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Risk assessment of beta-carotene in food supplements

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

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Assessed and approved

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

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Competence of VKM experts

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

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Summary

Key words: VKM, risk assessment, Norwegian Scientific Committee for Food Safety, beta-carotene, food supplement, upper level, tentative upper level.

Beta-carotene is a provitamin, i.e. a precursor of vitamin A (retinol), which is classified as an essential nutrient for humans. Beta-carotene is one of many carotenoids found in plants, fungi and bacteria. Carotenoids are therefore predominantly obtained through foods of plant origin or food supplements. Carrots contribute approximately half of the total beta-carotene intake in the Norwegian diet, followed by mixed frozen vegetables, tomatoes, fruits and berries. VKM emphasises that this opinion on upper level (UL) for beta-carotene addresses beta-carotene in food supplements only. Beta-carotene from regular foods such as vegetables and fruits is not considered to be a health concern.

In 2002, the Scientific Committee on Food (SCF) established a tolerable upper intake level (UL) for vitamin A (SCF, 2002). However, the SCF opinion covers only retinol compounds (various forms of vitamin A). The bioconversion of carotenoids to vitamin A in the body is well regulated and therefore only intake of vitamin A has been considered relevant for vitamin A toxicity (Blomhoff et al., 2003; EFSA, 2008). The Norwegian Food Safety Authority is considering whether beta-carotene should be regulated separately from retinol compounds.

Beta-carotene seems to have a carcinogenic effect in smokers. A number of studies have been published where possible mechanisms of this negative health effect are discussed. The suggested mechanisms are either related to effects on cytochrome P450-related activities, altered retinoid signalling or to a pro-oxidant activity of beta-carotene.

No UL has been established for beta-carotene. Several risk-assessment bodies have, however, previously attempted to establish safe levels or temporary guidelines, summarised in the following table:

Previous reports	Conclusion
SCF, 2000, EU	No dose-response relationship could be derived. Supplementation of 20 mg beta-carotene per day or more is contraindicated for use in current heavy smokers. There is insufficient evidence to set an UL for beta-carotene.
IOM, 2000, USA	No UL was established for beta-carotene or carotenoids. Beta-carotene supplementation is not recommended in the general population.
EVM, 2003, UK	The LOAEL was set to 20 mg/day. An uncertainty factor of 3 was applied to extrapolate from LOAEL to a NOAEL. A Safe Upper Level for beta-carotene supplements was set at 7 mg/day (equivalent to 0.11 mg/kg body weight/day for a 60 kg adult).

Previous reports	Conclusion
NNR, 2012, Nordic countries	No specific beta-carotene recommendation or UL.
Rasmussen, 2006, Denmark	A Temporary Guidance Level for beta-carotene equal to the average dietary level of 5 mg/day for all age groups was suggested.

Seven randomised controlled trials (RCTs) have been included in this VKM opinion, conducted either in Europe or the USA, with almost 47 100 participants in the beta-carotene groups. In six of these RCTs there were no observed increased risk of cancer, but the large Finnish ATBC study found an increased risk of lung cancer in the beta-carotene group.

Two prospective studies were included, one Danish and one from the USA, with 125 000 participants all together. The Danish study found that risk of lung cancer increased in smokers with increasing doses of beta-carotene supplements.

In addition, eleven meta-analyses were included; one with age-related macular degeneration as endpoint, one with a mixture of cardiovascular disease (CVD) and cancer as endpoints, four on cancer as only endpoint, one on a mixture of CVD and all-cause mortality as endpoints and four on all-cause mortality alone. One of the meta-analyses on all-cause mortality was later excluded.

There were no significant findings in the meta-analysis on macula degeneration. One of the two meta-analyses on CVD found a small increased risk in the beta-carotene arm (Vivekananthan et al., 2003). The combined CVD and cancer meta-analysis did not have sufficient statistical power to get significant results, but found a probable increase in lung cancer incidence in high-risk subgroups (smokers and asbestos workers (Fortmann et al., 2013)).

In the five meta-analyses studying cancer, there were no effect on other cancer forms than lung cancer.

The four meta-analyses on all-cause mortality used information from the same RCTs as included in this VKM-opinion. They extracted information on numbers of death in each study and used these numbers to analyse risk of death in the beta-carotene versus placebo groups. Alarmingly, they all found an increased risk of all-cause mortality. These meta-analyses have been discussed thoroughly.

To complete the risk characterisation of beta-carotene, VKM has followed the steps 1 – 4 as suggested by SCF in their Guidelines for the development of tolerable upper intake levels for vitamins and minerals (SCF, 2000a).

Step 1 and 2. Hazard identification and characterisation.

Up until two decades ago, beta-carotene was thought to be harmless even in large doses. In the wake of the Finnish ATBC study which found an increased risk of lung cancer and death in male smokers, animal studies have indicated three possible mechanisms for such a

detrimental effect. Although conclusive mechanistic explanations for the negative effects have not yet been agreed upon, there is a scientific rationale for the argument that population groups with vulnerable lungs may also have increased risk from beta-carotene supplements.

The dose used in the Finnish ATBC study was 20 mg beta-carotene/day. The effect was only observed during the intervention period; in follow-up studies conducted after the active period was finished, the risk declined and was no longer significant. 20 mg beta-carotene may thus be considered as a LOAEL.

The Danish prospective study found a dose-dependent increase in lung cancer risk with increased intake of supplemental beta-carotene. Unfortunately, the paper does not allow for setting a NOAEL or LOAEL based on the published data.

In the four meta-analyses on all-cause mortality, all found a 6-7% increased risk of death. One of the meta-analyses also found an increased risk of CVD in smokers. However, all results were driven, statistically, by the ATBC study. Studies with a more mixed population (both men and women) and with a more typical prevalence of smokers (10–20%), found no such increased risk.

Step 2, continued: Derive at a UL, taking into account the scientific uncertainties in the data. ULs may be derived for various life-stage groups within the population.

VKM found it extraordinary challenging to decide which uncertainty factor to use for beta-carotene. The present SCF guidelines for establishment of tolerable upper intake levels do not give clear guidance/advice in deciding the numeric level of the uncertainty factor. This seems to leave the decision to scientific judgement.

. If the NOAEL is based on human data, an uncertainty factor of 10 is recommended as a starting point to encompass inter-individual variation and sensitivity. The SCF guidelines state that a small uncertainty factor is to be used if the judgement is that little population variability is expected for the adverse effects, and a larger uncertainty factor (close to 10) may be used if variability is expected to be large.

For beta-carotene, a NOAEL is not available, and an uncertainty factor may be applied to account for the uncertainty in deriving a UL from the LOAEL. The size of the uncertainty factor involves a judgement based on the severity and incidence of the observed effect at the LOAEL and the steepness (slope) of the dose response, if this is possible to estimate. For beta-carotene, we have not found the data necessary to make a dose-response curve.

In addition, the following considerations were discussed before deciding on an uncertainty factor:

- The study which found a negative effect of beta-carotene supplementation (the Finnish ATBC study) was very large (n=29 133) which indicates that it encompasses

inter-individual variation and sensitivity. Additionally, the most vulnerable groups, in this case smokers, was an inclusion criteria. Both these factors indicate that the uncertainty factor can be in the lower end.

- The meta-analyses for the endpoint "increased risk of all-cause mortality" found an increased risk of death in the beta-carotene groups. This is severe, and indicates that a maximum uncertainty factor should be applied. However, as all results in the all-cause mortality meta-analyses were driven statistically, by the ATBC study on smokers, we choose to use a lower factor.

Based on the above considerations, VKM has chosen to use 5 as an uncertainty factor for beta-carotene.

An UL for beta-carotene cannot be derived, but a tentative upper level (TUL) is set at 4 mg/day, based on a LOAEL of 20 mg and the uncertainty factor of 5.

Smokers and anyone else in the population with vulnerable lungs (e.g. asthmatics, COPD patients) should be discouraged from taking beta-carotene containing supplements all together.

Step 3. Exposure assessment – evaluates the distribution of usual total daily nutrient intakes among members of the general population.

In the food survey Småbarnskost 2007, the mean intake of beta-carotene in 2-year-olds was 1.5 mg/day. In Norkost 3, the estimated mean intake in adults was 2.4 mg/day and 6.9 mg/day in the 95th percentile. About 3% of the adults reported use of beta-carotene supplements. The use of tanning pills containing beta-carotene may have been underreported.

Beta-carotene from regular foods such as vegetables and fruits is, however, not considered to be of any health concern. Negative health effects from beta-carotene in natural foods have never been reported. On the contrary, the consumption of vegetables and fruits should be increased, and the recommendation of "5 a day" should be achieved in all age groups of the population.

Step 4. Risk characterisation – analyses of the conclusions from steps 1 through 3 and characterises the risk. The risk will depend on the fraction of the population exceeding the UL and the magnitude and duration of excessive intake.

As beta-carotene from food, irrespective of amount, is considered innocuous, it is only intake of beta-carotene from supplements that is considered as relevant

VKM concludes that with a LOAL of 20 mg beta-carotene/day and a safety factor of 5, 4 mg beta-carotene/day is an appropriate tentative upper level for supplemental beta-carotene.

Sammendrag på norsk

Betakaroten er et pro-vitamin, det vil si det kan omdannes til vitamin A (retinol) som er et essensielt næringsstoff. Betakaroten er et av mange karotenoider som finnes i planter, sopp og bakterier. Karotenoider får vi derfor hovedsakelig fra frukt og grønnsaker, eller kosttilskudd. Gulrøtter bidrar med omtrent halvparten av det totale betakaroten inntaket i norsk kosthold, etterfulgt av blandede frosne grønnsaker, tomater, frukt og bær. VKM understreker at denne risikovurderingen om tolerabelt øvre inntaksnivå (UL) for betakaroten kun omhandler betakaroten i kosttilskudd. Betakaroten fra vanlige matvarer som grønnsaker og frukt medfører ingen helserisiko.

EUs vitenskapskomité (Scientific Committee on Food) har fastsatt UL for vitamin A (SCF, 2002). SCF- vurderingen omfatter imidlertid bare retinolforbindelser. Omdanningen av karotenoider til vitamin A i kroppen er velregulert, og det er derfor bare inntak av vitamin A som er relevant for toksisiteten til vitamin A (Blomhoff et al., 2003; EFSA, 2008). Mattilsynet ønsker å vurdere om det er behov for å regulere betakaroten adskilt fra forskjellige retinolforbindelser.

Betakaroten ser ut til å øke risikoen for lungekreft hos røykere. Det er publisert en rekke studier hvor mulige mekanismer for denne negative helseeffekten blir diskutert. De foreslåtte mekanismene er enten knyttet til effekter på cytokrom P450-relaterte aktiviteter, endret retinoid-signalisering eller en pro-oksidantaktivitet hos betakaroten.

Det er ikke fastsatt en UL for betakaroten. Flere risikovurderingsorganer har imidlertid forsøkt å fastsette UL eller gi uttalelser om hvilke nivåer som kan anses som trygge, oppsummert i tabellen nedenfor:

Tidligere rapporter	Konklusjoner
SCF, 2000, EU	Ingen dose-respons kunne utledes. Tilskudd på 20 mg betakaroten per dag eller mer bør frarådes røykere Det er ikke tilstrekkelig data til å fastsette en UL for betakaroten.
IOM, 2000, USA	Ikke fastsatt UL for betakaroten eller karotenoider. Betakarotentilskudd anbefales ikke til den generelle befolkningen.
EVM, 2003	LOAEL fastsatt til 20 mg/dag. En usikkerhetsfaktor på 3 ble brukt for denne LOAELen. Fastsatte et "Safe Upper Level" for betakarotentilskudd på 7 mg/dag (tilsvarende 0,11 mg/kg kroppsvekt/dag for voksen 60 kg).
NNR, 2012, Norden	Ingen spesifikke anbefalinger for betakaroten.
Rasmussen, 2006, Danmark	Foreslo en "Temporary Guidance Level" for betakaroten som tilsvarte gjennomsnittlig daglig inntak fra kosten (5 mg/dag) I alle aldersgrupper.

Sju randomiserte kontrollerte studier (RCT) fra Europa og USA er inkludert i denne VKM-vurderingen. Til sammen er det nesten 47 100 deltakere som har fått betakaroten som eneste antioksidant i disse studiene. I tillegg ble to prospektive studier inkludert, en fra Danmark og en fra USA, med 125 000 deltakere.

I seks av de randomiserte kontrollerte studiene ble det verken observert økt eller redusert risiko for forskjellige former for kreft. Unntaket er lungekreft, der den store finske Alpha-Tocopherol, Beta-Carotene prevention study (ATBC studien) fant en økt risiko for lungekreft blant de som fikk betakaroten.

Også i den danske prospektive studien fant man at risikoen for lungekreft var høyere hos røykere med økt inntak av betakarotentilskudd.

I tillegg er elleve metaanalyser inkludert i denne VKM-vurderingen; en med aldersrelatert makuladegenerasjon (ARMD) som endepunkt, en med både kardiovaskulær sykdom (CVD) og kreft som endepunkter, fire med kreft som eneste endepunkt, en med både CVD og total dødelighet som endepunkter og fire med total dødelighet som eneste endepunkt. En av metaanalyser med total dødelighet som eneste endepunkt ble senere ekskludert.

Det var ingen økt risiko i metaanalysen for ARMD. En av de to metaanalyser på CVD fant en liten økt risiko i betakarotengruppen (Vivekananthan et al., 2003). Metaanalysen som hadde både CVD og kreft som endepunkt hadde ikke nok styrke til å kunne gi signifikante resultater, men de fant en mulig økning i forekomsten av lungekreft i undergrupper med høy risiko (røykere og asbestarbeidere (Fortmann et al., 2013)).

I de fem metaanalysene på kreft var det ingen økt risiko for andre kreftformer enn lungekreft.

