract only
Dower, Geleijnse, Gijsbers, Schalkwijk, et al., 2015	Same study as Dower et al., 2015, from Am. J. Clin. Nutr.
Duan et al., 2012	No mention of adverse effects or any safety parameters measured
Dumke et al., 2009	No mention of adverse effects or any safety parameters measured
Duranti et al., 2018	No mention of adverse effects or any safety parameters measured
Egert et al., 2011	No mention of adverse effects or any safety parameters measured
Egert et al., 2012	Identical doses in both groups with different matrix. No control
Eghtesadi et al., 2015	Abstract only
Erlund et al., 2001	Abstract only
Forconi et al., 1980	No mention of adverse effects or any safety parameters measured
Frandoli et al., 1972	No mention of adverse effects or any safety parameters measured
Gallasch et al., 1985	German. No mention of safety in title or abstract
Ghiasvand et al., 2013	Abstract only
Grinder-Pedersen et al., 2003	Flavonoids in organic vs conventionally produced foods
Guo et al., 2013	The intervention was not quercetin
Hassan et al., 2018	No mention of adverse effects
Heinz, Henson, Austin et al., 2010[1]	Results for quercetin could not be separated from other components
Heinz, Henson, Nieman, et al., 2010[1]	Results for quercetin could not be separated from other components
Henson et al., 2008	No mention of adverse effects
Hezaveh et al., 2019	Results for quercetin could not be separated from other components
Jin et al., 2010[1]	Results for quercetin could not be separated from other components
Junchi et al., 2014	Abstract only
Knab, Shanely, Henson et al., 2011[1]	Results for quercetin could not be separated from other components
Knab, Shanely, Jin et al., 2010[1]	Results for quercetin could not be separated from other components
Kawai et al., 2009	Overlap with Hirano et al., 2009 (included)
Kim et al., 2009	No mention of adverse effects or any safety parameters measured
Kim & Park, 2009	Abstract only
Kooshyar et al., 2017	Patients with cancer
Kuennen et al., 2011	No mention of adverse effects
Le Devehat et al., 1988	No mention of adverse effects
Le Devehat, Vimeux & Bondoux, 1989	Same study Le Devehat et al., 1988
Le Devehat, Vimeux, Bondoux et al., 1989	Same article as Le Devehat et al., 1988 (article from 1988, not 1989)
Lee et al., 2011	Results for quercetin could not be separated from other components
Mantovani et al., 2007	Wrong publication type, no results
McAnulty et al., 2008	No mention of adverse effects or any safety parameters measured

Authors, year	Reason for exclusion
Mitra et al., 2022	No mention of adverse effects or any safety parameters measured
Moonikh et al., 2020	Persian. No mention of safety in title or abstract
Nieman, Henson, Davis, Dumke, et al., 2007	Results for quercetin could not be separated from other components
Nieman, Henson, Davis, Murphy et al., 2007	Results for quercetin could not be separated from other components
Nieman, Henson, Gross, et al. 2007	Results for quercetin could not be separated from other components
Nieman et al., 2009	Results for quercetin could not be separated from other components
Nieman et al., 2010	No mention of adverse effects or any safety parameters measured
Omi et al., 2023	No mention of adverse effects or any safety parameters measured
Pasley et al., 2009	Abstract only
Patrizio et al., 2018	No mention of adverse effects or any safety parameters measured
Prysyazhnyuk et al., 2022	Abstract only
Quindry et al., 2008	Results for quercetin could not be separated from other components
Rashid et al., 1993	Results for quercetin could not be separated from other components
Rezvan et al., 2018	No mention of adverse effects or any safety parameters measured
Rondanelli et al., 2022	No mention of adverse effects or any safety parameters measured
Shanley et al., 2010[1]	Results for quercetin could not be separated from other components
Scholten & Sergeev, 2013	No mention of adverse effects or any safety parameters measured
Sesink et al., 2001	No mention of adverse effects or any safety parameters measured
Sgro et al., 2021	No mention of adverse effects or any safety parameters measured
Sharp et al., 2012	No mention of adverse effects or any safety parameters measured
Shi & Williamson, 2015	Results for quercetin could not be separated from other components
Stopa et al., 2017	Not RCT
Taliou et al., 2013[2]	Results for rutin could not be separated from other components
Tsao et al., 2022	No mention of adverse effects or any safety parameters measured
Utter et al., 2009	No mention of adverse effects or any safety parameters measured
Van den Eynde et al., 2018	No mention of adverse effects or any safety parameters measured
Wang et al., 2013	No mention of adverse effects or any safety parameters measured
Watanabe & Holobar, 2021	No mention of adverse effects or any safety parameters measured
Wieslander et al., 2011	No mention of adverse effects or any safety parameters measured
Wieslander et al., 2012	Results for rutin could not be separated from other components
Xiao et al., 2014	No mention of adverse effects or any safety parameters measured
Ying, 2023	No mention of adverse effects or any safety parameters measured
Zahedi et al., 2013b	Same study as Zahedi et al., 2013a

[1]These publications were excluded from our systematic review since they failed to meet all our inclusion criteria, but they were included as supporting evidence because of the high number of participants (n = 1002) in the cohort these studies reported results from (see Chapters 5.4.2 and 12.2).

[2]Since this study was the only study on children, it was included as supporting evidence, although it did not completely meet our inclusion criteria (see Chapter 5.4.3).

11.2 Category 3 publications

Table 11-2 Category 3 publications.

Author, year
Amirchaghmaghi et al., 2015
Ashigai et al., 2019
Bazyar et al., 2022
Bondonno et al., 2016
Cesarone et al., 2002
Cesarone et al., 2006b
Cheuvront et al., 2009
Dehghani et al., 2021
Dehghani et al., 2023
Di Pierro et al., 2023
Dower et al., 2015
Hosseinikia et al., 2020
Incandela et al., 2002
Kuipers et al., 2023
Larson et al., 2012
Mathrani et al., 2023
Nocker et al., 1989
Olson et al., 2010
Perez et al., 2014
Rezvan et al., 2017
Solnier et al., 2023
Zahedi et al., 2013a

12 Appendix III

12.1 Beneficial effects studied in the included publications

Table 12-1. Beneficial effects examined in addition to adverse effects reported in other tables.

Reference (authors, year)	Substance, dose(s), exposure length, study participants	Parameters examined for evaluation of beneficial effects (some also with relevance for evaluation of adverse effects)
Annoni et al., 1986	*O*-(β-hydroxyethyl)-rutosides (HER, Venoruton®), 4000 mg per day, 28 days. Patients with haemorrhoids	Severity scores for pain, bleeding, itching, secretion, edema and inflammation caused by haemorrhoids.
Bazyar et al., 2023	Rutin, 500 mg per day, 3 months. Patients with type 2 diabetes mellitus	Mean arterial pressure (MAP), heart rate (HR), pulse pressure (PP), systolic and diastolic blood pressure (SBP and DBP), serum levels of antioxidant enzymes (catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD)) and quality of life (QOL) parameters (emotional limitations, energy and freshness, mental health, social performance, physical performance, physical limitations, physical pain and general health).
Bergqvist et al., 1981	*O*-(13-hydroxyethyl)-rutosides (HER, Venoruton®), a single i.v. injection of 1000 mg and 1500 mg per day orally for 4 weeks. Patients with chronic venous insufficiency	Subjective symptoms (pain, day cramps, night cramps, tired legs, swollen legs, pruritus, increased «troubles» before menses) were assessed using a standardised questionnaire. Plethysmographic parameters (calf circumference, reserve venous volume (RVV), venous emptying (VE) and capillary filtration rate (CFR)). At the end of the trial, the patients stated their global opinion on the effect of the treatment and there were no significant differences in these statements.
Boyle et al., 2000	Rutin (quercetin-3-*O*-β-rutinoside), 500 mg per day, 6 weeks. Healthy non-obese normocholesterolaemic female volunteers	Plasma flavonoids, ascorbic acid, tocopherols and carotenoids, plasma antioxidant capacity (FRAP assay), lymphocyte DNA damage (Comet assay), blood chemistry and haematology, liver function tests (alkaline phosphatase, lactate dehydrogenase, γ-glutamyl transferase and alanine aspartame transaminase), urinary malondialdehyde (MA), oxidized glutathione (GSSG), 8-hydroxy-2′-deoxyguanosine (8OHdG), 8-iso-prostaglandin $F_{2\alpha}$, urinary thromboxane B2 (TXB2, a non-invasive index of *in vivo* platelet activation).
Cappelli et al., 1987	*O*-(β-hydroxyethyl)-rutosides (HER, Venoruton®), 3000 mg per day, 28 days. Women taking oral contraceptives and suffering from venous insufficiency of the lower limbs	Strain gauge plethysmography (7 parameters), improvement of subjective symptomatology of the lower limbs (pain, "heavy legs", sense of swelling, restless legs, tinglings).
Di Pierro et al., 2021	Quercetin Phytosome® (QP), 1000 mg per day (400 mg per day quercetin), 30 days. COVID-19 outpatients	Need and length of hospitalization, need of non-invasive oxygen therapy, progression to intensive care units, death. Most patients of the QP group reported clear beneficial effects, including reduction of fatigue and tiredness, and appetite improvement.
Egert et al., 2008	Quercetin dihydrate, 50, 100 or 150 mg per day, 2 weeks. Healthy volunteers	Dietary intake, plasma concentrations of quercetin and metabolites, plasma antioxidant capacity (ferric reducing antioxidant potential (FRAP) and oxygen radical absorbance capacity (ORAC)), serum uric acid, total cholesterol and