De fire metaanalysene på total dødelighet er basert på data fra de samme RCT studiene som er inkludert i denne VKM-vurderingen. De ekstraherte data for dødstall i hver studie og brukte disse dataene til å analysere risiko for død i betakarotengruppene versus placebogruppene. I alle disse fire metaanalysene ble det funnet en økt risiko for total dødelighet. Disse metaanalysene er blitt grundig diskutert.

I denne risikovurdering av betakaroten i kosttilskudd, har VKM fulgt 4 trinn som beskrevet i SCF sine retningslinjer for fastsettelse av øvre tolerabelt inntaksnivå for vitaminer og mineraler (SCF, 2000a).

Trinn 1 og 2: Fareidentifikasjon og karakterisering.

Inntil for 20 år siden ble betakaroten betraktet som ufarlig selv i store doser. I kjølvannet av den finske ATBC studien som fant en økt risiko for kreft og død hos mannlige røykere, har dyrestudier indikerte tre mulige mekanismer for en slik skadelig effekt fra betakaroten. Selv om man ikke med sikkerhet kan si hva som er mekanismen bak at betakaroten har gitt disse skadelige effektene, er det vitenskapelig grunnlag for å hevde at befolkningsgrupper med sårbare lunger vil kunne ha økt risiko fra betakarotentilskudd.

Dosen i den finske ATBC studien var 20 mg beta-karoten per dag. Økt risiko ble bare observert under selve intervensjonsperioden. I oppfølgingsstudier utført etter at den aktive perioden var avsluttet, falt risikoen og var ikke lenger signifikant. 20 mg beta-karoten kan

således betraktes som det laveste nivået der effekt er observert (lowest observed adverse effect level (LOAEL)).

Den danske prospektive studien fant en doserelatert økning av risiko for lungekreft med økt inntak av betakarotentilskudd. Dessverre er det ikke mulig ut fra de publiserte dataene å sette et null-effekt-nivå (no observed adverse effect level) NOAEL eller LOAEL.

De fire metaanalysene på total dødelighet fant alle 6-7 % økt risiko for død. En av metaanalyser fant også økt risiko for hjerte- og karsykdom hos røykere. Imidlertid er alle resultatene drevet statistisk av ATBC studien. Studier med en mer blandet befolkning (både menn og kvinner) og med en mer normal forekomst av røykere (10-20 %), fant ingen slik økt risiko.

Trinn 2, forts.: Fastsette en UL som tar hensyn til usikkerheten i de vitenskapelige dataene. UL kan utledes fra ulike grupper i befolkningen.

VKM har funnet det særdeles utfordrende å bestemme hvilken usikkerhetsfaktor som vil være mest korrekt å bruke for betakaroten. SCFs retningslinjer for fastsettelse av tolerable øvre inntaksnivåer overlater valg av usikkerhetsfaktor til faglig skjønn. Når NOAEL er utledet fra humandata antas at en sikkerhetsfaktor på 10 å være tilstrekkelig til å ivareta interindividuell variasjon og følsomhet. Retningslinjene fra SCF beskriver videre at en mindre usikkerhetsfaktor kan brukes hvis det sannsynligvis er liten populasjonsvariabilitet for den negative helseeffekten. En større usikkerhetsfaktor (nær 10) kan brukes hvis variasjonen antas å være stor.

For betakaroten er det ingen tilgjengelig NOAEL, men kun LOAEL. Usikkerhetsfaktoren må derfor ta høyde for den usikkerheten som ligger i å utlede en UL fra LOAEL. Størrelsen på usikkerhetsfaktoren bør være avhengig av alvorligheten og hyppigheten av den observerte effekten på LOAEL og dose-respons stigningskurven hvis en slik er mulig å utlede. For betakaroten har vi ikke funnet data som gjø det mulig å lage en dose-responskurve.

I tillegg ble følgende momenter diskutert før en usikkerhetsfaktor ble valgt:

• Studien som fant en negativ effekt fra betakaroten tilskudd (ATBC) var svært stor (n = 29 133), noe som indikerer at populasjonen i studien omfatter interindividuell variasjon og følsomhet. I tillegg inkluderer populasjonen i denne studien de mest sårbare gruppene, i dette tilfellet røykere. Begge disse forholdene indikerer at usikkerhetsfaktoren kan være i den nedre enden av skalaen.

• Metaanalysene for endepunktet "økt risiko for total dødelighet" fant altså en økt risiko for dødelighet i betakaroten-gruppene. Dette er alvorlig, og indikerer en maksimalt høy usikkerhetsfaktor. Imidlertid er alle resultatene for økt total dødelighet i disse metaanalysene drevet statistisk av ATBC studien med røykere. Vi velger derfor å bruke en lavere faktor.

Basert på momentene diskutert ovenfor, velger VKM å bruke 5 som en usikkerhetsfaktor for betakaroten.

En UL for beta-karoten kan ikke utledes, men et tentativ øvre nivå (TUL) blir satt til 4 mg/dag, basert på en LOAEL på 20 mg og usikkerhetsfaktor på 5.

Røykere og andre i befolkningen med sårbare lunger (for eksempel astmatikere og KOLS-pasienter) bør frarådes å ta betakarotentilskudd.

Trinn 3: **Eksponeringsvurdering - en vurdering inntaket av betakaroten i den generelle befolkningen.**

I kostholdsundersøkelsen Småbarnskost (2007) er det gjennomsnittlige inntaket av betakaroten blant 2-åringer 1,5 mg/dag, og i Norkost 3 er det gjennomsnittlige inntaket for voksne 2,4 mg/dag og inntaket i 95-persentilen er 6,9 mg/dag.

Bare ca. 3 % av de voksne har rapportert bruk av betakarotentilskudd. Det er mulig at bruk av bruningspiller har blitt underrapportert i Norkost 3.

Betakaroten i vanlige matvarer som grønnsaker og frukt er ikke ansett for å kunne medføre negative helseeffekter. Det er aldri rapportert om negative helseeffekter av betakaroten i vanlig mat. Tvert imot bør forbruket av grønnsaker og frukt økes, slik at anbefalingen "5 om dagen" oppnås i alle grupper i befolkningen.

Trinn 4. Risikokarakterisering - analyse av konklusjonene fra trinn 1 til 3, og karakterisering av risikoen. Risikoen vil avhenge av om noen grupper i befolkningen overskrider UL samt størrelsen og varigheten av overskridelsen.

Ettersom betakaroten i mat, uavhengig av mengde, anses som uproblematisk, er det kun inntak av betakaroten fra kosttilskudd som er relevant.

Basert på en LOAEL på 20 mg betakaroten per dag og en usikkerhetsfaktor på 5 konkluderer derfor VKM med at 4 mg betakaroten per dag er et egnet tentativ øvre nivå for tilsatt betakaroten.

Abbreviations and glossary

ARM	– age-related maculopathy
ARMD	– age-related macular degeneration
AREDS	– Age Related Eye Disease Study
ATBC study	– Alpha-Tocopherol Beta-Carotene prevention study, Finland
bw	– body weight
CARET	– Carotene and Retinol Efficacy Trial
CBP	– carotenoid breakdown product
CI	– confidence interval
COPD	– chronic obstructive pulmonary disease
CYPs	– cytochromes P450
CVD	– cardiovascular disease
DCH	– Danish Diet, Cancer and Health cohort study
EFSA	– European Food Safety Authority
EMBASE	– Excerpta Medica dataBASE
EVM	– Expert group on vitamins and minerals of the Food Standard Agency, UK
FFQ	– food frequency questionnaire
HPS	– Heart Protection Study, USA
HR	– hazard ratio
IRR	– incidence rate ratio
LOAEL	– lowest observed adverse effect level
Maculopathy	– pathological condition of the macula, an area at the centre of the retina
MEDLINE	– Medical Literature Analysis and Retrieval System Online
NNR	– Nordic Nutrition Recommendations
NOAEL	– no observed adverse effect level
NSCPS	– Nambour Skin Cancer Prevention Study, Australia
NSAS	– Nambour Skin Aging Study, Australia
IOM	– Institute of medicine, USA
OR	– odds ratio
PHS	– Physicians' Health Study, USA
PPS	– Polyp Prevention Study
RARs	– retinoic acid receptors
RCT	– randomised controlled trial
RDA	– recommended daily intake
RE	– retinol equivalent
RR	– relative risk
SCF	– Scientific Committee on Food, EU
SCPS (SCP)	– Skin Cancer Prevention Study, USA
TGL	– temporary guidance level
TUL	– tentative tolerable upper intake level
UL	– tolerable upper intake level
VITAL study	– Vitamins and Lifestyle cohort study

VKM – Norwegian Scientific Committee For Food Safety
WACS – Women's Antioxidant Cardiovascular Study, USA
WHS – Women Health Study, USA

Primary prevention trial – study investigating delay or prevention of onset of a disease or condition.
Secondary prevention trial – study investigating subjects with a disease or condition to prevent recurrence or exacerbation.

Background as provided by the Norwegian Food Safety Authority/ Norwegian Environment Agency

Directive 2002/46/EC on food supplements was implemented in Norwegian law in 2004 in Regulation 20 May 2004 No. 755 on food supplements. Pursuant to directive 2002/46/EC, common maximum and minimum levels of vitamins and minerals in food supplements shall be set.

The national maximum limits for vitamins and minerals were established in the former regulation on vitamin and mineral supplements from 1986 and were continued in the 2004 regulation. These maximum limits apply until common limits are established in the EU.

The European Commission started to establish common limits in 2006, but the work was temporarily put on standstill in 2009. The time frame for the further work is not known.

Maximum limits for levels of vitamins and minerals in food supplements shall be set on the basis of the following criteria, pursuant to article 5 in Directive 2002/46/EC:

- Upper safe levels of vitamins and minerals established by scientific risk assessment based on generally accepted scientific data, taking into account, as appropriate, the varying degrees of sensitivity of different consumer groups
- Intake of vitamins and minerals from other dietary sources

When the maximum levels are set, due account should also be taken of reference intakes of vitamins and minerals for the population.

Pending establishment of common maximums limits in the EU, the Norwegian Food Safety Authority is evaluating the national maximum limits for vitamins and minerals in food supplements.

Assessment of beta-carotene

Vitamin A includes all forms having the same biological activity as retinol. Retinol is present in several foods of animal origin. Vitamin A also exists in plants in the form of carotenoids, which are converted to vitamin A (retinol) in the body.

Pursuant to the Norwegian regulation on food supplements, the minimum and maximum limit for vitamin A in food supplements is 200 µg RE and 1500 µg RE per daily dose, respectively. Beta-carotene is one of four permitted vitamin A forms in food supplements (retinol, retinyl palmitate, retinyl acetate and beta-carotene).

The vitamin A activity of beta-carotene depends on whether beta-carotene is purified or in foods (IOM, 2000; NNR Project Group, 2012). The NNR Project Group (2012) indicates that 1 retinol equivalent (RE)=1 µg retinol=2 µg of supplemental beta-carotene=12 µg of dietary beta-carotene=24 µg of other dietary provitamin A carotenoids.

In 2002, the Scientific Committee on Food (SCF) established a tolerable upper intake level (UL) for vitamin A (SCF, 2002). However, this opinion covers only retinol compounds (pre-formed vitamin A). The bioconversion of carotenoids to pre-formed vitamin A in the body is well regulated and therefore only intake of pre-formed vitamin A is considered relevant for vitamin A toxicity (Blomhoff et al., 2003; EFSA, 2008). Serious adverse health effects from beta-carotene not related to its conversion to retinol have however been described in both the SCF opinion (2000), in the EFSA statement on beta-carotene (EFSA, 2012) and in the Nordic Nutrition Recommendation 5th update (NNR Project Group, 2012).

The Norwegian Food Safety Authority is therefore considering whether beta-carotene should be regulated separately from retinol compounds. A maximum limit for beta-carotene should be set considering the criteria listed above.

Relevant background documents

- Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of Beta Carotene (SCF, 2000)
- Statement on the safety of β-carotene use in heavy smokers (EFSA, November 2012)
- Nordic Nutrition Recommendations 5the update (NNR Project Group, 2012)
- Comments from the Norwegian Scientific Committee for Food Safety, Panel on Nutrition, dietetic products, Novel Food and Allergy (Panel 7) on the setting of maximum limits for vitamins and minerals in foods (VKM, 2007)
- Safe Upper Levels for Vitamins and Minerals, UK (EVM, 2003)
- A safe strategy for addition of vitamins and minerals to foods (Rasmussen, 2005)

Terms of reference as provided by the Norwegian Food Safety Authority

The Norwegian Food Safety Authority requests the Norwegian Scientific Committee for Food Safety (VKM) to assess the risk of beta-carotene in food supplements. The risk assessment should address the following aspects and questions:

- Because of insufficient data, neither SCF nor EFSA have been able to establish a tolerable upper intake level (UL) for beta-carotene. Other expert groups have set indicative or temporary upper guidance levels (EVM, IOM, Rasmussen et al., 2005). What upper safe level for beta-carotene should be used as basis for establishing a maximum limit in food supplements? A description of the adverse health effects related to this upper safe level shall be included. Are there particular circumstances in Norway that should be taken into consideration in this context? Are there any circumstances that may result in toxic effects from conversion of carotenoids to retinol?
- It is important to ensure that the total intake of vitamins and minerals from all sources does not exceed the UL. VKM is therefore requested to estimate the intake of beta-carotene from the diet, in all age groups in the population above 1 year.
- VKM is requested to conduct scenario estimations to illustrate the consequence of a possible maximum limit for beta-carotene in food supplements.

Assessment

1 Introduction

Beta-carotene is a provitamin, i.e. a precursor of vitamin A (retinol), which is classified as an essential nutrient for humans. Being an essential nutrient implies that we get deficiency symptoms if we are provided with too little of the substance. Vitamin A has multiple functions: it is important for growth and development, for the maintenance of the immune system and good vision. Vitamin A is needed by the retina of the eye in the form of retinal, which combines with protein opsin to form rhodopsin, the light-absorbing molecule necessary for both low-light (scotopic vision) and colour vision. Vitamin A deficiency is a leading cause of blindness worldwide.

Vitamin A also functions in a very different role as retinoic acid (an irreversibly oxidised form of retinol), which is an important hormone-like growth factor for epithelial and other cells.

Retinol has a narrow range between recommended daily intake and upper level, the upper level being only 2-3 times above the recommendation. Beta-carotene, on the other hand, which chemically speaking may be considered as a "double-retinol", was regarded as innocuous until some decades ago. A simple cleavage in the middle changes one molecule of retinol into two molecules of beta-carotene. In 1994, results from the Finnish ATBC-study indicated that beta-carotene from supplements increased the risk of lung cancer in male smokers. In the wake of this and other later findings, it seems pertinent to perform a risk assessment of beta-carotene, especially because the serious adverse effects of beta-carotene in the form of supplements do not seem to be related to its conversion to retinol.

VKM emphasises that this opinion on UL for beta-carotene is addressing beta-carotene in food supplements. Beta-carotene from regular foods such as vegetables and fruits is not considered to be of health concern.

2 Hazard identification and characterisation

2.1 Chemistry, absorption and metabolism

The paragraphs in this chapter (2.1) are mostly obtained directly from the following sources: 1) Mammalia Metabolism of beta-carotene: Gaps in knowledge (Shete and Quadro, 2013) 2), SCF (2000b) and 3) Modern Nutrition in Health and Disease (Ross et al., 2012).

Occurrence

Carotenoids are found in plants, fungi and bacteria. Carotenoids are therefore predominantly obtained through foods of plant origin or food supplements. In plants, these compounds accumulate in the plastids, giving the characteristic colours yellow, red and orange to many fruits and vegetables (Shete and Quadro, 2013). More than 600 carotenoids have been isolated from natural sources; nearly 60 of them have been detected in the human diet. Beta-carotene is the most abundant carotenoid found in the human diet and plant foods are the major source. Carrots contribute with approximately half of the total beta-carotene intake in the Norwegian diet, followed by mixed frozen vegetables, tomatoes, fruits and berries and to some extent dairy products.

In plants, they function as structural and functional assistants to the photosynthetic apparatus, specifically to serve as light-harvesting pigments and protect against photo-oxidative stress. Carotenoids obtained through the diet have several beneficial functions in mammals, due to their antioxidant properties, their ability to generate vitamin A. In addition, emerging knowledge indicates that beta-carotene metabolites might have crucial signalling functions (Kaulmann and Bohn, 2014).

Chemistry

Based on their chemical structure, carotenoids can be classified as carotenes and xanthophylls. Carotenes (like beta-carotene, alpha-carotene and beta-cryptoxanthin) are non-oxygenated carotenoids that may be linear or possess cyclic hydrocarbons at one or both ends of the molecule. Beta-carotene contains 40 carbons with 15 conjugated double bonds and 2 β-ionone rings at both ends of the molecule. These structural properties make beta-carotene highly hydrophobic and non-polar. In nature it is predominantly found as all-*trans* beta-carotene.

Metabolism

Beta-carotene and some other carotenoids also serve as precursors of vitamin A, thus allowing their classification as provitamin A carotenoids. Provitamin A carotenoids yield vitamin A and its metabolites (retinoids) upon enzymatic and non-enzymatic cleavage.

Beta-carotene can yield two molecules of retinaldehyde upon its symmetrical cleavage by the enzyme β-carotene-15,15'-oxygenase (CMOI or BCMO1 or BCO1). However, it can also be cleaved asymmetrically by the enzyme β-carotene-9',10'-oxygenase (CMOII or BCDO2 or BCO2), to generate a β-ionone ring and apo-carotenals, which can be ultimately converted to one molecule of retinaldehyde. The mechanism of the latter conversion has not been completely elucidated. Retinaldehyde formed upon the cleavage of provitamin A can be oxidised by the action of enzymes of the retinaldehyde dehydrogenase family (RALDH or ALDH 1 family) to generate all-*trans* retinoic acid, the biologically active form of vitamin A. The metabolism of beta-carotene is illustrated in Figure 2.1-1.

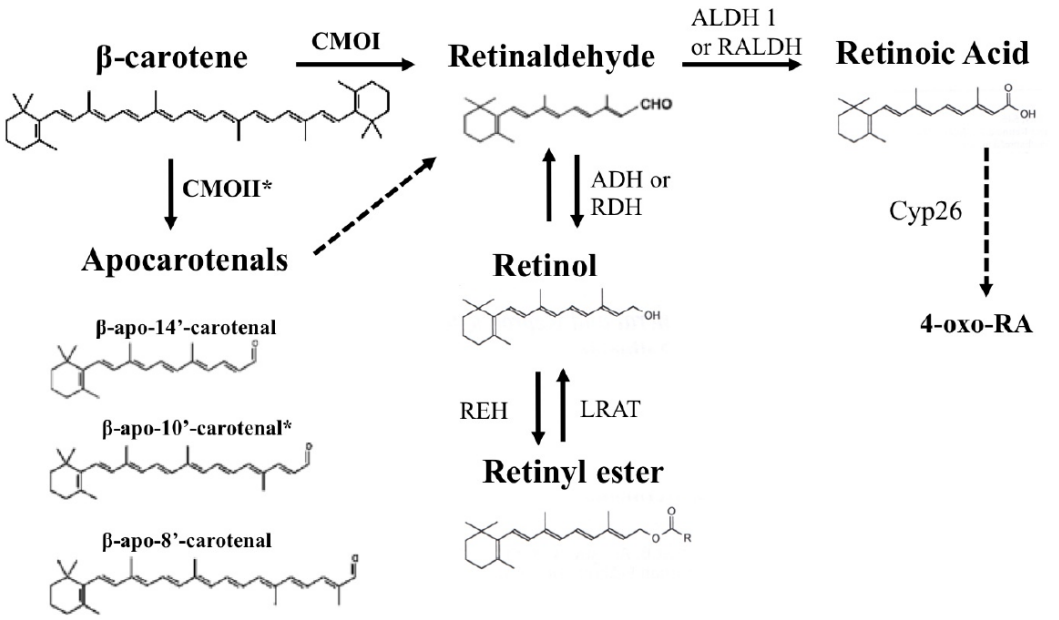


Figure 2.1-1 Metabolism of beta-carotene. Note that the CMOI conversion from beta-carotene to retinaldehyde is tightly regulated.

Absorption

Beta-carotene is absorbed in the small intestine with lipids. About 17-45% of the ingested beta-carotene is released into the blood circulation uncleaved even though CMOI is abundant in the small intestine. In humans, the concentration of intact beta-carotene in plasma is a good indicator of the absorption of ingested beta-carotene. In addition to genetic factors, the bioavailability of beta-carotene is affected by the nature of food matrix, fat content of the diet, type of fat, digestibility of fat-soluble components in the diet, bile acids, interactions with other carotenoids and individual variations due to endogenous activity of the digestive

enzymes. The absorption/bioavailability of beta-carotene from supplements is higher than the bioavailability of beta-carotene from regular foods.

Transport and storage

Beta-carotene is transported from the intestine to various tissues with lipoproteins. Circulating carotenoid concentrations are found to be lower in smokers than in non-smokers, due in part to the depletion of these compounds by components of cigarette smoke (SCF, 2000 citing Handelman et al., 1996).

Animal models

Animal models may be useful for studying human nutrient metabolism provided that the model has physiologic similarities to humans with respect to the particular issue being studied. Finding appropriate animal models for beta-carotene has proved difficult. According to a review by Lee et al. (Lee et al., 1999a), an ideal animal model would be one having the following main characteristics: 1) absorb a variety of carotenoids intact at physiologic levels, similarly to humans; 2) have carotenoid distribution in tissues and serum similar to that of humans; 3) represent an appropriate model for the disease state of interest.

There are no animal models fulfilling all these criteria and thus having the features required to represent an adequate model of human absorption and metabolism of beta-carotene. Gerbils, ferrets and pre-ruminant calves are most similar to humans. Only gerbils and calves convert beta-carotene to vitamin A with efficiency similar to that of humans. However, these models are not well established for the endpoints which have investigated in epidemiologic studies with carotenoids. Mice and rats are more established for the study of cancer, immune function and vitamin A deficiency. Mice and rats efficiently convert beta-carotene to vitamin A, but beta-carotene can only be detected in their circulation if the intake is very high.

Therefore, results from animal model studies were not included in the literature search done for this report. Animal model studies have, however, been considered in discussions regarding mechanisms of actions.

Interaction with vitamin A

Intestinal uptake and beta-carotene conversion into retinoids have been shown to be regulated by a feedback mechanism depending upon the vitamin A status. These processes are attenuated when dietary vitamin A is in excess to prevent accumulation of toxic levels of retinoids.

Little is known regarding how beta-carotene is transported within mammalian cells, despite the importance of this process which might influence intracellular accumulation and metabolism. Most of the current knowledge in this area pertains to the intracellular trafficking of carotenoids other than beta-carotene and even in these cases, many questions remain unanswered.

2.1.1 Mechanism of carcinogenic action

As will be described in greater detail in the following chapters, beta-carotene seems to have a carcinogenic effect in smokers. A number of studies have been published where possible mechanisms for this effect are discussed. The suggested mechanisms in the sections below are elaborated on in SCF (2000b), and are just briefly mentioned here.

Effects on cytochrome P450-related activities

It has been suggested that beta-carotene may exert its carcinogenic effects by inducing cytochrome P450 (CYP) activities (in particular CYP 1A1/2), with a consequent increase in the metabolism of cigarette smoke constituents. Moreover, beta-carotene has also been shown to induce phase I carcinogen-bioactivating enzymes. Supplementation with beta-carotene may thus increase the risk of lung cancer in smokers both due to the co-carcinogenic properties of beta-carotene and its capacity to generate oxidative stress.

Altered retinoid signalling

In animals fed a beta-carotene supplement and simultaneously exposed to cigarette smoke, supplementation with beta-carotene led to lower levels of retinoic acid in lung tissue and reduced gene expression of one of the retinoic acid receptors (RARs)(the RAR-beta). Moreover, animals given beta-carotene supplement and exposed to tobacco smoke had three- to fourfold elevated expression of the *c-jun* and *c-fos* genes. The decreased lung concentration of retinoic acid may cause diminished retinoic signalling, enhanced lung cell proliferation, and potential tumour formation. The retinoic acid levels were lowered in lung tissue as a result of beta-carotene supplementation, in spite of the animals having increased levels of beta-carotene. It has been suggested that some of the eccentric cleavage products of beta-carotene may act as ligands and interfere with RARs, however, this has not been documented. But it is possible that beta-carotene supplementation in itself might modify beta-carotene metabolism.

The pro-oxidant activity of beta-carotene

Carotenoids can act as antioxidants and can scavenge peroxyl radicals. Their role in vivo is, however, unclear. As with all antioxidants, beta-carotene can switch to a pro-oxidant form. The switch from antioxidant to pro-oxidant behavior can be a function of oxygen concentration. The pro-antioxidant activity of beta-carotene has been demonstrated at high partial pressure of oxygen (Zhang and Omaye, 2001), as may occur in the outermost cells of the lung. These cells might be particularly vulnerable to the pro-oxidative effect of beta-carotene. Further, the anti- and pro-oxidant effects of beta-carotene are concentration dependent, and high doses of beta-carotene (like some other vitamins) may exert a pro-oxidant effect and cause inflammation (de Oliveira et al., 2012). It seems appropriate in this context to mention that certain anti-oxidant vitamins, including beta-carotene, have been reported to induce DNA damage also by mechanisms unrelated to oxygen radical formation (Veloso et al., 2013).

2.2 Previous reports on safe upper level for beta-carotene

2.2.1 Scientific Committee on Food (EU), 2000

The SCF (2000b) report on the tolerable upper intake level (UL) of beta-carotene focused on safety related to intake levels in European countries. Available literature was reviewed for estimating the intake levels. The committee reported that natural food sources may contribute 2-5 mg beta-carotene per day, with potentially large variations within populations due to variations in fruit and vegetable consumption, while food additives (as e.g. colour agent) may contribute 1-2 mg per person per day.

Animal studies and human studies published up to year 2000 were reviewed with focus on safety. Concerning animal studies, the committee stated that no adverse effects of high-dose oral beta-carotene supplementation have been observed in several standard toxicological studies in various experimental animals. However, they noted that animal studies do not represent appropriate models for the effect in humans, due to their intestinal conversion of carotenoids to retinol, leading to negligible concentrations of beta-carotene in the circulation. The ferret studies are discussed.

Concerning human studies, six large prevention trials in humans did not support a preventive effect of supplementation with beta-carotene for preventing cancer and cardiovascular disease, as suggested by older observational studies. Three of these are described in detail: The Alpha-Tocopherol Beta-carotene Cancer prevention study (ATBC-study) (The Alpha-Tocopherol-Group, 1994), the Carotene and Retinol Efficacy Trial (CARET), and the Physicians' Health Study (PHS).

Based on the available studies, no dose-response relationship could be derived. The SCF (2000b) concluded that beta-carotene supplementation of 20 mg per day or more is contraindicated for use in current heavy smokers, while there is insufficient evidence to set an UL for beta-carotene.

2.2.2 Institute of Medicine (USA), 2000

The IOM (2000) evaluation regarding tolerable upper intake levels for beta-carotene starts with stating that no adverse health effects have been observed after intake of beta-carotene, except for carotenodermia (orange discolouration of the outer skin) with doses >30 mg/day. Carotenodermia is reversible and a cosmetic problem only. Beta-carotene is used therapeutically in very high doses (180 mg/day and similar) to treat erythropoietic porphyria. No hypervitaminosis A or other toxic effects have been observed with these doses. There are no indications that beta-carotene is teratogenic, mutagenic, or carcinogenic in long-term studies in animals. However, two recent (at that time) studies are mentioned that open the possibility of an increased risk for lung cancer in active smokers who take beta-carotene supplements.