Reference (authors, year)	Substance, dose(s), exposure length, study participants	Parameters examined for evaluation of beneficial effects (some also with relevance for evaluation of adverse effects)
		triacylglycerols, LDL-cholesterol, plasma α- and γ-tocopherols, plasma oxidized LDL, and tumor necrosis factor (TNF-α), systolic and diastolic blood pressure, plasma glucose, body composition, height, weight and resting energy expenditure.
Egert et al., 2009	Quercetin dihydrate, 150 mg per day, 6 weeks. Overweight subjects with a high-cardiovascular disease risk phenotype (metabolic syndrome)	Nutritional status, plasma quercetin and metabolite concentrations, systolic and diastolic blood pressure, resting pulse rate, serum HDL-cholesterol, total cholesterol, TAG and the LDL:HDL-cholesterol and TAG:HDL-cholesterol ratios, glucose and uric acid, plasma concentrations of atherogenic oxidised LDL, TNF-α, hs-CRP: high-sensitivity C-reactive protein, markers of oxidative stress (plasma oxidised LDL), plasma antioxidant capacity, and body composition, height, weight, waist and hip circumference, BMI.
Erlund et al., 2000	Cross-over design with quercetin aglycone (8, 20, 50 mg) and rutin (16, 40 and 100 mg), single doses, with doses having equimolar quercetin aglycone), in total, 78 mg aglycone and 156 mg rutin, 12 weeks. Healthy volunteers	Pharmacokinetic parameters. Plasma total quercetin, including unconjugated quercetin, quercetin conjugated with glucuronic acid, sulfate or glycoside groups, and quercetin either bound to protein or not, plasma unconjugated rutin.
Ganio et al., 2010	Quercetin-supplemented food bar, 1000 mg per day of quercetin, 5 days. Sedentary and untrained volunteers	Quercetin in blood and urine. Maximal oxygen uptake (VO_{2max}), oxygen consumption, tidal volume, respiratory rate, expired volume, respiratory exchange ratio, blood lactate. Heart rate, rating of perceived exertion, perceived muscle pain, delayed-onset muscle soreness, duration of the test.
Han et al., 2020	Quercetin chews, 500, 1000 or 2000 mg per day in a dose-escalation manner, 7 days. Patients with chronic obstructive pulmonary disease (COPD)	Patients recorded symptoms daily in 'COPD Assessment Test', a standardised questionnaire. Lung functions tested were forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and FEV1/FVC. Blood counts were white blood cells and platelets. Fasting glucose levels.
Hirano et al., 2009	Enzymatically modified isoquercitrin (EMIQ), 100 mg per day, 8 weeks. Subjects with Japanese cedar pollinosis	Serum concentrations of quercetin and its methylated derivatives. Ocular symptom scores, ocular congestion scores, ocular itching scores, lacrimation scores, ocular congestion scores, nasal symptoms, serum concentrations of total IgE and specific IgE for Japanese cedar pollen. Quality of life (QOL) assessed by the Japanese allergic rhinitis QOL questionnaire (JRQLQ), comprised questions about rhinorrhea, sneezing, nasal obstruction, nasal and ocular itching, lacrimation, general fatigue, irritability, depression and difficulties with daily activities such as working, housekeeping, studying, reading, doing sports, going outdoors, sleeping and having conversations.

Reference (authors, year)	Substance, dose(s), exposure length, study participants	Parameters examined for evaluation of beneficial effects (some also with relevance for evaluation of adverse effects)
Kienzler et al., 2002	*O*-(β-hydroxyethyl)-rutosides (HER, Venoruton®), given as 500 mg Venoruton® powder, 1000 mg Venoruton® powder, 2000 mg Venoruton® powder, and 4000 mg Venoruton® powder, administered as a drinking solution (single-dose, four treatments, four-period cross-over design). Healthy volunteers	Plasma mono-3'-HER and mono-4'-HER derivatives (pharmacokinetic parameters).
Nakamura et al., 2022	Enzymatically modified quercetin glycoside (EMIQ), 110 mg per day, as isoquercitrin, 40 weeks. Healthy volunteers	Cognitive function assessment by Mini-Mental State Examination (MMSE) and Cognitrax, cerebral blood flow (CBF) by tNIRS-1 (near-infrared spectroscopy), health-related quality of life by the Short Form Health Survey and physical parameters (blood pressure, pulse), biological and hematological parameters (white blood cells, red blood cells, Hb, hematocrit, platelet count test), liver function indicators (aspartate aminotransferase, alanine transaminase, γ-glutamyl transferase, alkaline phosphatase and lactate dehydrogenase levels), renal function indicators (blood urea nitrogen and creatinine levels, urinalysis and estimated glomerular filtration rate), blood lipid indicators (total cholesterol, low-density lipoprotein LDL cholesterol, high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol, apolipoprotein B, apolipoprotein AI, triglycerides), glycaemic index (fasting glucose, glycated haemoglobin, glycoalbumin and fasting insulin) and serum proteins (total protein, albumin), insulin-like growth factor-1 (IGF-1), plasma amyloid-β 40, amyloid-β 42, Tau, neurofilament light chain, tumor necrosis factor-α, interleukin-6 and interferon-γ and lateral index. Height, body weight, body fat percentage, body mass index (BMI). Total hemoglobin (total Hb) concentration, oxygenated hemoglobin (O_2Hb) concentration and StO_2. Total Hb is an index reflecting the cerebral blood volume (CBV), whereas O_2Hb reflects the CBF and brain activity.
Pfeuffer et al., 2013	Quercetin dihydrate, 150 mg per day, 8 weeks. Healthy men with varying apolipoprotein E (APOE) genotypes	Plasma quercetin concentration. Examined atherosclerosis, endothelial function, systolic and diastolic blood pressure, anthropometry (body weight, waist circumference, body mass index (BMI), metabolic parameters (glucose, insulin, HOMA-IR, total (TC), high density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) cholesterol, triacylglycerols) and inflammatory parameters (high-sensitive C-reactive protein (hs-CRP), TNFα, soluble adhesion molecules s-VCAM, s-ICAM, s-E-selectin and 8-iso-PGF2α. Total glutathione. APOE genotype-dependent effects were seen only on waist circumference and BMI.
Riva et al., 2019	Quercetin (500 mg), Quercetin Phytosome® (QP) (250 or 500 mg, which are 100 or 200 mg quercetin), single-dose, randomized, six-sequence/three-period cross-over study. Healthy volunteers	Pharmacokinetic parameters (to compare the two quercetin preparations).

Reference (authors, year)	Substance, dose(s), exposure length, study participants	Parameters examined for evaluation of beneficial effects (some also with relevance for evaluation of adverse effects)
Shatylo et al., 2021	Quercetin, 240 mg per day, 3 months. Elderly patients with metabolic syndrome	Body weight, body mass index (BMI), systolic and diastolic blood pressure, serum total cholesterol, high and low-density lipoprotein cholesterol, triglyceride, fasting plasma insulin and glucose levels, oral glucose tolerance test (OGTT), HOMA-IR, markers of oxidative stress (plasma glutathione level, catalase (CAT), superoxide dismutase (SOD), telomere length and plasma advanced glycation end products (AGEs)).
Shi & Williamson, 2016	Quercetin dihydrate aglycone, 500 (544) mg per day, 4 weeks. Men with non-optimal but still healthy, blood uric acid levels	Weight, height. Plasma and urine uric acid levels, 24-h urinary excretion of uric acid, resting blood pressure and fasting plasma glucose.
Shoshkes et al., 1999	Quercetin, 1000 mg per day, 1 month. Men with prostate-related pelvic pain	They used the validated National Institutes of Health (NIH) chronic prostatitis symptom score, which comprised nine questions covering pain, voiding dysfunction and impact on quality of life. Forty-seven percent of patients taking placebo had a worsening of the symptom score versus only 13% of the quercetin patients (P = 0.05).
Yamada et al., 2022	Quercetin Phytosome®, 200 mg per day (80 mg quercetin per day), 4 weeks. Subjects with allergic symptoms of pollinosis	Blood tests (white blood cells, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, differential leukocyte count (percentage and count of neutrophils, lymphocytes, monocytes, eosinophils and basophils), aspartate aminotransferase, alanine transaminase, γ-glutamyl transferase, alkaline phosphatase, lactate dehydrogenase, leukocyte alkaline phosphatase, total bilirubin, direct bilirubin, indirect bilirubin, cholinesterase, total protein, urea nitrogen, creatinine, uric acid, creatine kinase (CK), calcium, serum amylase, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, glycoalbumin, serum iron (Fe), sodium (Na), potassium (K), chlorine (Cl), inorganic phosphorus (P), glucose and hemoglobin A1c (HbA1c). Urinalysis covered protein, glucose, urobilinogen, bilirubin, occult blood reaction, ketone body, specific gravity and pH. Virological and immunoserological tests (hemoglobin antigen, hepatitis C virus antibody III, HIV antigen/antibody, syphilis. The Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ) scores (i.a. allergic symptoms, such as eye itching, sneezing, nasal discharge and sleep disorder), total score, sleep score and physical score. Height, body weight, BMI, body-fat ratio, blood pressure, heart rate. Severity grading of allergic rhinitis symptoms, nasal discharge test (nasal discharge eosinophil count), blood test (unspecific IgE, specific IgE (*Dermatophagoides pteronyssinus*, Japanese cedar, Hinoki cypress, house dust) and a homemade questionnaire.
Yasutake et al., 2015	Quercetin glycosides (enzyme-treated **isoquercitrin** (EMIQ), 330 mg per day, for 4 weeks). Healthy adults 20-64 years with BMI ≥ 18.5 and < 30.0 kg/m^2	Safety was the primary objective of the study.
Yoshimura et al., 2008 (Study 2)	Enzyme-treated isoquercitrin (EMIQ) (275 mg per day, 12 weeks). Overweight and obese men and women 20-65 years with BMI 24-31 kg/m^2	Body fat accumulation (abdominal total fat area, visceral fat area, s.c. fat area, waist circumference) were reduced significantly in the EMIQ group versus placebo group.

Reference (authors, year)	Substance, dose(s), exposure length, study participants	Parameters examined for evaluation of beneficial effects (some also with relevance for evaluation of adverse effects)
Yoshimura et al., 2012	Enzyme-treated isoquercitrin (EMIQ) (110 mg per day, 24 weeks). Overweight and obese men and women 20-66 years with BMI ≥25 - <30 kg/m^2	Abdominal total fat area, visceral fat area and subcutaneous fat area were reduced.

Abbreviations: AGEs: advanced glycation end products, ALP: alkaline phosphatase, ALT: alanine transaminase, APOE: apolipoprotein E, AST: aspartate aminotransferase, BMI: body mass index, CAT: catalase, CBF: cerebral blood flow, CBV: cerebral blood volume, CFR: capillary filtration rate, CK: creatine kinase, COPD: chronic obstructive pulmonary disease, COVID-19: coronavirus disease 2019, CPK: creatine phosphokinase, DBP: diastolic blood pressure, EMIQ: enzyme-treated isoquercitrin, FEV1: forced expiratory volume in one second, FRAP: ferric reducing antioxidant potential, FVP: forced vital capacity, GPx: glutathione peroxidase, GSSG: oxidized glutathione, γ-GTP: γ-glutamyl transpeptidase, Hb: hemoglobin, HbA1c: hemoglobin A1c, HDL: high density lipoprotein, HER: *O*-(β-hydroxyethyl)-rutoside, HOMA-IR: homeostasis model assessment-insulin resistance, HR: heart rate, Ig: immunoglobulin, IGF-1: insulin-like growth factor-1, 8-iso-PGF2α: 8-epimer of prostaglandin F2α, JRQLQ: Japanese allergic rhinitis QOL questionnaire, LDH: lactate dehydrogenase, LDL: low density lipoprotein, MA: malondialdehyde, MAP: mean arterial pressure, MMSE: Mini-Mental State Examination, O_2Hb: oxygenated hemoglobin, OGGT: oral glucose tolerance test, 8OHdG: 8-hydroxy-2'-deoxyguanosine, ORAC: oxygen radical absorbance capacity, PP: pulse pressure, QOL: quality of life, QP: Quercetin Phytosome®, NIH: National Institutes of Health, USA, RVV: reserve venous volume, SBP: systolic blood pressure, s.c.: subcutaneous, s-ICAM: soluble intercellular adhesion molecule, SOD: superoxide dismutase, StO_2: tissue oxygen saturation, s-VCAM: soluble vascular cell adhesion molecule, TAG: triacylglycerol, TC: total cholesterol, TNF-α: tumor necrosis factor-α, tNIRS-1: near-infrared spectroscopy, TXB2: thromboxane B2, VE: venous emptying, VO_{2max}: maximal oxygen uptake.

12.2 The beneficial effects that were evaluated within the cohort by David C. Nieman and colleagues

David C. Nieman and colleagues performed a double-blinded randomized controlled clinical study to identify potential beneficial effects of quercetin supplementation. Shanely et al. (2010) was the first of a total eight publications from this study. Participants received a placebo, 500 mg quercetin per day or 1000 mg quercetin per day for 12 weeks (n = 1002), who completed the study). Quercetin was given together with vitamin C and niacin, while these substances were not included in the placebo. See the main text in Chapter 5.4.2 for further details.

Quercetin uptake and metabolism: The study gives valuable information on plasma quercetin levels after continuous supplementation. Plasma levels of quercetin (overnight fasted) were ~100 µg/l for all groups at the start of the intervention and increased to ~150 µg/l in the placebo group, ~400 µg/l in the group receiving 500 mg quercetin per day and ~600 µg/l in the group receiving 1000 mg quercetin per day. Fluctuations in plasma quercetin levels pre- and post-study were considerably lower in the placebo group compared to the two quercetin supplemental groups. Individual variations in plasma quercetin levels ranged from nearly 0 - >2500 µg/l for several individuals receiving 500 or 1000 mg quercetin per day, but were unrelated to age, gender, BMI, fitness levels or diet. These findings demonstrated large individual variations in the uptake and/or metabolism of quercetin (Jin et al., 2010).

Antioxidant capacity: Plasma F2-isoprostanes and plasma oxidized low-density lipoprotein (LDL), both measures of oxidative stress, were unaltered. The content of reduced glutathione (GSH) in red blood cells, the ferric-reducing ability of plasma (FRAP) and the total antioxidant capabilities in plasma were unaltered (Shanley et al., 2010).

Upper respiratory tract infection (URTI) rates: Quercetin supplementation had no significant influence on URTI rates for all subjects combined or when analyzed separately by gender, body mass index and age categories. Regression analysis revealed an interaction effect with self-reported fitness level. A separate analysis of subjects 40 years of age and older rating themselves in the top half of the entire group for fitness level (n = 325) showed lower URTI severity (36% reduction, P = 0.020) and URTI total sick days (31% reduction, P = 0.048) for the group receiving 1000 mg quercetin per day compared to the placebo group (Heinz, Henson, Austin, et al., 2010).