The IOM report gives a brief summary of the ATBC study (The Alpha-Tocopherol-Group, 1994) (see chapter 2.4.2.1) and the CARET trial. In both studies, the occurrence of lung cancer was found to be increased in active smokers taking beta-carotene.

IOM then refers to the PHS study in which supplementation with beta-carotene was given, without being followed by a change in cancer incidence or mortality (Hennekens et al., 1996). Finally, the IOM report (2000) mentions a study by Blot et al., 1993, in which participants were given different supplement combinations, one of them containing beta-carotene. Reduced total mortality, cancer mortality, and reduced gastric and oesophageal cancer occurrence were reported in this study from China.

IOM concluded that there are conflicting results in different studies with regard to the effect of beta-carotene on lung cancer in smokers, and that available data were insufficient for dose-response evaluation and for setting of a UL for this outcome. Beta-carotene as a food supplement could not be recommended for the general population.

2.2.3 Expert Group on Vitamins and Minerals (UK), 2003

After a general introduction about the chemistry, bioavailability, content in foods and toxicity of beta-carotene, the UK report from the Expert Group on Vitamins and Minerals (EVM, 2003) focuses on studies of particular importance in a risk assessment. Animal studies are mentioned and it is emphasised that no animal models are ideal for studying beta-carotene because animals metabolise beta-carotene very differently from humans. The ferret model seems to be the best so far, but also this has several limitations.

Four intervention studies with beta-carotene are described in detail (the ATBC study, the CARET trial, the US PHS study and the Heart Protection Study).

When EVM (2003) established a safe upper level, they put most emphasis on the ATBC study because this was the study to show adverse effects at the lowest level of supplemental intake. The lowest observed effect level (LOAEL) from this study was 20 mg/day. Applying an uncertainty factor of 3 to extrapolate from a LOAEL to a no observed adverse effect level (NOAEL) resulted in a safe upper level for supplementation of 7 mg/day. This is equivalent to 0.11 mg/kg bw/day for a 60 kg adult. It is stressed that this safe upper level applies to supplements only, as there is no evidence to suggest that current levels of beta-carotene intake from foods result in adverse effects. The choice of size of the safety factor was not explained.

2.2.4 Nordic Nutrition Recommendations, 2012

In the Nordic Nutrition Recommendations (NNR Project Group, 2012), dietary antioxidants (including beta-carotene) are described in a separate chapter. The chapter describes the concept of oxidative stress and describes food sources with and anti-oxidative properties. Among these are carotenoids including beta-carotene, alpha-carotene and beta-cryptoxanthin.

They describe studies investigating effects of anti-oxidative food components. These are mainly cell culture studies which are difficult to extrapolate from. A major limitation in intervention studies is that currently there is no single "gold-standard" method for measurement of biomarkers of oxidative stress. Some intervention studies (mainly animal studies) are described that have investigated the effect of antioxidant rich foods (such as strawberries, spinach, blueberries, raspberries, walnuts, pomegranate, brussels sprouts, onions, and tomatoes) on different markers of oxidative stress.

The main human antioxidant supplementation trials where beta-carotene has been investigated are also described. The NNR Project Group (2012) refers to several meta-analyses which have shown no protective effects on cardiovascular diseases (CVDs), gastrointestinal cancer or mortality. Finally, a large meta-analysis by Bjelakovic et al. from 2007 including 47 high-quality studies is described, which found a significant increased mortality after beta-carotene supplementation (7%) (see chapter 2.4.4).

No evidence of vitamin A toxicity or increased plasma retinol concentrations has been shown with doses up to 180 mg beta-carotene /day as supplements.

The NNR Project Group (2012) concluded that "there is a large body of evidence that diet rich in fruits, berries, vegetables, pulses and seeds reduces the risk of CVD, cancer and other chronic diseases associated with major oxidative stress". However they also conclude that "recommendations for specific antioxidant-rich fruit and vegetables beyond the ordinary recommendations cannot be given at this time".

With respect to antioxidant supplements they conclude that "there is a large body of evidence suggesting that elevated intakes of certain supplements, mainly vitamins with antioxidative properties might increase the risk of certain adverse health effects. Thus there is no justification for using supplements as a tool for adjusting an unbalanced diet".

2.2.5 Danish strategy for vitamins and minerals, 2006

Rasmussen et al published the following article in 2006 in the European Journal of Nutrition: A safe strategy for addition of vitamins and minerals to foods (Rasmussen et al., 2006).

The article has its focus on food fortification and dosages of vitamins and minerals which are safe to add to foods, but have some reflections on upper intakes of nutrients and also has a small paragraph on beta-carotene. The article was pivotal for establishing Norwegian regulations on food fortification and their beta-carotene paragraph is therefore quoted here:

"Although the adverse effects of beta-carotene on human lung cancer risk have only been observed in groups at elevated risk for lung cancer, the studies available with cohorts at lower risk have insufficient power to exclude that adverse effects of high doses of beta-carotene may also affect others. Animal studies in ferrets indicate that beta-carotene *per se* affects cell differentiation and proliferation in the bronchus and that substances in cigarette smoke enhance this effect. Sources of beta-carotene in the diet include its natural

abundance in many foods, the use of beta-carotene as a colorant and as a provitamin A in supplements. The difference in dose between the 95% confidence interval for dietary levels (5-9 mg/day) and the adverse intake levels is quite narrow, although differences in bioavailability of beta-carotene from foods and from supplements may cause a larger difference in plasma levels. Moreover, the mechanisms behind the adverse effects are not fully understood and may affect population groups and target organs other than those presently known. Based on these uncertainties, we suggest avoiding the use of beta-carotene for food fortification in Europe, i.e., setting the Temporary Guidance Level (TGL) for beta-carotene equal to the average dietary level of 5 mg/day for all age groups leading to a zero allowance for fortification of food with beta-carotene."

2.2.6 Summary of previous reports on beta-carotene

Conclusions from previous reports on UL for beta-carotene are summarised in Table 2.2.6-1.

Table 2.2.6-1 Conclusions from previous reports on UL for beta-carotene.

Previous reports	Conclusion
SCF, 2000, EU	No dose-response relationship could be derived. Supplementation of 20 mg beta-carotene per day or more is contraindicated for use in current heavy smokers. There is insufficient evidence to set an UL for beta-carotene.
IOM, 2000, USA	No UL was established for beta-carotene or carotenoids. Beta-carotene supplementation is not recommended in the general population.
EVM, 2003, UK	The LOAEL was set to 20 mg/day. An uncertainty factor of 3 was applied to extrapolate from LOAEL to a NOAEL. A Safe Upper Level for beta-carotene supplements was set at 7 mg/day (equivalent to 0.11 mg/kg bw day for a 60 kg adult).
NNR, 2012, Nordic countries	No specific beta-carotene recommendation or UL.
Rasmussen, 2006, Denmark	A Temporary Guidance Level for beta-carotene equal to the average dietary level of 5 mg/day for all age groups was suggested.

2.3 Literature search

A literature search was conducted to obtain and assess new knowledge about adverse health effects from high intakes of supplemental beta-carotene. The search aimed to retrieve studies addressing high intakes of beta-carotene in food supplements.

2.3.1 Search strategy

In order to retrieve relevant publications addressing high intakes of supplementary beta-carotene and health outcomes, systematic literature searches in MEDLINE and EMBASE were conducted (28 September 2014). Both databases were used in order to ensure comprehensive study retrieval. The strategy for the searches was discussed within the project group and with a librarian who also performed the searches.

The search included different terms for beta-carotene and carotenoids, different terms for adverse health effects and different terms for food supplements. Both animal and human studies were included in the search terms. However, animal studies were later excluded. For view of the search terms use, see Appendix I.

The search period was limited to publications from 2002 till September 28, 2014, the argument being that we could use previous reports from competent bodies on the establishment of UL for beta-carotene as a starting point, e.g. the report published in 2003 by the Expert Group on Vitamins and Minerals, UK. The search was further limited to include publications written in English or Scandinavian languages (Danish, Swedish and Norwegian) only.

2.3.2 Publication selection

The study types for inclusion in this opinion were systematic reviews and meta-analyses of human studies, randomised controlled trials and prospective cohort studies presenting good data for beta-carotene supplementation in at least one subgroup. The criteria for inclusion were:

- Beta-carotene in relation to health outcomes was the main issue (or one of the main issues) in the article.
- Results for beta-carotene could be separated from results from other antioxidants or carotenoids, i.e. there had to be a pure beta-carotene arm in randomised controlled trials (RCTs) and there had to be good information on the amount of beta-carotene-data obtained from single supplements in prospective studies.
- The effects of beta-carotene supplements could be compared to a placebo group
- Study population representative for the general population (e.g. some specific patients groups such as patients with HIV were excluded).

- To reduce the possibility of confounding from inclusion of nutritionally deficient populations, we only included studies from populations from developed countries without overt evidence of vitamin deficiencies.

Animal- and in vitro studies were excluded (see chapter 2.1), as were position papers, conference abstracts/summaries, editorial comments and various dietary guidelines.

The literature searches identified 353 articles after removal of duplicates.

Study titles and abstracts were independently reviewed by two persons from the project group according to the above mentioned inclusion criteria. Titles were selected if chosen by one of the experts and resulted in 45 full text publications which were distributed in the project group for full text examination. This resulted in the inclusion of 16 publications.

Additionally, 13 publications resulting from hand searching/retrieval of relevant literature cited in the full-text papers have been included.

A final total of 28 publications were identified and included in the results in this report (see Figure 2.3.2-1).

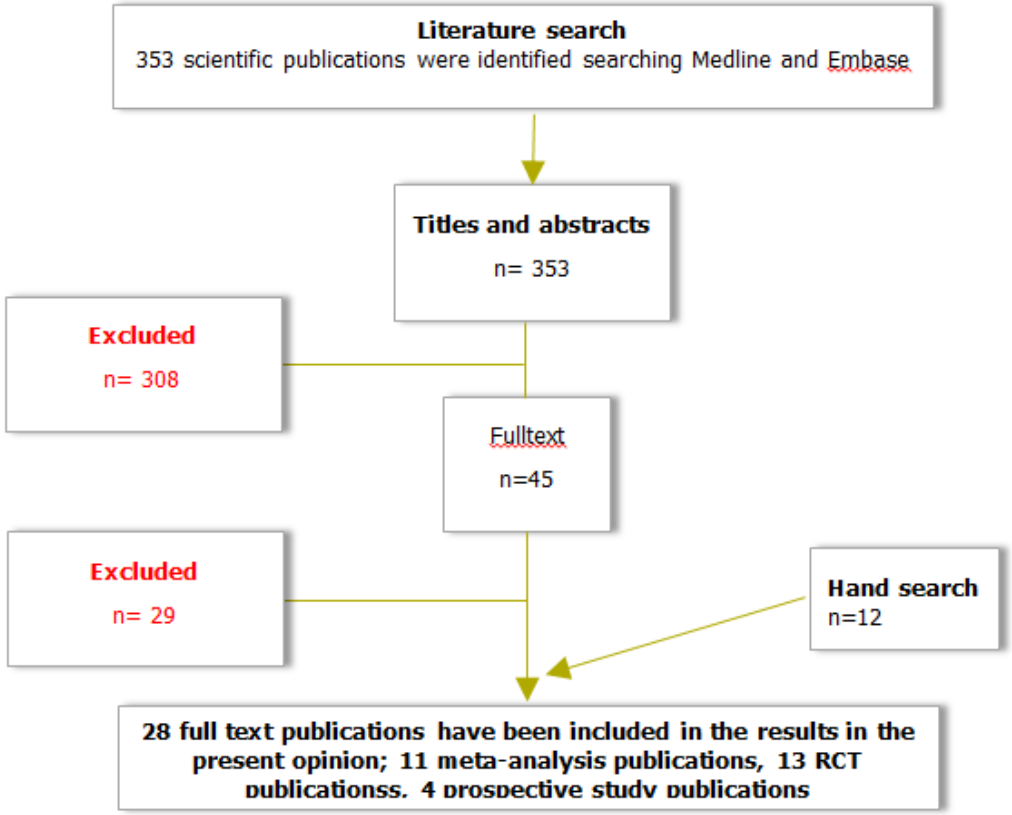


Figure 2.3.2-1 Flowchart for the literature search for supplemental beta-carotene and associated health outcomes and the subsequent selection of publications.

2.3.3 Data extraction and relevance

The majority of the RCTs retrieved in the literature search investigated possible beneficial effects of beta-carotene or other antioxidants on coronary heart disease, cancer or prevention of other conditions. The study design of these "effect" studies might not be suitable for detecting possible negative health effects, but are, however the best at hand.

Relevance for the purpose of this opinion has been evaluated for each included paper, and is stated in the Summary Tables in Appendix III.

2.4 Human studies investigating supplementary beta-carotene and health effects

2.4.1 Chapter introduction

This chapter has a brief review of human studies with beta-carotene. Almost all studies were conducted in the 1980s and 1990s in the wake of the new understanding that free radical damage was involved in the early stages of atherosclerosis and may contribute to cancer, vision loss, and many other chronic conditions. Several large studies showed that people with low intakes of antioxidant-rich fruits and vegetables were at greater risk for developing these chronic conditions than were people with higher fruit and vegetable intakes. This triggered the onset of clinical trials which began testing the impact of single substances, especially beta-carotene and vitamin E, as prevention or treatment for heart disease, cancer, and other conditions. When results started appearing, mostly showing no effect, or even detrimental effects, such studies stopped abruptly, and we have not found any relevant, randomised controlled trial (RCT) with beta-carotene supplements conducted after the year 2000.