Innate immune function and inflammation: In what seems to be a sub-group analysis of female subjects (n = 120) who participated in the full study (n = 1002), quercetin supplementation revealed no influence on measures of innate immune function or inflammation in community-dwelling adult females. Plasma total leucocyte, neutrophil and lymphocyte cells were unaffected by quercetin supplementation, as well as plasma levels of the inflammatory markers IL-6 and TNFα. In a subset of the female subjects, granulocyte phagocytosis ability, granulocyte oxidative burst activity, natural killer cell activity, as well as levels of blood T-lymphocytes, B-lymphocytes or natural killer lymphocytes, were also unaffected by quercetin supplementation (Heinz, Henson, Nieman, et al., 2010).

Measurements of disease risk factors: Plasma hemoglobin, hematocrit, glucose, C-reactive protein (CRP) and the cytokines monocyte chemoattractant protein 1 (MPC-1), granulocyte colony-stimulating factor (GCSF), tumor necrosis factor-alpha (TNFα) interleukin 6 (IL6) and IL10 were unaffected by quercetin supplementation. A marginal reduction in plasma creatinine levels and increased glomerular filtration rate was observed with quercetin supplementation. Systolic and diastolic blood pressure were unaffected, but a minor reduction in mean arterial blood pressure was observed with quercetin supplementation. Triglyceride levels were unaltered, but a minor reduction in total cholesterol levels was seen with 500 mg quercetin per day compared to placebo (Knab, Shanely, Henson, et al., 2011).

Adipose tissue expansion: No significant differences in body mass or body composition were found with quercetin supplementation in either males or females or in subgroups of overweight or obese subjects (Knab, Shanely, Jin, et al., 2010).

Cognitive function: Out of all participants that completed the study (n = 1002), the majority completed full study requirements, including cognitive testing at baseline and post-treatment (n = 941). Data collected from the individuals were used to determine the effects on cognitive functions. Neurocognitive Index (NCI), psychomotor speed, reaction time and cognitive flexibility were increased from pre- to post-treatment but were not affected by quercetin supplementation. Memory and attention were not affected. Subgroup analysis of older participants (from 60 years of age, n = 217) revealed the same result (Broman-Fulks et al., 2012) This publication was not obtained in our literature search, but the participants were apparently from the same cohort as in the other publications.

Metabolic profiling: In a smaller sub-group of individuals (placebo (n = 37), 500 mg quercetin per day (n = 32) and 1000 mg quercetin per day (n = 31)), two mass spectrometry platforms were used to analyze a large number of metabolites in samples collected at 0, 4 weeks and 12 weeks (end) of the study. After accounting for age, sex and BMI, quercetin supplementation was associated with significant shifts in 163 out of 2153 measured metabolites. Metabolic shifts were apparent for individuals receiving 1000 mg quercetin per day. Many unknown (structure unidentified) and various quercetin conjugates (e.g. quercetin-3-glucuronide, isorhamnetin-3-glucuronide, quercetin diglucuronide and quercetin-3'-sulphate) were amongst metabolites that were altered with quercetin supplementation. The top five metabolites with an increase in serum were 2-methoxyphenol (guaiacol), 2-oxo-4-methylthiobutanoic acid, allocystathionine and two bile acids. Inflammatory and oxidative stress metabolites were not affected by quercetin supplementation. Across the affected metabolites, no common theme emerged, suggesting that quercetin exerts disparate and wide-ranging metabolic effects. The authors concluded that further research will be required to understand any potential health implications of these alterations (Cialdella-Kam et al., 2012).

13 Appendix IV – OHAT scoring

Reference: Annoni F, Boccasanta P, Chiurazzi D, Mozzi E, Oberhauser V (1986) Treatment of acute symptoms of hemorrhoid disease with high-dose oral O-(β-hydroxyethyl)-rutosides. Minerva Med, 77(37), 1663-1668. URL: https://pubmed.ncbi.nlm.nih.gov/3531924/.			
Tier of internal validity: Tier 2			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	The two treatments were assigned according to a randomized scheme, but the method of randomization was not stated.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	It was not mentioned how allocation was done.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**NR**	There is no information about drop-outs and the total numbers are not given in the tables, so we cannot say.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**- -**	There is no information about purity or stability of HR, and the producer is not stated. There is no information about compliance to treatment.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**+**	Great attention was paid to the possible appearance of indirect effects, questioning patients at each visit. The side-effects were mild, so no patients had to interrupt treatment, even temporarily. Laboratory tests performed before and at the end of the therapy did not show any significant changes. No data was shown.
Selective reporting	Q10: Were all measured outcomes reported?	**+**	Apparently, but the safety data was not shown.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**+**	The statistical methods appear o.k., but we cannot say if they are used for the safety data. Protocol is not mentioned. There is no information on funding.

Reference: Bazyar H, Javid AZ, Ahangarpour A, Zaman F, Hosseini SA, Zohoori V, Aghamohammadi V, Yazdanfar S, Cheshmeh MGD (2023) The effects of rutin supplement on blood pressure markers, some serum antioxidant enzymes, and quality of life in patients with type 2 diabetes mellitus compared with placebo. Front Nutr, 10, 1214420. URL: https://doi.org/10.3389/fnut.2023.1214420.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	++	Randomization was performed using "Random Allocation Software" (RAS) with block randomization protocol (6 blocks with 4 codes).
Selection	Q2: Was allocation to study groups adequately concealed?	++	Allocation concealment was performed using 2 codes, A and B. Each can containing supplement or placebo tablets (both cans and tablets looked similar) received a code A or B (coding was done by someone who was out of the study but had information about the research work).
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	++	The study was double-blind. The researcher, patients and physician (clinical consultant) were blinded to the supplementation. In addition, the person who performed the laboratory tests did not know the details of the study.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	++	The researcher monitored the use of tablets by the patients twice a month through phone call or text message. The ITT approach was applied to compensate for the withdrawal. Two patients in the rutin group were excluded due to coronavirus disease and unwillingness to continue and two patients in the control group were excluded because of coronavirus disease. Finally, four patients re-entered the analysis using ITT approach and results were analyzed in each group with 25 patients.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	+	The purity or stability of rutin was not stated, and no information was found on the producer's website. Rutin and placebo appeared to be taken at the same time points. The compliance assessment was done according to the number of returned tablets. Patients who had taken less than 90% of the tablets were not evaluated for the second stage and were excluded from the study process.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	+	The side-effects of rutin consumption were assessed during the study, apparently by reporting from the patients when the researcher monitored the use of tablets twice a month through phone call or text message.
Selective reporting	Q10: Were all measured outcomes reported?	+	Yes, apparently. No side-effects were reported.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	+	Appropriate statistical methods were used (but not for side-effects, since none were reported). Protocol was mentioned, but not provided, and thus, compliance with this could not be evaluated.

Reference: Bergqvist D, Hallböök T, Lindblad AD, Lindhagen A (1981) VASA, 10(3), 253-260. PMID: 7025500. URL: https://pubmed.ncbi.nlm.nih.gov/7025500/.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	The patients with chronic venous insufficiency (CVI) were randomly allocated to treatments with O-(β-hydroethyl)-rutoside (HER), but the method for randomisation was not explained.
Selection	Q2: Was allocation to study groups adequately concealed?	**++**	Stratification for diagnostic and other criteria was made at the end of the trial, but before the opening of the code.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**++**	Yes, since drop-outs were explained. The 'exclusions and dropouts' refer to those who did not satisfactorily complete the four week oral treatment phase, as indicated by a large number of tablets returned for pill counting (4 patients), or to those who stopped treatment because of side-effects (2 patients, both placebo).
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	No information was given about purity or stability of HR, not even information about the producer. Compliance: 4 patients dropped out of the study because they did not satisfactorily complete the four week oral treatment phase, as indicated by a large number of tablets returned for pill counting. ITT analysis was not used.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**+**	Based on a 'active standardized questionnaire', the HER group reported in total 98 side-effects, and the placebo group reported 93 (no difference).
Selective reporting	Q10: Were all measured outcomes reported?	**++**	Yes. Numbers of each category of side-effects were shown in a table.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**+**	The statistical methods were o.k., but apparently not used for side-effects. A protocol was not mentioned. There was no information about funding and the authors did not appear to belong to a interested company.