A list of excluded articles can be found in Appendix II.

2.4.2 Randomised controlled trials

The literature search revealed that a limited number of RCTs laid the foundation for the published reviews and meta-analyses, almost irrespective of outcome. Table 2.4.2-1 gives an overview of these trials. The reference column only cites one or two articles from each study, while in reality a number of articles have been published from each of them. In the Summary Tables in Appendix III, there is one Summary Table per article.

The first trial included smokers only and is listed first, one study was a secondary prevention trial of people diagnosed with skin cancer, this comes next, while four studies included mostly healthy people and are listed last. Table 2.4.2-1 is followed by a closer description of each study. As pointed out earlier, an inclusion criterion for this report was for an RCT to have a pure beta-carotene arm. Because of this, several well-known studies were excluded, e.g. the CARET study (gave retinol and beta-carotene in combination) and the Heart Protection Study (HPS) (gave vitamin C, E and beta-carotene in combination).

The papers from Lin et al. (2009) and Song et al. (2009) are both from the same study (WACS) and have been given separate rows to show the number of participants and results more clearly.

Table 2.4.2-1 Overview of all randomised controlled trials included in this opinion.

Reference	Study	Participant character-istics	Country (study start)	Design	Number in treatment group; beta-carotene/ placebo	Dose	Main endpoint and result	Intervention duration/ follow-up years
The Alpha-Tocopherol-Group (1994), Teikari et al. (1998), Virtamo et al. (2003), Wright et al. (2010)	**ATBC***	29 133 male smokers, aged 50-69y	Finland (1985-88)	2x2 factorial design, vit E, b-carotene, vit E + b-carotene, placebo	7282/7287	20 mg/d	Lung cancer. Increased risk for lung cancer (18%) in the bc group after 6 y, but not for other cancers.	6.1y/14.1y
Greenberg et al. (1990)	**SCPS***	1805 elderly skin cancer patients	USA (1983)	b-carotene vs placebo	878/870	50 mg/d	Skin cancer. No difference between groups after 5 y	5y/5y
Green et al (1999)	**NSCPS***	1621, 20-69y.	Australia (1986)	2x2 factorial design, b-carotene vs sunscreen	416/393	30 mg/d	Skin cancer. No difference between groups after 4.5 y	4.5y/4.5y
Hughes et al. (2013)	**NSAS***	903 young adults	Australia (1992)	2x2 factorial design, sunscreen vs b-carotene	447/439	30 mg/d	Skin aging. No difference between groups after 4.5 y	4.5y
Hennekens et al. (1996), Christen et al. (2007), Liu et al. (2009)	**PHS***	22 071 male physicians age 40-84y. 11% were current smokers	USA (1982)	2x2 factorial design, aspirin vs b-carotene	11 036/ 11 035	50 mg every second day	Lung cancer, deaths from cancer, CVD, myocardial infarction, stroke. No difference between groups after 12 y	12y/12.9y
Lee et al. (1999b)	**WHS***	Women age > 45y, no history of cancer or CVD, 13% smokers at start	USA (1993)	2x2x2 factorial design, aspirin, vit E and/or b-carotene	19 939/ 19 937	50 mg every second day	Cancers, deaths from cancer, CVD, myocardial infarction or stroke. The β-carotene part terminated after 2.1 y.	2.1y/10.1y
Lin et al. (2009)	**WACS***	8171 female health professionals ≥40y with a history of CVD or ≥3 CVD risk factors	USA, study start 1995	2x2x2 factorial design, vit C, Vit E, b-carotene or placebo	3807/3820	50 mg every second day	Cancer risk	9.4y/9.4y
Song et al. (2009)	As in Lin, 2009	6574 female as above who were free of diabetes at baseline	As above	As above	3284/3290	As above	Type 2 diabetes	As above

*ATBC: The Alpha-Tocopherol Beta Carotene Cancer Prevention Study, SCPS: Skin Cancer Prevention Study, USA, NSCPS: Nambour Skin Cancer Prevention Study, Australia, NSAS: Nambour Skin Aging

Study: PHS: Physicians Health Study, WHS: Women's Health Study, WACS: Women's Antioxidant Cardiovascular Study.

2.4.2.1 The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study

The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) was a randomised, double-blind, placebo-controlled trial with a 2x2 factorial design conducted in Finland between 1985 and 1993 (duration of the intervention 5 to 8 years, median 6.1 years) (The Alpha-Tocopherol-Group, 1994). Post-intervention follow-up for cause-specific deaths lasted for 6 years until 1999 (2001 for all-cause mortality) (Virtamo et al., 2003). Participants randomised were 29 133 males; inclusion criteria were male smokers (five or more cigarettes daily), aged 50 to 69 years (average at study entry 57.2 years). Exclusion criteria were prior cancer or presence of other serious illness, or use of vitamin E, vitamin A, or beta-carotene in excess of pre-defined doses, or anticoagulants. There were no losses to follow-up.

Participants were randomly assigned in four groups to receive:
group 1: alpha-tocopherol 50 mg (n=7286);
group 2: beta-carotene 20 mg (n=7282);
group 3: alpha-tocopherol and beta-carotene, (n=7278);
group 4: placebo (n=7287);

The primary outcome was incidence of lung cancer, secondary outcomes were occurrence of other major cancers, overall and cause specific mortality, and occurrence of other diseases. At the end of the intervention phase, a higher incidence of lung cancer was observed among the men who received beta-carotene than among those who did not (change in incidence 18%; 95% CI 3-36%). Beta-carotene had little or no effect on the incidence of other cancers than lung cancer. Total mortality was 8% higher (95% CI 1-16%) among those men who received beta-carotene than among those who did not, primarily due to more deaths from lung cancer and ischemic heart disease.

At post-intervention follow-up (Virtamo et al., 2003), the relative risk (RR) for lung cancer (n=1037) among beta-carotene recipients compared to non-recipients was 1.06 (95% CI 0.94-1.20). No significant overall difference in lung cancer incidence was observed between beta-carotene recipients and non-recipients during the post-intervention follow-up period (RR 1.03; 95% CI 0.91-1.20). There were no late preventive effects on other cancers. Relative risk of dying for beta-carotene recipients vs non-recipients was 1.07 (95% CI 1.02-1.12). The higher mortality rate of beta-carotene recipients compared with non-recipients returned toward null four to six years after discontinuation of supplementation. Thus, the adverse effects of beta-carotene supplementation were gradually reduced during the post-intervention follow-up.

Wright et al. (2010) investigated molecular markers in normal epithelium in tumour specimens from 52 men randomised to receive 20 mg of beta-carotene per day and 30 men randomised to the placebo arm. They reported that male smokers taking beta-carotene supplementation may have an increased risk of cancer because of aberrant cell growth as

indicated by positive staining for cyclin D1 (while zero percent for the placebo group); however the number of cases was low and observed effects were small.

In a random subsample of 941 men aged 65 and older, an ophthalmologic examination was performed at the end of the follow-up to compare the prevalence of age-related maculopathy (ARM) in the intervention groups (Teikari et al., 1998). The examination identified 68 cases in the beta-carotene group (29.1%) and 53 cases in the placebo group (24.9%). Based on logistic regression analysis controlling for risk factors, there was no statistically significant effect of 20 mg/day beta-carotene vs. placebo for median 6.1 years on ARM in older male smokers: Odds ratio (OR) 1.01 (95% CI 0.77-1.03).

2.4.2.2 Skin Cancer Prevention Study

The Skin Cancer Prevention Study (SCPS) from USA is a multicentre study where beta-carotene was randomly assigned to 1805 patients who had had a recent non-melanoma skin cancer: they received either 50 mg of beta-carotene or placebo per day and underwent annual skin examinations to determine the occurrence of new non-melanoma skin cancer (Greenberg et al., 1990).

Patients were randomly assigned to receive:
group 1: beta-carotene 50 mg (n=913);
group 2: placebo (n=892);

The study lasted for five years and the duration of follow-up was another five years.

Adherence to the prescribed treatment was good, and after one year the beta-carotene group's median plasma beta-carotene level (3021 nmol per liter) was much higher than that of the control group (354 nmol per liter). After five years of intervention, however, there was no difference between the groups in the incidence of the first new non-melanoma skin cancer (relative rate 1.05; 95% CI 0.91-1.22). In subgroup analyses, active treatment showed no efficacy either in the patients whose initial plasma beta-carotene level was in the lowest quartile or in current smokers. There was also no significant difference between treated and control groups in the mean number of new non-melanoma skin cancers per patient-year.

The authors concluded that in persons with a previous non-melanoma skin cancer, treatment with beta-carotene did not reduce the occurrence of new skin cancers over a five-year period of treatment and observation.

2.4.2.3 Nambour Skin Cancer Prevention Study

The Nambour Skin Cancer Prevention Study (NSCP) investigated sunscreens' versus beta-carotenes' ability to prevent skin cancers among 1621 residents of Nambour in south-east Queensland, Australia (Green et al., 1999).

Patients were randomly assigned to receive:

group 1: sunscreen and beta-carotene (30 mg) (n=404);
group 2: sunscreen and oral placebo (n=408);
group 3: beta-carotene (n=416);
group 4: oral placebo (n=393);
one tablet daily for a period of 4.5 years.

In a community-based randomised trial with a 2x2 factorial design, individuals were assigned to four treatment groups: daily application of a sun protection factor 15-plus sunscreen to the head, neck, arms, and hands, and beta-carotene supplementation (30 mg per day); sunscreen plus placebo tablets; beta-carotene only; or placebo only. The endpoints after 4.5 years of intervention were the incidence of basal-cell and squamous-cell carcinomas both in terms of people treated for newly diagnosed disease and in terms of the numbers of tumours that occurred. Analysis of the effect of sunscreen was based only on skin cancers that developed on sites of daily application. All analyses were by intention-to-treat.

1383 participants underwent full skin examination by a dermatologist in the follow-up period. 250 of them developed 758 new skin cancers during the follow-up period. There were no significant differences in the incidence of first new skin cancers between groups randomly assigned to daily sunscreen and no daily sunscreen (basal-cell carcinoma 2588 vs 2509 per 100 000; (rate ratio 1.03; 95% CI 0.73-1.46; squamous-cell carcinoma 876 vs 996 per 100 000; rate ratio 0.88 [0.50-1.56]). Similarly, there was no significant difference between the beta-carotene and placebo groups in incidence of either cancer (basal-cell carcinoma 3954 vs 3806 per 100 000; 1.04 [0.73-1.27]; squamous-cell carcinoma 1508 vs 1146 per 100 000; 1.35 [0.84-2.19]). In terms of the number of tumours, there was no effect on incidence of basal-cell carcinoma by sunscreen use or by beta-carotene, but the incidence of squamous-cell carcinoma was significantly lower in the sunscreen group than in the no daily sunscreen group (1115 vs 1832 per 100 000; 0.61 [0.46-0.81]).

There was no harmful effect of daily use of sunscreen in this study. Cutaneous squamous-cell carcinoma, but not basal-cell carcinoma, seems to be amenable to prevention through the routine use of sunscreen by adults for 4.5 years. There was no beneficial or harmful effect on the rates of either type of skin cancer as a result of beta-carotene supplementation (Green et al., 1999).

2.4.2.4 The Australian skin aging study

The aim of the Australian skin aging study (NSAS) was to determine whether regular use of sunscreen compared with discretionary use or beta-carotene supplements compared with placebo retard skin aging, measured by degree of photoaging. The study is a follow-up of the Australian skin cancer study in the way that they recruited participants from the same group, but only invited persons younger than 55 years old (Hughes et al., 2013).

903 adults younger than 55 years were randomly assigned into four groups: daily use of broad-spectrum sunscreen and 30 mg of beta-carotene, daily use of sunscreen and placebo,

discretionary use of sunscreen and 30 mg of beta-carotene, and discretionary use of sunscreen and placebo.

They investigated change in microtopography between 1992 and 1996 in the sunscreen and beta-carotene groups and compared with controls, graded by assessors blinded to treatment allocation.

The daily sunscreen group showed no detectable increase in skin aging after 4.5 years. Skin aging from baseline to the end of the trial was 24% less in the daily sunscreen group than in the discretionary sunscreen group (relative odds 0.76; 95% CI 0.59-0.98]). Beta-carotene supplementation had no overall effect on skin aging, although contrasting associations were seen in subgroups with different severity of aging at baseline. Regular sunscreen use retards skin aging in healthy, middle-aged men and women. No overall effect of beta-carotene on skin aging was identified.

2.4.2.5 Physicians' Health Study

The Physicians' Health Study (PHS) was a 12-year randomised, double-blind, placebo-controlled trial with a 2x2 factorial design that tested aspirin and beta-carotene supplementation in the primary prevention of cardiovascular disease and cancer (Hennekens et al., 1996). Twenty-two thousand US male physicians aged 40-84 years were enrolled in 1982. The participants constituted a healthy population with no history of cancer, myocardial infarction, stroke, or transient cerebral ischemia. Half were never-smokers at enrolment, 39% were former smokers and 11% were current smokers. The aspirin component of the study was terminated after five years due to a clear preventive effect of aspirin on myocardial infarction, while the randomised beta-carotene component continued uninterrupted for 12 years. The intervention group (n=11 036) received 50 mg beta-carotene on alternate days, while n=11 035 received placebo.

group 1 (n approx. 5500): active beta-carotene 50 mg on alternate days plus aspirin placebo
group 2 (n approx. 5500): active aspirin 325 mg on alternate days plus beta-carotene placebo
group 3 (n=5536): both active ingredients
group 4 (n approx. 5500): both placebo

The primary endpoint was any type of malignant neoplasm except non-melanoma skin cancer. Secondary outcomes were cardiovascular disease and overall mortality. Endpoints were reported through annual postal questionnaires and confirmed in medical records.

No effect of beta-carotene was found on risk of any malignant neoplasm (1273 vs. 1293 cases, RR 0.98, 95% CI 0.91-1.06). There was no difference between the beta-carotene group and the placebo group in the number of cases of lung cancer (82 vs. 88 cases), deaths from cancer, cardiovascular disease, myocardial infarction, or stroke. Statistical analyses restricted to former or current smokers revealed no effect of beta-carotene within these groups. The authors concluded that 12 years of supplementation with beta-carotene

produced neither benefit nor harm in terms of the incidence of malignant neoplasms, cardiovascular disease, or death from all causes.

Liu et al. (2009) investigated molecular markers in cancer tissue from 39 cases in the PHS study. CYP1A1 protein expression in lung cancer tissue from cases assigned to receive beta-carotene tended to be reduced (fewer CYP1A1-positive cases) (OR 0.2; 95% CI 0.1-1.1; p=0.06), however, the low number of subjects should be noted (6-13 per group). Otherwise, there was no significant difference in the molecular markers examined between cancer cases assigned to beta-carotene and placebo.

The effect of beta-carotene supplementation on ARM was published in 2007 (Christen et al., 2007). This diagnosis was self-reported on the 7-year questionnaire and annually thereafter, and the analysis included all those who survived the first seven years of follow-up and provided information concerning ARM. Five-hundred-and forty-nine incident cases were identified and confirmed in medical records. Additional information concerning date of diagnosis, clinical characteristics and severity was obtained from ophthalmologists and optometrists. The authors found no effect of 50 mg beta-carotene supplementation every other day on the risk of ARM: RR 0.96 (95% CI 0.78-1.20) in the beta-carotene vs. placebo group.

2.4.2.6 The Women's Health Study

The Women's Health Study (WHS) was designed as a randomised, double-blind, placebo-controlled trial testing benefits and risks of aspirin, vitamin E and beta-carotene in the primary prevention of cancer and cardiovascular disease, using a 2x2x2 factorial design (Lee et al., 1999b). The participants were 39 876 US female health professionals, aged 45 years or older, without a history of cancer, coronary heart disease or cerebrovascular disease were randomly assigned to one of the eight treatment groups beginning in April 1993:

Participants were randomly assigned to one of the eight treatment groups. The active agents were 100 mg of aspirin, given on alternate days; 600 IU of vitamin E, given on alternate days; and 50 mg of beta-carotene, given on alternate days.

group 1: aspirin 100 mg, beta-carotene 50 mg, vitamin E 600 IU;
group 2: aspirin 100 mg, beta-carotene 50 mg, vitamin E placebo;
group 3: aspirin 100 mg, beta-carotene 50 mg placebo, vitamin E 600 IU;
group 4: aspirin 100 mg, beta-carotene placebo, vitamin E placebo;
group 5: aspirin placebo, beta-carotene 50 mg, vitamin E 600 IU;
group 6: aspirin placebo, beta-carotene 50mg, vitamin E placebo;
group 7: aspirin placebo, beta-carotene placebo, vitamin E 600 IU;
group 8: aspirin placebo, beta-carotene placebo, vitamin E placebo;

A total of 19 939 were assigned at random to receive beta-carotene and 19 937 were randomly assigned to receive placebo. In both groups, 13% of the women were cigarette smokers at base-line. The beta-carotene component of the study was terminated in after 2.75 years; a) because of null findings in the parallel Physicians Health Study and b) because

of two other trials reported possible harmful effects (the ATBC study and the CARET study). Median treatment period was 2.1 years.

There were no overall effects of beta-carotene supplementation on risk of cancer or CVD after a median treatment duration of 2.1 years and a median total follow-up of 4.1 years. For individual endpoints such as site-specific cancer, myocardial infarction or stroke, also no significant benefit or harm was observed. The same lack of effect was found when looking at the 13% of smokers among the participants.

However, results from the pure beta-carotene compared to placebo have not been published.

2.4.2.7 Women's Antioxidant Cardiovascular Study

In the US Woman's Antioxidant Cardiovascular Study (WACS) 7627 women who were free of cancer before random assignment were selected for this study. Diagnoses and deaths from cancer at a specific site were confirmed by use of hospital reports and the National Death Index (Lin et al., 2009).

Participants were randomly assigned to receive:
group 1: vitamin C 500 mg daily, vitamin E 600 IU and beta-carotene 50 mg, every other day (n=1020);
group 2: vitamin C 500 mg daily, vitamin E 600 IU and placebo beta-carotene 50 mg, every other day (n=1021);
group 3: active vitamin C 500 mg daily, placebo vitamin E and beta-carotene 50 mg, every other day (n=1023);
group 4: vitamin C 500 mg daily, placebo vitamin E and placebo beta-carotene, every other day (n=1023);
group 5: placebo vitamin C daily, vitamin E 600 IU every and beta-carotene 50 mg, every other day (n=1021);
group 6: placebo vitamin C daily, vitamin E 600 IU and placebo beta-carotene every other day (n=1021);
group 7: placebo vitamin C daily, placebo vitamin E and beta-carotene 50 mg, every other day (n=1020);
group 8: placebo vitamin C daily, placebo vitamin E and placebo beta-carotene every other day (n=1022); for a mean period of 9.4 years (range, 8.3 to 10.1 years).

Cox proportional hazards regression models were used to assess hazard ratios (represented as relative risks [RRs]) of common cancers associated with use of antioxidants, either individually or in combination. Subgroup analyses were conducted to determine if duration of use modified the association of supplement use with cancer risk (Song et al., 2009).

During an average 9.4 years of treatment, 624 women developed incident invasive cancer and 176 women died from cancer. There were no statistically significant effects of use of any

antioxidant on total cancer incidence. Compared with the placebo group, the RR in the beta-carotene groups was 1.00 (95% CI 0.85-1.17). Similarly, no effects of these antioxidants were observed on cancer mortality. Compared with the placebo group (n = 3820), the RR in the beta-carotene group (n=3807) was 0.84 (95% CI 0.62-1.13). Also, when separating the supplementation and follow-up time, no significant associations of the three antioxidants (taken singly or combined) or follow-up time (1-5 years or 6-10 years) with total cancer incidence or mortality were found.

The authors' conclusion was that supplementation with vitamin C, vitamin E, or beta-carotene offers no overall benefits in the primary prevention of total cancer incidence or cancer mortality.

In another study from the WACS, they investigated the effect of beta-carotene supplementation on primary prevention of diabetes type 2 (Song et al., 2009). The same exclusion criteria as above were used. However, in addition women with prevalent diabetes at baseline were also excluded, leaving a total of 6574 women at high risk of CVD and who were free of diabetes at baseline included in this study. The design is similar as described above.

During an average 9.2 years of treatment, 895 incident cases of diabetes occurred among the 6574 women who were free of diabetes at baseline. Compared to placebo (n=3290), no significant effect was observed for beta-carotene treatment (n=3284) (RRs 0.97 95% CI 0.85 1.11). Separate analysis was also performed in which the effects of each of the combinations of active agents were compared with the group receiving all 3 placebos (n=822), and no significant differences in diabetes risk was found for the beta-carotene group (n=819).

The authors concluded that there were no significant effects of supplementation with beta-carotene on risk on developing diabetes in women with high risk of CVD.

2.4.2.8 Is beta-carotene supplementation refleted in blood plasma levels?

As mentioned in chapter 2.1, plasma beta-carotene levels reflect intake levels well and can thus be used as a marker of intake, and in the case of the RCT studies, as a marker of compliance with the interventions. Compliance was tested this way in most of the RCTs described above, but mostly in a random manner in some, and not in all participants. However, the ATBC study examined results by tertiles of serum beta-carotene concentration after reaching a stable treatment level, and found no evidence of increased risk of lung cancers or deaths in the highest tertile of beta-carotene Omenn (2007).

In the ATBC study, there was a trend toward lower lung cancer incidence among persons with higher base-line serum concentrations (Albanes et al., 1996). However, this was associated with only limited modification of the beta-carotene intervention effects. No significant association was found between change in serum beta-carotene concentrations (divided into tertiles) after three years of intervention and lung cancer incidence after three

years (tertile cuts in µg/L increase: <2060, 2060-3467, and >3467). Analysis of the absolute beta-carotene serum concentrations gave similar results. From the ATBC study also an analysis of beta-carotene serum levels at the beginning of the study and risk for colorectal cancer has been published (Malila et al., 2002). No statistically significant association between baseline serum beta-carotene levels and colorectal cancer risk was observed.

2.4.3 Prospective studies

An overview of prospective studies included in this report is given in Table 2.4.3-1.

Table 2.4.3-1 Overview of prospective studies included in this report.

Reference	Study	Participant characteristic	Country	Participant number	Beta-carotene from supplements	Main endpoint and results	Length of follow-up
Asgari et al. (2009), Asgari et al. (2012)	VITAL*	Both sexes	USA	69 635	Highest category:>= 3 mg/d in 2009 paper; >0.6 mg/d in 2012 paper	Melanoma. No reduction in incidence in the group consuming more than 3 mg beta-carotene/day for 10 years	5-6y
Roswall et al. (2010)	DCH*	Both sexes, middle-aged	Denmark	55 453	Median intake: 2.2 mg/d, 95 percentile: 15.3 mg/d, range 0.0 - 40.5 mg/d	Lung cancer. A significantly higher risk for lung cancer (721 cases) was observed with supplemental beta-carotene for users vs. non-users	3-7y
Roswall et al. (2012)	DCH	As above	As above	As above	As above	All-cause mortality. No difference between beta-carotene supplement users and non-users.	Median follow-up period: 13.8 years

*VITAL: Vitamins and Lifestyle cohort study, DCH: The Danish Diet, Cancer and Health cohort study.

2.4.3.1 The VITAL Study

The Vitamins and Lifestyle (VITAL) prospective cohort study was carried out in middle-aged and older men and women residing in Western Washington State, USA (Asgari et al., 2009). The objective was to investigate the association between long-term antioxidant supplement use and risk of melanoma. More than 69 000 men and women aged 50 to 76 years completed self-administered questionnaires in 2000-02 providing information about use (duration and dose) of dietary supplements, including multivitamins and beta-carotene supplements, as well as various risk factors for melanoma. Average daily supplemental nutrient intake across 10 years was calculated as (dose per day) x (days per week/7) x (years/10). Incident melanoma cases up to 31.12.2006 (n=461) were identified through linkage to the Surveillance, Epidemiology, and End Results cancer registry.

The calculated ten-year average beta-carotene intake from individual and multivitamin supplements was divided into four categories: 0, >0-600, >600 to <3000, and >=3000 µg per day. While 34% of the cohort reported no supplements containing beta-carotene during the previous 10 years, 27% had an average daily dose of 600-3000 µg/day, and 3% had an average dose of >=3000 µg/day.

The reason for defining the highest category of beta-carotene starting at 3000 µg was to mimic the beta-carotene dose given in a previous supplementation study (Hercberg et al., 2007) which reported a 4-fold higher melanoma risk in women randomised to receive antioxidant supplements containing beta-carotene, vitamin C, vitamin E, selenium and zinc. There is no information on the distribution or upper dose in the ">=3000 µg/day" category.

No association was found between the calculated average daily intake of supplemental beta-carotene across 10 years and incident melanoma, p(trend)=0.38. RR 0.87 (95% CI 0.48-1.56) for >=3000 µg/day compared with none, and RR 1.17 (0.93-1.48) for 600-3000 µg/day compared with none, after adjustment for major melanoma risk factors.

In a follow-up paper from the same study, now including 566 incident melanoma cases diagnosed up to 31.12.2007, the authors reported associations between melanoma risk and various baseline characteristics of participants, including retinol and carotenoid intakes (Asgari et al., 2012). In this analysis, beta-carotene supplementation at baseline was assessed in terms of current, former or never-use, and in terms of daily supplemental dose (including the contribution from multivitamin supplements) categorised into no use, 6-600 µg/day and >600 µg/day. No association was found between beta-carotene supplementation and risk of melanoma, nor between total beta-carotene supply from diet and supplements and risk of melanoma.

In summary, the VITAL cohort study found no increased melanoma risk of an average daily supply of more than 3 mg beta-carotene from supplements across 10 years. Danish cohort

2.4.3.2 Danish cohort

Roswall et al. (2010) reported on a prospective cohort study in Denmark (DCH) with the aim to evaluate the association between intake of beta-carotene, folate, vitamin C, vitamin E and risk of lung cancer, focusing on source-specific effects of dietary and supplemental intake. Participants numbered 57 053 middle-aged subjects recruited from the general population, of which 55 557 were available for analysis in the present study. Dietary intake of beta-carotene, based on food frequency questionnaire (FFQ), ranged from 36 to 94 667 µg/day (median 3213 µg), supplemental intake from 1 to 40 500 µg/day (median 2255 µg). A significantly higher risk for lung cancer (721 cases) was observed with supplemental beta-carotene (IRR 1.64; 95% CI 1.20-2.23 for users vs. non-users). A dose-response effect was observed. No effect was observed from dietary beta-carotene intake. Regarding sub-types of lung cancer, no statistical significance was achieved for any individual sub-type. No statistically significant interaction between any micronutrient and smoking status was observed.

A follow-up study on all-cause mortality was published in 2012, comprising 19 573 never smokers (1223 cases/18 350 non-cases), 15 733 former smokers (1630 cases/14 103 non-cases) and 20 147 current smokers (3924 cases/16 233 non-cases) (Roswall et al., 2012). 6767 deaths were identified and incidence rate ratios (IRRs) of mortality related to micronutrient intake were calculated using Cox proportional hazards models.

The study found no effect of dietary vitamin C, E, folate, or beta-carotene in relation to mortality. Effect modification by smoking and alcohol intake, but not body mass index (BMI), was suggested in relation to some dietary micronutrients. The effect of supplements did not differ in groups defined by dietary micronutrient intake.