Reference: Boyle SP, Dobson VL, Duthie SJ, Hinselwood DC, Kyle JAM, Collins AR (2000) Bioavailability and efficiency of rutin as an antioxidant: a human supplementation study. Eur J Clin Nutr, 54(10), 774-782. URL: https://doi.org/10.1038/sj.ejcn.1601090.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	+	The treatment was randomized, but it was not stated how randomization was done.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	There is no information about how allocation was done.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	+	This study was a single-blind placebo-controlled trial, and apparently the participants were blinded.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	+	Apparently, since the data are reported for 8 participants, the same number as was exposed.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	+	There was no information on purity or stability of rutin, either in the publication or on the producer's website. There was no direct information on compliance, but apparently all eight subjects completed the 6-week treatment.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	+	It was stated that rutin supplementation did not induce any adverse changes in blood chemistry or indices of liver function, but no data were shown.
Selective reporting	Q10: Were all measured outcomes reported?	+	Yes, apparently.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	+	No numerical data on adverse effects were shown, and thus statistical methods were probably not used on such data. A protocol was mentioned, but not available. There was no information about funding and CoI.

Reference: Cappelli R, Pecchi S, Oberhauser V, Forconi S, Di Perri T (1987) Efficacy of O-(β-hydroxyethyl)-rutocides at high dosage in counteracting the unwanted activity of oral contraceptives on venous function. Int J Clin Pharm Res, 7(4), 291-299. URL: https://pubmed.ncbi.nlm.nih.gov/3596872/.			
Tier of internal validity: Tier 2			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**++**	The participants were randomly assigned to the treatment groups, and the boxes with *O*-(β-hydroxyethyl}-rutosides (HER) or placebo were numbered in random sequence and given to the patients i chronological order according to the numbering.
Selection	Q2: Was allocation to study groups adequately concealed?	**++**	The boxes containing the drug or the placebo were numbered in random sequence and were given to the patients in chronological order according to the numbering. The code for this enumeration was sealed until the conclusion of the trial.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**++**	Yes, the results were given for the same number of participants (and their legs) before and after treatment.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**- -**	No information on purity or stability of HER was stated in the publication, and the producer's name was not given. There was no information of compliance, i.e. whether the patients took all tree sachets per day of HER for 28 days.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**+**	In order to estimate the tolerability of the drug, before and after the treatment, the following parameters were checked: azotaemia, glycaemia, protrombin activity, bilirubin, transaminases, alkaline phosphatase, haemochrome and urine analysis. However, no data were shown on these outcomes.
Selective reporting	Q10: Were all measured outcomes reported?	**+**	Apparently, since they report that the laboratory tests performed at the end of the treatment did not show changes of any parameters, and that no side-effects were observed. However, we cannot know for sure since no data were shown.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**+**	The statistical method used for adverse effects was o.k. A protocol was not mentioned. No information was given on funding or CoI.

Reference: Di Pierro F, Derosa G, Maffioli P, Bertuccioli A, Togni S, Riva A, Allegrini P, Khan A, Khan S, Khan BA, Altaf N, Zahid M, Ujjan ID, Nigar R, Khushk MI, Phulpoto M, Lail A, Devrajani BR, Ahmed S (2021). Possible therapeutic effects of adjuvant quercetin supplementation against early-stage COVID-19 infection: A prospective, randomized, controlled, and open-label study. International Journal of General Medicine,14, 2359–2366. https://doi.org/10.2147/IJGM.S318720.			
Tier of internal validity: Tier 3			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	It is stated that the participants were randomized to the two treatment groups, but the method for randomization was not stated.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	Since this is an open-label, pilot study, there is no concealment of allocation.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**-**	Since this is an open-label, pilot study, there is no blinding either of the study researchers or the patients.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**NR**	Yes, apparently, but we cannot know for certain since the numbers having each side-effect were not stated.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	No information was given on purity or stability of quercetin phytosome (QP), either in the text or on the producer's website. It is not stated if exposure was in the same time frame in both treatment groups (QP and no QP, both also standard care), but it is assumed. Compliance was >95% for treatment with QP.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**+**	The secondary outcomes tolerability and side-effects were self-reported, and the lack of blinding could have affected the result.
Selective reporting	Q10: Were all measured outcomes reported?	**NR**	Yes apparently, but the numbers of each adverse event was not stated for the two treatment groups.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**-**	The statistical methods appear to be appropriate (but were not used for the side-effects). A study protocol is mentioned, but adherence to it cannot be evaluated since it is not described. The authors reported conflicts of interest in this work; as several authors are either employed by, are members of the Scientific Board of, or give advice to the company producing QP, or report a pending patent for QP. The company producing QP also supported this study.

Reference: Egert S, Wolffram S, Bosy-Westphal A, Boesch-Saadatmandi C, Wagner AE, Frank J, Rimbach G, Mueller MJ (2008) Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. J Nutr, 138(9), 1615–1621. URL: https://doi.org/https://doi.org/10.1093/jn/138.9.1615.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	The subjects were randomly assigned to the supplementation groups, but it is not stated how the randomization was done.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	There was no mentioning of how allocation was done.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was conducted double-blind, with parallel design.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**++**	Yes, apparently, and there was mentioning of one drop-out for which the reason was given.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	No information was given on purity or stability, and no such information was found on the producer's website. The doses were likely to be given within the same time frame. Compliance was monitored by capsule count at the end of the study and by instructing subjects to document capsule consumption.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**+**	The participants were instructed to document observed side-effects, deviations from their normal physical activity or any other observations considered relevant in a study diary.
Selective reporting	Q10: Were all measured outcomes reported?	**+**	Yes, apparently. No adverse effects of quercetin dihydrate intake was reported.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**+**	The statistical methods were appropriate (but were not used on the side-effects). Protocol was mentioned, but not provided, and thus, compliance with this could not be evaluated.

Reference: Egert S, Bosy-Westphal A, Seiberl J, Kürbitz C, Settler U, Plachta-Danielzik S, Wagner AE, Frank J, Schrezenmeir J, Rimbach G, Wolffram S, Müller MJ (2009) Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: a double-blinded, placebo-controlled cross-over study. Br J Nutr, 102(7), 1065–1074. URL: https://doi.org/10.1017/S0007114509359127			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**++**	Subjects were assigned to placebo or quercetin groups by blocked randomisation procedure, separately for men and women.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	It was not mentioned how allocation was done.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**++**	It was reported that two subjects dropped out because they found the study protocol too demanding and one subject was excluded due to gastrointestinal symptoms (the drop-outs were in both groups, because of the cross-over design).
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	The purity and stability of quercetin dihydrate was not stated, either in the publication or on the producer's website. Capsules (quercetin or placebo) were handed out at days 0 and 21 of each treatment period and leftovers were collected at days 21 and 42. Compliance was monitored by determining quercetin plasma concentrations, by capsule count at the end of the study and by instructing subjects to document capsule consumption. Compliance was high: 97.9 and 98.1% during quercetin and placebo consumption, respectively, and was not significantly different between the treatment groups and periods. Quercetin supplementation was also confirmed by a marked increase in plasma quercetin concentrations by 349% ($P > 0.001$) in subjects taking quercetin, but not placebo capsules.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**+**	The subjects were instructed to document observed side-effects or any other observations considered relevant in a study diary, but these results were not reported. Blood parameters of liver and kidney function (alanine transaminase, aspartate transaminase, γ-glutamyl-transpeptidase, alkaline phosphatase, cholesteryl esterase, creatinine), haematology and serum electrolytes were all within normal ranges at all times and no differences were observed between the groups. The data was not shown.
Selective reporting	Q10: Were all measured outcomes reported?	**-**	Probably, but this cannot be assessed since the safety data were not shown. The results of any self-reported side-effects were not described.

Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	+	The statistical methods used were o.k., but it is not known if they were used on the safety data. A protocol was mentioned, but not available for scrutiny of compliance. No CoIs were apparent.

Reference: Erlund I, Kosonen T, Alfthan G, Mäenpää G, Perttunen K, Kenraali J, Parantainen J, Aro A (2000) Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. Eur J Clin Pharmacol, 56(8), 545-553. URL: https://doi.org/10.1007/s002280000197.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	The participants in the study were randomized into two groups, but it was not stated how randomization was done.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	There was no information about how allocation was done.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**++**	Yes. The main approach was the intent-to-treat analysis in which all randomised subjects were included in the analysis. With both substances, two persons (one of each sex) discontinued the study, and the reasons were stated, and claimed not to be related to the substances.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	No information was given on purity or stability, not even producer's name, of either quercetin aglycone or rutin. A study nurse administered the capsules at the study site, ensuring compliance.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**+**	Yes, blood and urine samples were taken for assessment of safety (haematology, biochemical profile, urinalysis, electrocardiogram (ECG), blood pressure and heart rate) before and after the invention. Subjects were asked to record adverse events in their patients diaries that were collected daily.
Selective reporting	Q10: Were all measured outcomes reported?	**+**	Yes, apparently. Self-reported adverse effects, however, no adverse events related to the study compounds occurred during the study (no data shown).
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**+**	A protocol was mentioned, but since it was not available, adherence to it could not be evaluated. The statistical methods reported are irrelevant for our purpose (adverse effects), including the intent-to-treat analysis in which all randomised subjects were included in the analysis.