The all-cause mortality study suggests no effect of dietary micronutrients in relation to overall mortality. Supplemental folic acid was found to be associated with increased mortality, but further studies are required.

2.4.4 Systematic reviews and meta-analyses

They searched MEDLINE, Embase, Cochrane Central Register of Controlled Trials, Cochrane Database of systematic reviews of effects.

An overview of systematic reviews and meta-analyses included in this report is given in Table 2.4.4-1.

Table 2.4.4-1 An overview of systematic reviews and meta-analyses included in this report.

Reference	Design	Participant number in beta-carotene arms	Beta-carotene from supplement	Main endpoint	Results
Evans and Lawrenson (2012)	RCTs	10 819	20-25 mg/d	Age-related macular degeneration	No difference between intervention and placebo groups
Fortmann et al. (2013)	RCTs	47 266 (all-cause mortality), 30 973 (CVD and cancer incidence)	20-50 mg/d	CVD and cancer (primary prevention)	No differences between groups, but a probable increase in lung cancer incidence in high-risk subgroups (smokers and asbestos workers)
Bjelakovic et al. (2008b), Bjelakovic et al. (2008c)	RCTs	19 455	6-30 mg/d	Gastrointestinal cancer (primary and secondary prevention)	No difference between intervention and placebo groups
Bjelakovic et al. (2006)	RCTs		15-25 mg/d	Colorectal adenoma (secondary prevention)	No difference between intervention and placebo groups
Jeon et al. (2011)	RCTs	20 290	30-75 mg/d	Cancer, (primary prevention)	No difference between intervention and placebo groups

Reference	Design	Participant number in beta-carotene arms	Beta-carotene from supplement	Main endpoint	Results
Bjelakovic et al. (2007), Bjelakovic et al. (2008a)	RCTS			All-cause mortality	7% higher risk of deaths in the beta-carotene groups; RR 1.07 (1.02-1.11)
Bjelakovic et al. (2012), Bjelakovic et al. (2013)	RCTs			All-cause mortality	6% higher risk of deaths at doses above 9.6 mg/d beta-carotene compared to doses below 9.6 mg/d; RR 1.06 (95% CI, 1.02-1.09)
Vivekananthan et al. (2003)	RCTs	138 113	15-50 mg/d	All-cause mortality	7% higher risk of deaths in the beta-carotene groups; RR 1.07 (1.02-1.11)

2.4.4.1 Age-related maculopathy and age related macular degeneration

The available evidence of beta-carotene supplementation on the prevention of age related macular degeneration (ARMD) was summarised and meta-analysed in 2012 (Evans and Lawrenson, 2012). The review included altogether four RCTs with 65 520 participants, conducted in Australia, Finland and the USA.

Only two high-quality RCTs included a pure beta-carotene supplementation group and are relevant for our purpose: The Finnish ATBC study in male smokers and the US Physicians' Health Study. In the meta-analysis of these two trials 21 589 participants (Evans and Lawrenson, 2012), there were 343 cases of ARMD in the beta-carotene groups and 327 in the control groups. There was no significant association between supplementation with beta-carotene vs. placebo and risk ratio of ARMD or advanced ARMD: RR 1.03 (95% CI 0.89-1.19) for ARMD, and RR 0.97 (95% CI 0.69-1.36) for advanced ARMD. The results of the trials were consistent (I^2=0% for both ARMD and advanced ARMD). The evidence was graded as high quality, indicating that "further research is very unlikely to change the authors' confidence in the estimate of effect". According to these two large high-quality randomised trials, there is no evidence of a harmful effect on age-related maculopathy of an average daily dose of 20-25 mg beta-carotene taken for 6-12 years in middle-aged and older men. According to the authors of the Cochrane review, none of these trials reported eye-related adverse effects. A limitation is the population studied, consisting of men only. The healthy physicians constitute the vast majority (97.7%) of the participants included in the meta-analysis, since the study of older male smokers (here constituting 2.1%) was based on a small subsample of the ATBC participants. The physicians represent a healthy selection with 89% non-smokers and no history of cardiovascular disease or cancer.

2.4.4.2 Cardiovascular disease and cancer

Fortmann et al. (2013) systematically reviewed the evidence for the benefit and harm of vitamin and mineral supplements in community-dwelling, nutrient sufficient adults for the

primary prevention of CVD and cancer and all-cause mortality. They included 103 articles (26 studies). Six of the studies examined the benefits and harms of individual and paired supplementation with beta-carotene (Green et al., 1999; Greenberg et al., 1990; Hennekens et al., 1996; Lee et al., 1999b; Omenn et al., 1996; The Alpha-Tocopherol-Group, 1994). The doses given were in the range of 20-50 mg/day, and the duration of most studies was less than 10 years. The study showed consistent null results for CVD incidence and cancer incidence across 6 trials of beta-carotene. They found a probable increase in lung cancer incidence in high-risk subgroups (smokers and asbestos workers) and the review also confirmed the established harm of beta-carotene supplementation on lung cancer incidence and death for individuals at high risk for lung cancer.

A limitation of the review is that the analysis included only primary prevention studies in adults without known nutritional deficiencies and that the studies were conducted in older individuals and included various supplements and doses.

2.4.4.3 Cancer

Bjelakovic et al. (2008b) (Cochrane) and Bjelakovic et al. (2008c) are meta-analyses of the same 12 RCTs investigating antioxidants in the prevention of gastrointestinal cancer (eight primary prevention (p), four secondary prevention (s)). Two larger studies used beta-carotene alone as the intervention. (ATBC study (p), n=7282 in the beta-carotene arm; PHS (p) 1996, n=11 036 in beta-carotene arm. In addition were included two small studies, Correa et al (2000)(s), n=117 in the beta-carotene arm and WACS (p), n=1020 in the beta-carotene arm). Doses were 6-30 mg/day. Bjelakovic et al. found that beta-carotene supplements given alone did not significantly influence gastrointestinal cancers (RR 1.04; 95% CI 0.80-1.35).

(Bjelakovic et al., 2006) present meta-analyses of eight RCTs on antioxidants and colorectal adenoma (secondary prevention). One RCT included in this meta-analysis used beta-carotene supplementation as the single intervention (MacLennan et al., 1995). Doses were 15 to 25 mg/day. Neither the fixed effects (RR 0.93; 95% CI0.81-1.1) nor the random effect model meta-analyses (RR 0.82, 95% CI 0.60-1.1) showed statistically significant effects on colorectal adenoma occurrence (secondary prevention except for one trial) of supplementation with beta-carotene, alone or in combination with other antioxidants.

Jeon et al. (2011) investigated by meta-analysis the effect of beta-carotene supplements given alone on cancer prevention as reported by RCTs (primary prevention (p), three trials; secondary prevention (s), three trials). All trials had pure beta-carotene arms. Number of cases/number of participants in the beta-carotene group were: Mayne et al. (2001)(s) 33/135; ATBC (Virtamo et al., 2003)(p) 602/7282; Toma et al. (2003)(s) 15/104; Greenberg et al. (1990)(s) 362/913; Green et al. (1999)(p) 142/820; PHS (Cook et al., 2000)(p) 1314/11036. Doses were 30-75 mg/day. The main outcome measures were cancer incidence overall and mortality, with subgroup analyses of types of cancer. No evidence of a preventive effect of beta-carotene supplementation on cancer incidence and mortality (primary (three trials) and secondary prevention (three trials)) was found.

2.4.4.4 All-Cause Mortality

There are altogether five publications investigating beta-carotene and all-cause mortality. Four of them have Goran Bjelakovic as first author. Two of Bjelakovic's publications are Cochrane meta-analyses (published in 2008 and 2012) and two are scientific articles based on these two Cochrane reports (published in 2007 and 2013).

Vivekananthan et al. (2003) performed a meta-analysis to assess the effect of vitamin E, beta-carotene, or both, on all-cause mortality and cardiovascular death. They analysed seven randomised trials of vitamin E treatment and, separately, eight of beta-carotene treatment (AREDS, ATBC, CARET, HPS, NSCP, PHS, SCP, WHS, see Table 2.4.4-1 for further description of trials with pure beta-carotene arms); all trials included 1000 or more patients. The dose range for beta-carotene was 20-50 mg/day. The intervention period ranged from 1.4 to 12.0 years.

The beta-carotene trials involved 138 113 participants in the all-cause mortality analyses. Beta-carotene led to a small but significant increase in all-cause mortality (7.4 vs 7.0%, OR 1.07 [1.02-1.11] p=0.003) and with a slight increase in cardiovascular death (3.4 vs 3.1%, OR 1.10 [1.03-1.17] p=0.003). No significant heterogeneity was noted for any analysis. The lack of a salutary effect was seen consistently for various doses of vitamins in diverse populations. As described earlier, this report does not include results from the AREDS and CARET studies because they had no pure beta-carotene arms. They found a small, but significant increased risk of CVD in the beta-carotene groups. The result was very much driven by the CARET study which did not have a pure beta-carotene arm.

In 2008 Bjelakovic et al published a large Cochrane review called "Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases" (Bjelakovic et al., 2008a). They analysed RCTs with a number of different antioxidant combination, including beta-carotene.

In the trials with a low risk of bias, the antioxidant supplements significantly increased mortality. When the different antioxidants were assessed separately, analyses including trials with a low risk of bias and excluding selenium trials found significantly increased mortality by beta-carotene (RR 1.07, 95% CI 1.02 to 1.11.

The authors conclude that supplemental vitamin A, beta-carotene, and vitamin E may increase mortality.

Bjelakovic et al. (2013) published another meta-regression analyses, meta-analyses and trial sequential analyses of the effects of supplementation with beta-carotene and other antioxidants, singly or in different combinations, on all-cause mortality.

The study is based on a 2012 Cochrane systematic review (Bjelakovic et al., 2012) which updates the 2008 study (Bjelakovic et al., 2008a) and again, analyses beneficial and harmful effects of antioxidant supplements in adults. Using random-effects meta-analyses, meta-regression analyses, and trial sequential analyses, they this time examined the association

between beta-carotene, vitamin A, and vitamin E, and mortality according to their daily doses and doses below and above the recommended daily allowances (RDA). They included seven randomised trials with low risk of bias where beta-carotene was assessed singly (43 019 participants when only counting the participants in the beta-carotene arm + placebo/no intervention arm. The studies were SPCS (1990), PPS (1994), PHS (1996), NSCPS (1990), Correa et al. (2000), ATBC (2003) and WACS (2007). The doses were from 20-50 mg/day.

As there is no RDA for beta-carotene, they take the IOMs (US Institute of Medicine) proposed value of 12 mg beta-carotene as a starting point, which is equivalent to the activity of 1000 µg of all-trans retinol. This translates to 9.6 mg beta-carotene if the RDA for vitamin A is 800 µg.

Based on a beta-carotene dose of 9.6 mg, they found a significantly increased mortality in RCTs with doses above this value (relative risk (RR) 1.06, 95% CI 1.02 to 1.09, I(2)=13%). Doses below the RDAs did not affect mortality, but data were sparse.

2.4.5 Summary human studies

Summary, RCTs and prospective studies

A full-scale literature search was conducted and studies were selected according to standard procedures for systematic reviews. An important inclusion criterion was that a study had to include information on beta-carotene intake, either in the form of pure beta-carotene arms in RCTs, or, in prospective studies, very specific questions on beta-carotene supplements.

All included studies are described in Summary Tables, see Appendix III. Excluded articles, with a short explanation for their exclusion, are listed in Appendix II.

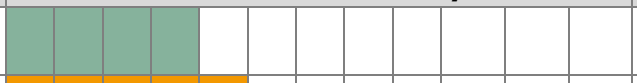
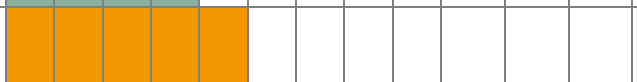
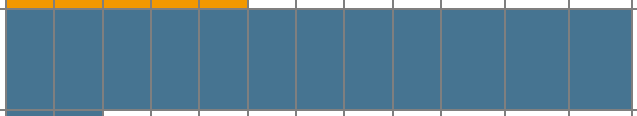
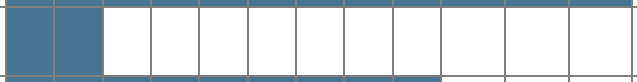
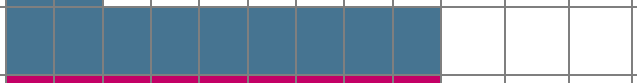
Seven different RCTs were included, conducted either in the USA or Europe, and including almost 47 100 participants in the beta-carotene arms alone.

Two prospective studies were included, one Danish and one from the USA, with 125 000 participants all together.

A brief summary of main findings is given in Table 2.4.5-1 below.

Table 2.4.5-1 Summary of main findings in randomised controlled trials with single beta-carotene arms.

Country (study start)	Study	Intervention duration in years												Results	Reference
		1	2	3	4	5	6	7	8	9	10	11	12		
Finland (1985-88)	ATBC													Increased risk of lung cancer	(Virtamo et al., 2003; Wright et al., 2010)
USA (1983)	SCPS													ND	(Greenberg et al., 1990)

Country	Study	Intervention duration in years	Results	Reference
Australia (1986)	NSCPS		ND	(Green et al., 1999)
Australia (1992)	NSAS		ND	(Green et al., 1999; Hughes et al., 2013)
USA (1982)	PHS		ND	(Hennekens et al., 1996; Liu et al., 2009)
USA (1993)	WHS		ND	(Lee et al., 1999b)
USA (1995)	WACS		ND	(Lin et al., 2009)
USA (1995)	WACS		ND	(Song et al., 2009)

Endpoint	Lung cancer
Endpoint	Skin cancer
Endpoint	Skin aging
Endpoint	Multiple outcomes e.g. deaths from cancer, cancer risk, CVD, myocardial infarction or stroke
Endpoint	Type 2 diabetes

ND: No difference between intervention and placebo groups.