Reference: Ganio MS, Armstrong LE, Johnson EC, Klau JF, Ballard KD, Mihniak-Kohn B, Kaushik D, Maresh CM (2010) Effect of quercetin supplementation on maximal oxygen uptake in men and women. J Sports Sci, 28(2), 201-208. URL: https://doi.org/10.1080/02640410903428558.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	+	The treatments were randomly assigned and counterbalanced so that an equal number of participants received placebo and quercetin-supplementation in each phase of testing, but the method for randomisation was not mentioned.
Selection	Q2: Was allocation to study groups adequately concealed?	++	The treatment code was revealed only after all experiments were completed.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	++	The bars were indistinguishable in taste and appearance and were only identified with an alphabetical code that was blinded to the investigators and participants. This code was revealed only after all experiments were completed. Thus, the study was double-blind. In addition, the code on plasma and urine samples was unknown to the technicians who conducted the quercetin analyses.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	+	It was stated that one male left the study due to time constraints, and data analysis was completed on 11 participants (5 males, 6 females).
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	+	The purity and stability of the quercetin was not stated in the publication, and not found on the producer's website. The compliance was apparently 100% since the treatments were given to the participants at the laboratory, who ingested the bars with quercetin or placebo in the presence of an investigator.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	+	No participant exhibited or self-reported side-effects due to food bar consumption.
Selective reporting	Q10: Were all measured outcomes reported?	+	Yes, apparently all data were reported, except that no data about the results of processing of unknowns in plasma and urine were given. But this does not affect the safety evaluation.
Other sources of bias	Q11: 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 but was not used on the side-effects, since none were reported. A protocol is not mentioned, but the participants signed a consent document approved by the institutional local review board for human studies.

Reference: Han MK, Barreto TA, Martinez FJ, Cornstock AT, Sajjan US (2020) Randomised clinical trial to determine the safety of quercetin supplementation in patients with chronic obstructive pulmonary disease. BMJ Open Resp Res, 7, e000392. URL: https://doi.org/10.1136/bmjresp-2018-000392.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	++	Enrolled participants were divided into three cohorts, and within each cohort, the subjects were allocated to placebo or three doses of quercetin as per randomisation codes that were generated by a biostatistician prior to project enrolment.
Selection	Q2: Was allocation to study groups adequately concealed?	+	Enrolled participants were divided into three cohorts, and within each cohort, the subjects were allocated to placebo or three doses of quercetin as per randomisation codes that were generated by a biostatistician prior to project enrolment.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	++	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	+	It was explained that one subject dropped out because he was unable to come to study visits after being determined to be eligible, and therefore was not randomised.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	+	The purity or stability of quercetin was not stated either in the publication or on the producer's website. Compliance was determined on the basis of the study drug log and by counting the remaining study drug chews.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	+	Safety was determined by assessing postbronchodilator FEV1, blood profile (complete blood count and comprehensive metabolic panel) and patient-reported adverse events. The patients recorded symptoms daily in 'COPD Assessment Test', a standardised questionnaire. Abnormal laboratory results and the patient-reported symptoms were reviewed by the clinical principal investigator to determine the severity of the abnormality and the causality of the study participation. The clinical investigator informed the participants if the abnormalities were clinically significant.
Selective reporting	Q10: Were all measured outcomes reported?	+	Data on FEV1, FEV1/FVC, white blood cells, platelets and fasting glucose were shown. Patient-reported adverse events were gastro-oesophageal reflux disease (GERD), stomach upset, breathlessness, chest congestion, headache and nausea. Not all the safety data was shown, some were only described in the text.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and	-	Due to small numbers (2 or 3 subjects) in each group, the authors were not able to perform statistical analysis. A protocol was not mentioned, but the study was approved by an institutional review board.

	researchers adhered to the study protocol?		

Reference: Hirano T, Kawai M, Arimitsu J, Ogawa M, Kuwahara Y, Hagihara K, Shima Y, Narazaki M, Ogata A, Koyanagi M, Kai T, Shimizu R, Moriwaki M, Suzuki Y, Ogino S, Kawase I, Tanaka T (2009) Preventative effect of a flavonoid, enzymatically modified isoquercitrin on ocular symptoms of Japanese cedar pollinosis. Allergol Int, 58(3), 373-382. URL: https://doi.org/10.2332/allergolint.08-OA-0070.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	The participants were randomised to the treatment groups, but it was not stated how randomisation was done.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	There was no information about how allocation was done.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**++**	All participants completed the study. All results were reported.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	The purity or stability of quercetin was not stated either in the publication or on the producer's website. The mean percentage of intake for the EMIQ and placebo groups was 96.9% and 97.8%, respectively.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**+**	No side effects, such as gastrointestinal symptoms, allergic reactions or cardiovascular symptoms, were observed during the entire period.
Selective reporting	Q10: Were all measured outcomes reported?	**+**	Yes, probably, but we cannot know since no adverse effects were observed.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**-**	The statistical methods were probably appropriate, but we cannot know since there was no adverse effects. The study protocols were approved by an Ethics Committee. Five of the authors were employed in the company producing the EMIQ used in this study.

Reference: Kienzler J-L, Sallin D, Schifflers M-H, Ghika A (2002) Pharmacokinetics of mono-3'- and mono-4'-O-(β-hydroxyethyl)-rutoside derivatives, after single doses of Venoruton powder in healthy volunteers. Eur J Clin Pharmacol, 58(6), 395-402. URL: https://doi.org/10.1007/s00228-002-0472-3.			
Tier of internal validity: Tier 2			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	The subjects were administered all four treatments in a randomised order using a cross-over design. The method of randomisation was not stated.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	The selection procedure took place 14 days before the start of the trial, but since this was an open study the allocation was not concealed.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**- -**	This was an open study and therefore both researchers and participants were probably aware of their treatment.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**+**	All the subjects having received at least one study treatment were considered in the analysis of safety.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	There was no information on purity or stability of the HER used, and the name of the producer was not stated. The subjects were confined in the clinical centre before and after the test substance administration in each of the four periods of exposure, ensuring compliance.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**++**	At screen and at the completion of the study, blood and urine samples were taken for assessment of the safety parameters: haematology, biochemical profile, urinalysis; an ECG was performed, and blood pressure and heart rate were measured. During all periods of residence at the clinic, constant medical monitoring was maintained. All adverse events were completely documented and reported. The subjects were asked after each blood sampling, in an open manner without prompting, if they experienced unusual or adverse events. Any condition that was not present at the initial examination was recorded.
Selective reporting	Q10: Were all measured outcomes reported?	**+**	There was no modification of vital signs, ECG or safety laboratory results during the study. The different doses of the study medication were safe and well tolerated. However, the data was not shown.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**-**	The study protocol was approved, but was not available. Statistical methods for safety was not mentioned, and it cannot be known it statistical methods were used and were appropriate, since no safety data were shown. All authors work for Novartis Consumer Health, which is a company manufacturing, marketing and/or distributing medicines.

Reference: Nakamura Y, Watanabe H, Tanaka A, Nishihira J, Murayama N (2022) Effect of quercetin glycosides on cognitive functions and cerebral blood flow: a randomized, double-blind, and placebo-controlled study. Eur Rev Med Pharmacol Sci, 26(23), 8700-8712. URL: https://doi.org/10.26355/eurrev_202212_30541.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	++	The participants were randomly assigned to active or placebo group by the stratified block randomization method.
Selection	Q2: Was allocation to study groups adequately concealed?	++	A third-party allocation agency managed allocation information and ensured that it was maintained until the data were fixed.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	++	A third-party allocation agency ensured that double-blindness was maintained until the data were fixed.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	++	The reasons for withdrawals and exclusions between allocation and analysis were given.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	+	There was no information about purity or stability of the EMIQ, either in the text or on the producer's website. The intake of the test beverage was >99% in both groups, indicating high compliance.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	+	There was no specific mentioning of side-effects in Materials and methods, but among the reported main study outcomes it was stated that there was no safety issues in conducting the study or the intake of the test beverage. The only exception was a possible causal relationship between frequent urination and the treatment (because of increased water intake by the test beverage of 500 ml per day). No other adverse events were causally related to the study.
Selective reporting	Q10: Were all measured outcomes reported?	+	Yes, however, side-effects as such were not reported in Materials and methods or in Results.
Other sources of bias	Q11: 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 mainly o.k. but were not used for analysis of side-effects. A protocol was not mentioned, but the study was approved by an ethics committee. The study received no external funding. One author work for a beverage and food company.

Reference: Pfeuffer M, Auinger A, Bley U, Kraus-Stojanowic I, Laue C, Winkler P, Rüfer CE, Frank J, Bösch-Saadatmandi C, Rimbach G, Schrezenmeir J (2013) Effect of quercetin on traits of the metabolic syndrome, endothelial function and inflammation in men with different APOE isoforms. Nutrition, Metabolism & Cardiovascular Diseases, 23, 403-409. URL: http://dx.doi.org/10.1016/j.numecd.2011.08.010.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	Randomization was mentioned, but the method for randomisation was not stated.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	It was not mentioned anything about allocation.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**++**	Right at onset two subjects with APOE3/3 and one with APOE3/4 dropped out of the study, because of a herniated vertebral disc, inability to swallow the capsules and night sweat. Only the latter condition may be related to treatment.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	No information was given on purity or stability of quercetin dihydrate or placebo, and no information was found on the producer's website The exposure was likely to be given within the same time frame. Compliance was checked by counting returned capsules. It was considered sufficient if >85% of the capsules were consumed. All participants achieved this goal. Quercetin concentration in plasma was increased from 121.9 ± 7.5 to 193.8 ± 20.4 nmol/L following quercetin as compared to placebo consumption ($n = 49$, $P < 0.01$).
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**++**	It was not stated that side-effects would be reported in Materials and methods, and there was no results on side-effects, except that night sweat was given as reason for drop-out of one person and a moderate increase in TNFα, a biomarker of inflammation, in the main results were reported as potential adverse effects.
Selective reporting	Q10: Were all measured outcomes reported?	**++**	Yes, apparently. It was not stated that side-effects would be reported in Materials and methods, and there was no results on side-effects as such, but reported among the main results.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**+**	The statistical methods were appropriate. Protocol was mentioned, but not provided, and thus, compliance with this could not be evaluated. No information about funding or conflict of interest was given.

Reference: Riva A, Ronchi M, Petrangolini G, Bosisio S, Allegrini P (2019) Improved oral absorption of quercetin from Quercetin Phytosome®, a new delivery system based on food grade lecithin. Eur J Drug Metab Pharmacokinet, 44, 169–177. URL: https://doi.org/10.1007/s13318-018-0517-3.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	+	It is a randomized study, but it is not described how randomisation was done.
Selection	Q2: Was allocation to study groups adequately concealed?	- -	Treatments were known to the investigator (the clinical research products were identified as A, B or C).
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	+	Each volunteer received one film-coated tablet of quercetin 500 mg (treatment A), one film-coated tablet of Quercetin Phytosome 250 mg (treatment C) and two film-coated tablets of Quercetin Phytosome 250 mg (500 mg total; treatment B) on the three experimental days, administered according to a previously randomized sequence. The three treatments were given as 1, 2 or 2 tablets, so at least partly the participants knew their treatment. Treatments were known to the investigator.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	+	It was reported that there was no withdrawals from the trial.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	+	There were some differences between quercetin and Quercetin Phytosome® regarding food-grade excipients and lecithin (added only in the last tablets). There was no information on purity or stability of these products, neither on the producer's website. The tablets were given during hospitalization, so most likely there was 100% compliance.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	+	The outcomes were clinical safety (evaluation of vital signs and adverse systemic effects) and biological safety (evaluation of each subject's blood count and blood chemistry results) which were monitored to determine the tolerability of the treatments. The endpoints were not very likely to be affected subjectively. However, no data were shown.
Selective reporting	Q10: Were all measured outcomes reported?	+	Yes, it appears that all announced data were included.
Other sources of bias	Q11: 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 not reported (and probably not used) for safety. Protocol was not mentioned, but the study had ethical approval. The authors were all employed by the company producing these tables, and the company provided the tablets and funded this study.

Reference: Shatylo V, Antoniuk-Shcheglova I, Naskalova S, Bondarenko O, Havalko A, Krasnienkov D, Zabuga O, Kukharskyy V, Guryanov V, Vaiserman A (2021) Cardio-metabolic benefits of quercetin in elderly patients with metabolic syndrome. PharmaNutrition, 15, 100250. URL: https://doi.org/10.1016/j.phanu.2020.100250.			
Tier of internal validity: Tier 2			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	The study was stated to be randomized, but the randomisation method was not stated. However, to ensure that placebo and intervention groups were comparable for important characteristics such as age, sex ratio and LDL-C, the required subjects were randomly allocated into groups by minimization method that adapted the randomization process by taking baseline characteristics into account.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	There is no information about how allocation was done.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	It was stated that the study was double-blind, i.e. both the patients and the investigators remained blinded to the active medication.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**++**	Number and explanations were given for the excluded participants (they did not fully meet the inclusion criteria for MetS or refused to participate). During follow-up, 6 participants in the placebo group and 7 in the intervention group were excluded because of change in medication or because they refused to continue.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**- -**	There is no information on purity or stability of quercetin, neither on the producer's website. No information was found on compliance/adherence to the treatment.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**+**	The safety endpoints were not very likely to be affected subjectively, and it was a double-blind study. Effects of both quercetin and placebo were measured apparently at the same time points.
Selective reporting	Q10: Were all measured outcomes reported?	**+**	Yes, detailed parameters were monitored for safety, but numbers were not shown.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**+**	The statistical methods were o.k. but not used for safety parameters. The study protocol has been approved (but not presented, thus it cannot be checked it they adhered to it). The authors reported no conflicts of interest or any funding.

Reference: Shi Y & Williamson G (2016) Quercetin lowers plasma uric acid in pre-hyperuricaemic males: a randomised, double-blinded, placebo-controlled, cross-over trial. Br J Nutr, 115(5), 800-806. URL: https:/doi.org/10.1017/S0007114515005310.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	++	The participants were randomised by a coin toss and received a random three-digit code, which was kept secret from the researcher assessing outcomes.
Selection	Q2: Was allocation to study groups adequately concealed?	++	The decode list was kept secret by a third person from the researcher assessing outcomes and the subjects could not see which tablet had quercetin or placebo, therefore also the allocation was most likely concealed.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	++	The study was double-blind. The decode list with participant identification and subject code was kept secret from the researcher assessing outcomes and the subjects could not see which tablet had quercetin or placebo. Analyses of blood and urine samples (not for safety) were also done by a blinded researcher.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	+	It was shown that one person discontinued the intervention (subject withdrawn).
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	+	Purity and stability of quercetin dihydrate used were not stated, and there was no such information on the producer's website. Compliance was assessed at the end of each 4-week period by call-back questionnaires, recording date of missing dose (if any), use of exotic diet or non-routine medications and the occurrence of any side-effects. Subjects were also asked to return the unconsumed tablets at each follow-up visit. According to the returned unconsumed tablets, participant self-reports and urinary quercetin, none of the participants was classified as non-compliant.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	+	No adverse events after receiving quercetin or placebo were reported.
Selective reporting	Q10: Were all measured outcomes reported?	+	No adverse events after receiving quercetin or placebo were reported.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	+	No protocol was available for study, but all the procedures were approved by an ethics committee. The statistical methods appeared appropriate, but were not used on adverse effects, since none were reported. This work did not receive funding from any commercial organisation, but one author had recently, or currently, received other research funding from Nestle and Florida Department of Citrus and conducted consultancy for Nutrilite, USA.

Reference: Shoskes DA, Zeitlin SI, Shahed A, Rajfer J (1999) Quercetin in men with category III chronic prostatitis: A preliminary prospective, double-blind, placebo-controlled trial. Urology, 54(6), 960–963. URL: https://doi.org/10.1016/s0090-4295(99)00358-1.			
Tier of internal validity: Tier 3			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	The patients with chronic pelvis pain syndrome were randomly allocated to treatments with quercetin, but the method for randomisation was not explained.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	There was no information about allocation.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**+**	Yes, since drop-outs were explained. Two patients taking placebo did not complete the study because of worsening of their symptoms, whereas all 15 patients on quercetin completed.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**- -**	No information was given about purity or stability of quercetin, not even information about the producer. There was no information on compliance.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**+**	Yes. The National Institutes of Health (NIH) chronic prostatitis symptom score was used to score symptoms (pain, voiding dysfunction and impact on quality of life).
Selective reporting	Q10: Were all measured outcomes reported?	**-**	There was no information on how data on side-effect were recorded.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**-**	The statistical methods were o.k., except that there was no information about normality and equal variance. A protocol was not mentioned, but the study was approved by an institutional review board. Two authors owned stocks in companies that will benefit from sales of the supplements reported in this study.

Reference: Yamada S, Shirai M, Inaba Y, Takara T (2022) Effects of repeated oral intake of a quercetin-containing supplement on allergic reaction: a randomized, placebo-controlled, double-blind parallel-group study. Eur Rev Med Pharmacol Sci, 26(12), 4331-4345. URL: https://doi.org/10.26355/eurrev_202206_29072.			
Tier of internal validity: Tier 2			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	++	The management of study participants was assigned to a clinical research organization. The participants were randomly assigned to active or placebo group, but the randomisation method was not described.
Selection	Q2: Was allocation to study groups adequately concealed?	++	The allocation sequence was determined with Statlight #11 Ver.2.10. Individual subjects were randomly allocated, in a stratified ratio of 1:1, to the placebo food group or the test food group. The allocation table was sealed and stored until the completion of the study, and was unsealed after the completion of the study.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	++	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	++	Subjects who withdrew their consent to the study or ceased ingesting the test or placebo food were discontinued from the study. Discontinued treatment, protocol deviations and lost to follow-up were stated (total n = 6 for both groups). There was no significant difference between the two groups at the start of the study.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	-	There was no information about purity or stability, neither on the producer's website. There was no information on compliance, only that participants who ceased to ingest the test or placebo substances were discontinued from the study. It appears that exposure was done at the same time point to both groups.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	++	Each complication or adverse event reported by the subject was recorded. Large numbers of safety parameters were recorded and comprised adverse events and adverse reactions, which were evaluated based on physical tests, blood tests and urinalysis (mostly objective parameters).
Selective reporting	Q10: Were all measured outcomes reported?	+	Yes, apparently, but the numerical data on safety were not shown.
Other sources of bias	Q11: 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 o.k. In the safety analysis, an inter-group comparison was conducted on the data from physical measurement (excluding height), physical examination, and peripheral blood test at 2 and 4 weeks after starting test/placebo ingestion using ANCOVA in which the baseline served as the covariable, and the group served as a factor. A protocol was mentioned, but not shown. It was stated that it was not changed after approval. One author worked for Indena Japan Co. Ltd., Tokyo, Japan, which produced the QP and

			supported the study with editorial assistance. This study was conducted under assignment from Indena Japan Co., Ltd. and Indena S.p.A. at the cost of the assignors.

Reference: Yasutake, Y., Hori, H., Kitagawa, Y., & Sugimura, H. (2015). Safety evaluation of excessive intake of tea containing quercetin glucosides in healthy adults including obese subjects: A randomized, double-blind, placebo-controlled, parallel-group study. [Japanese]. Japanese Pharmacology and Therapeutics, 43(3), 389-396.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**++**	A controller responsible for allocation not involved in the study randomised subjects into two groups at a 1:1 allocation rate using BMI and gender at the pretest as stratification factors.
Selection	Q2: Was allocation to study groups adequately concealed?	**++**	The controller sealed the allocation table and kept it sealed until the allocation table was opened.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**++**	Yes, all patients completed the study and were included in the analyses.
Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	There was no information on the purity and stability of the EMIQ, and the producer was not stated. All patients had a 100% intake of the test beverage.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**++**	A medical interview was conducted by a doctor to investigate subjective symptoms and objective findings. The principal investigator reviewed the requests from the subjects, their daily diaries and test results to determine the occurrence of adverse events and their continuation in the study. Anthropometry, systolic and diastolic blood pressure and pulse rate, hematology, blood biochemical tests, including enzymes, lipids, blood sugar etc., and urinalysis, were determined.
Selective reporting	Q10: Were all measured outcomes reported?	**++**	Yes. The data were shown and analysed statistically.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**-**	The safety data were analysed statistically, and the methods appeared to be appropriate. The study was approved beforehand. Some of the authors were associated with the food and beverage company Suntory.

Reference: Yoshimura, M., Maeda, A., Abe, K., Ohta, H., Kiso, Y., Takehara, I., Fukuhara, I., & Sakane, N. (2008). Body fat reducing effect and safety of the beverage containing polyphenols derived from Japanese pagoda tree (enzymatically modified isoquercitrin) in overweight and obese subjects. [Japanese]. Japanese Pharmacology and Therapeutics, 36(10), 919-930.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	Subjects were randomly assigned to test groups based on pretest results. The method for randomization was not stated.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	There was no information on how allocation was done.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**++**	Yes, it was stated that one person in the test group discontinued because of deep vein thrombosis, but it was judged not related to the test drink since symptoms started earlier, and four persons were excluded because of non-compliance (two in the test group and two in the placebo group).
of Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	No information was given on purity or stability of the EMIQ, and the producer was not stated. There was information on compliance.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**++**	Anthropometry, systolic and diastolic blood pressure and pulse rate, hematology, blood biochemical tests, including enzymes, lipids, blood sugar etc., and urinalysis, were determined, and the participants reported adverse events during the study, which was reported in the publication with numbers.
Selective reporting	Q10: Were all measured outcomes reported?	**++**	Yes. The data were shown and analysed statistically.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**-**	The safety data were analysed statistically, and the methods appeared to be appropriate. The study was approved beforehand. Some of the authors is associated with the food and beverage company Suntory.

Reference: Yoshimura, M., Maeda, A., Nakamura, J., Kitagawa, Y., Shibata, H., & Fukuhara, I. (2012). Body fat reducing effect of continuous consumption of the beverage containing quercetin glucosides (enzymatically modified isoquercitrin) in obese subjects. [Japanese]. Japanese Pharmacology and Therapeutics, 40(10), 901-914.			
Tier of internal validity: Tier 1			
Internal validity appraisal			
Bias domain	**Question**	**Score**	**Reasons for scoring**
Selection	Q1: Was administered dose or exposure levels adequately randomised?	**+**	Subjects were randomly assigned to test groups with uniformity of age, gender, BMI, body fat percentage and abdominal fat. No further method for randomization was stated.
Selection	Q2: Was allocation to study groups adequately concealed?	**NR**	There was no information on how allocation was done.
Performance	**Key question:** Q6: Were the research personnel and/or human subjects blinded to the study groups?	**++**	The study was double-blind.
Attrition/ Exclusion	Q7: Were outcome data complete without attrition or exclusion from analysis?	**+**	During the study there were no persons who dropped out. However, 5 persons in the control group and 3 persons in the test group appeared to be excluded (including one person with Graves' disease, that may affect the evaluation of efficiency).
of Detection	**Key question:** Q8: Can we be confident in the exposure characterization	**+**	No information was given on purity or stability of the EMIQ, and the producer was not stated. The intake rate was 90% or more of the test drink among all subjects.
Detection	**Key question:** Q9: Can we be confident in the outcome assessment?	**++**	Anthropometry, systolic and diastolic blood volume, hematology, blood biochemical tests, including enzymes, lipids, blood sugar etc., and urinalysis, were determined, and the participants reported adverse events during the study, which was reported in the publication with numbers.
Selective reporting	Q10: Were all measured outcomes reported?	**++**	Yes. The data were shown and analysed statistically.
Other sources of bias	Q11: Were there no other potential threats to internal validity (e.g. statistical methods were appropriate and researchers adhered to the study protocol?	**-**	The safety data were analysed statistically, and the methods appeared to be appropriate. The study was approved beforehand. Some of the authors was associated with the food and beverage company Suntory.