The focus of the RCTs was to study possible preventive effects of antioxidant supplementation, including beta-carotene. It was a surprise, and probably also a disappointment, that none of the studies found a reduced risk on outcomes like cancer or CVD. More remarkable and surprising at the time though, was that the huge Finnish ATBC study found an increased risk of lung cancer in the intervention group.

Also the Danish prospective study found that risk of lung cancer increased in smokers with increased intake of beta-carotene supplements.

Summary, meta-analyses

Altogether we included eleven meta-analyses; one with age-related macular degeneration as endpoint, one with a mixture of CVD and cancer as endpoints, four on cancer only as endpoint, one on a mixture of CVD and all-cause mortality and an endpoint and four on all-cause mortality alone. There were null findings in the meta-analysis on macula-degeneration.

One of the two meta-analyses on CVD found a small increased risk in the beta-carotene arm (Vivekananthan et al., 2003). The result was very much driven by the CARET study which did not have a pure beta-carotene arm. The other CVD meta-analyses did not have enough power to get significant results, but found a probable increase in lung cancer incidence in high-risk subgroups (smokers and asbestos workers (Fortmann et al., 2013)).

In the five meta-analyses studying cancer, there were no effect on other cancer forms than lung cancer.

The five meta-analyses of all-cause mortality used information from almost all the hitherto mentioned RCT studies. They extracted information on death numbers in each study and used the numbers to analyse risk of death in the active versus placebo intervention groups. Alarmingly, they all found an increased risk of all-cause mortality. Because these meta-analyses on all-cause mortality are the only ones finding harmful effects besides the ones on lung cancer and CVD in smokers, they will have to be discussed more thoroughly than most of the other studies when setting an upper level, see chapter 4.

3 Exposure

In the terms of reference from the Norwegian Food Safety Authority, it is stated that it is important to ensure that the total intake of vitamins and minerals from all sources does not exceed the UL. VKM is therefore requested to estimate the intake of beta-carotene from the diet, in all age groups in the population above 1 year.

VKM, however, emphasises that this opinion on UL for beta-carotene is addressing beta-carotene in food supplements. Beta-carotene from regular foods such as vegetables and fruits is not considered to be of health concern. On the contrary, the consumption of vegetables and fruits should be increased, and the recommendation on "5 a day" should be achieved in all age groups of the population.

Intakes of beta-carotene in the diet have been calculated for 2-year-olds and adults 18 to 70 year of age. Data from the national food consumption survey UNGKOST 2000, with food consumption data for the age groups 4-, 9-, and 13-year-olds were considered too old to be used directly in this opinion.

3.1 Description of food consumption surveys

The estimated intakes of beta-carotene presented in this opinion are based on data from the national food consumption surveys for children (2-year-olds) and adults (18 to 70-years). The national food consumption surveys are conducted by the Department of Nutrition, University of Oslo in collaboration with the Directorate of Health and the Norwegian Food Safety Authority. Different methodologies were used in the two different surveys and thus direct comparisons between 2-year-old children and adults can be misleading.

A description of the food consumption surveys and the different methodologies used is given below:

2-year-old children; Småbarnskost 2007 is based on a semi-quantitative food frequency questionnaire (FFQ). In addition to predefined household units, food amounts were also estimated from photographs. The study was conducted in 2007, and a total of 1674 2-year-olds participated (participation rate 56%) (Kristiansen et al., 2009).

Adults; Norkost 3 is based on two 24-hour recalls by telephone at least one month apart. Food amounts were presented in household measures or estimated from photographs (Totland et al., 2012). The study was conducted in 2010/2011 and 925 women and 862 men aged 18 to 70 years participated (participation rate 37%).

For Småbarnskost 2007 and Norkost 3, the daily intake of beta-carotene was computed by using food databases in the software system (KBS) developed at the Institute of Basic Medical Sciences, Department of Nutrition, at the University of Oslo. The food databases are

mainly based on various versions of the official Norwegian food composition table (Rimestad et al., 2000) and are continuously supplemented with data on new food items.

In this chapter, all values are given in micrograms, because this is the norm when setting recommendations or estimating intake in the KBS system.

3.2 Intake of beta-carotene from regular food and food supplements

3.2.1 Two-year-olds

Intakes of beta-carotene in 2-year-olds were calculated without food supplements, as none of the food supplements listed in the questionnaire for the two-year olds contain beta-carotene, see Table 3.2.1-1.

Table 3.2.1-1 Intake of beta-carotene in 2-year-olds, all participants (n=1674), mean, median and 95-percentile intakes.

	Mean (SD), µg/day	Median, µg/day	95thperc, µg/day
Total beta-carotene in the diet	1521 (1099)	1270	3352

3.2.2 Adults

Intakes of beta-carotene in adults were calculated both with and without food supplements, see Tables 3.2.2-1 and 3.2.2-2. The intake of fruits and vegetables may differ between men and women, and calculations were therefore also separated by gender. Only about 3% of the participants in Norkost 3 have reported use of beta-carotene supplements. It might be that tanning pills have been considered not to be relevant for a food survey by the participants, thus underreporting of beta-carotene cannot be ruled out.

Table 3.2.2-1 Intake of beta-carotene in adults, all participants (n=1787), men (n=862) and women (n=925) mean, median and 95-percentile intakes.

	Mean (SD), µg/day	Median, µg/day	95thperc, µg/day
All participants, including supplements	2468 (2370)	1739	7021
All participants, without supplements	2423 (2338)	1680	6858
Men, including supplements	2468 (2511)	1588	7437
Men without supplements	2441 (2495)	1574	7421
Women, including supplements	2469 (2232)	1881	6590
Women without supplements	2406 (2182)	1813	6396

Table 3.2.2-2 Intake of beta-carotene from supplements in those who take supplements (users only).

	Mean, µg/day
Men and women (n=53)	1522
Men (n=16)	1443
Women (n=37)	1556

According to present regulations for food supplements, the maximum limit for vitamin A is 1500 µg per day. There is no specific maximum limit for beta-carotene per se. All vitamin A can be present as beta-carotene, and the Norwegian Food Safety Authority has practiced the conversion factor 2 for calculating maximum limit for beta-carotene – i.e. the maximum limit for beta-carotene as source of vitamin A is 3000 µg per day.

3.3 Summary exposure

Intakes of beta-carotene have been calculated for 2-year-olds and adults 18 to 70 year of age. Mean intake of beta-carotene in 2-year-olds is 1521 µg/day. Mean intake for adults is 2423 µg/day and in the 95th percentile 6859 µg/day.

Only about 3% of the adults reported use of beta-carotene supplements. It might be that use of tanning pills have been underreported in the food survey.

Beta-carotene from regular foods such as vegetables and fruits is, however, not considered to be of health concern. On the contrary, the consumption of vegetables and fruits should be increased, and the recommendation on "5 a day" should be achieved in all age groups of the population.

4 Risk characterisation

4.1 Introduction

Beta-carotene has been considered an innocuous precursor of vitamin A since its identification in 1930. The only known side effect was yellowing of the skin at high intakes, for example through drinking carrot juice regularly. The same yellowing is taken advantage of in tanning pills which presently are sold on the Norwegian market and contain 3 - 9 mg beta-carotene per daily dose. At the 9 mg dosage, the producer promises a deep and lasting brown glow to the skin, especially for people with a fair complexion (see www.vitamed.no/info_betakaroten).

From 1995 and onwards several RCT studies reported an increased risk of lung cancer in smokers or asbestos workers who received supplemental beta-carotene daily (20 mg/day in the Finnish ATBC). The same effect was found in a prospective Danish study, i.e. there was a dose-dependent association between use of beta-carotene supplements and risk of lung cancer.

The mechanism of this effect on the lungs has so far not been clarified, much because there does not seem to be a good animal model to study the phenomena. At present, three different explanatory hypotheses exist, all elaborated in chapter 2.1.

Meanwhile, scientists are looking for answers to these paradoxes when trying to understand beta-carotene: The provitamin is more than anything an antioxidant, and together with the vitamins C and E and selenium, it was earlier used to explain results from the many observational studies which suggested that diets rich in these substances reduced the risk of several age-related chronic diseases, including some forms of cancer, cardiovascular-, eye- and neurodegenerative diseases.

These relatively consistent and positive results from observational studies stimulated the initiation of impressive, large scale RCTs, including the ATBC study, WHS, PHS, NSCPS and others (see Table 2.4.2-1 in chapter 2.4.2 for closer description). The RCTs were mostly designed to test the efficacy of antioxidant supplementation in the primary or secondary prevention of cancer and/or cardiovascular disease. As described in chapter 2.4.2, with a few exceptions, there were null findings in most of these RCTs. One study from Australia (NSCP) found a non-significant reduced risk of skin cancer in the beta-carotene group, while the aforementioned Finnish study with smokers found a significantly increased risk of lung cancer. Also several RCTs where a mixture of anti-oxidants was given found increased risk of lung cancer in smokers. In 2012, EFSA published a separate opinion on the issue called "Statement on the safety of beta-carotene use in heavy smokers" (EFSA, 2012).

For ethical reasons, no RCT can be designed to study mortality. However, mortality can be studied in meta-analyses if all-cause mortality data are given in the separate studies. This

exercise has been done in several meta-analyses which have been published the last 10 years. Because their conclusions are both disturbing (increased risk of dying) and relevant for determining an upper level for beta-carotene, four of them are presented in more detail here, as a follow-up of chapter 2.4.4.4.

Vivekananthan et al. 2003

A general description of the meta-analysis by Vivekananthan et al. (2003) is given in chapter 2.4.4.4. As mentioned there, three of the eight RCTs included in the analysis did not have a pure beta-carotene arm and accordingly we have not given these studies specific attention (AREDS, CARET and HPS).

However, the three largest studies, ATBC (n=29 133), PHS (n=22 071) and WHS (n=39 876) remain in the analyses together with the two smaller ones; NSCP(S) (n=1805) and SCP(S) (n=1621). Of these, it was only the ATBC study which by itself found a significantly increased risk on all-cause mortality in the intervention group. The NSCP(S) study drew the results in the opposite direction; here, there was a significantly reduced risk in the beta-carotene group. Being very small, this study is not given much weight compared to the large studies. In Figure 4.1-1 the yellow marked lines shows the odds ratios for all-cause mortality for the RCTs with pure beta-carotene arms included in Vivekananthan et al. (2003).

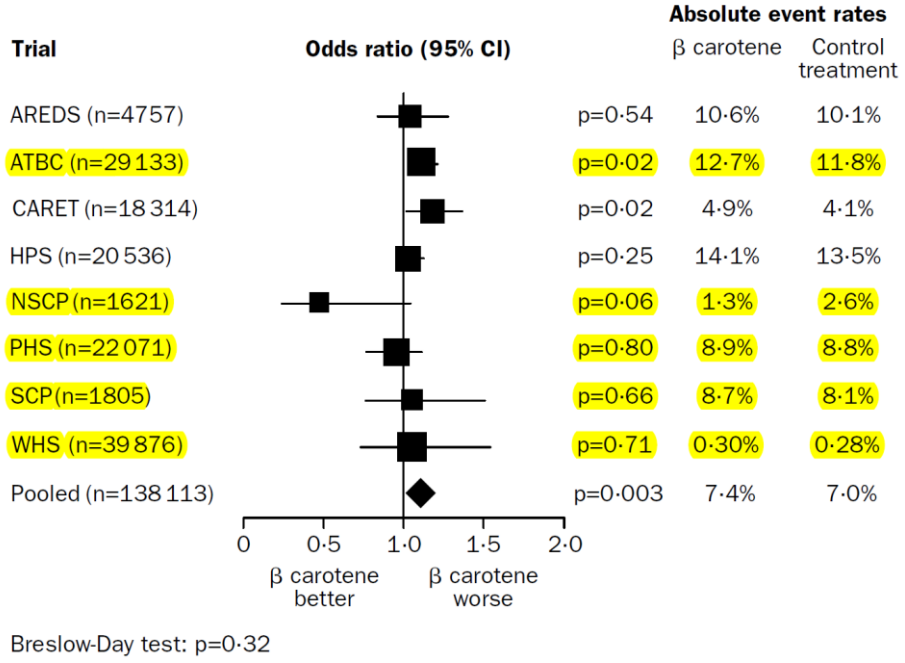


Figure 4.1-1 Odds ratios (95% CI) of all-cause mortality for individuals treated with beta-carotene or control therapy.

Vivekananthan et al. (2003) concludes that beta-carotene increases the risk of all-cause mortality, based on the finding of a small, but significant, increase in all-cause mortality (7.4 vs 7.0%, 1.07 [1.02-1.11] p=0.003) and with a slight increase in cardiovascular death (3.4 vs 3.1%, 1.1 [1.03-1.17] p=0.003).

Bjelakovic et al. 2008

As described in chapter 2.4.4.4, Bjelakovic et al. (2008a) performed a meta-analysis published in 2008 and did the same exercise as Vivekananthan et al. (2003) had done some years earlier. They included several small RCTs (less than 1000 participants) and thus the total number of RCTs included in the analysis is larger than the one of Vivekananthan et al.

For the purpose of the present report we focus on the studies which had a pure beta-carotene arm and which had more than 1000 participants altogether:

ATBC, NSCPS, PHS, SCPS, and WHS, i.e. five of the 12 studies included in the Bjelakovic analysis fulfilled our inclusion criteria. The others are either characterised by not having a pure beta-carotene arm (AREDS, CARET, HPS) or having less than 1000 participants (Correa 2000, Jacobson 2000, PPS).

Their results from Bjelakovic et al 2008 are shown in Figure 4.1-2: The yellow marked lines show the risk ratios for all-cause mortality for the RCTs with pure beta-carotene arms included in Bjelakovic et al. (2008a).

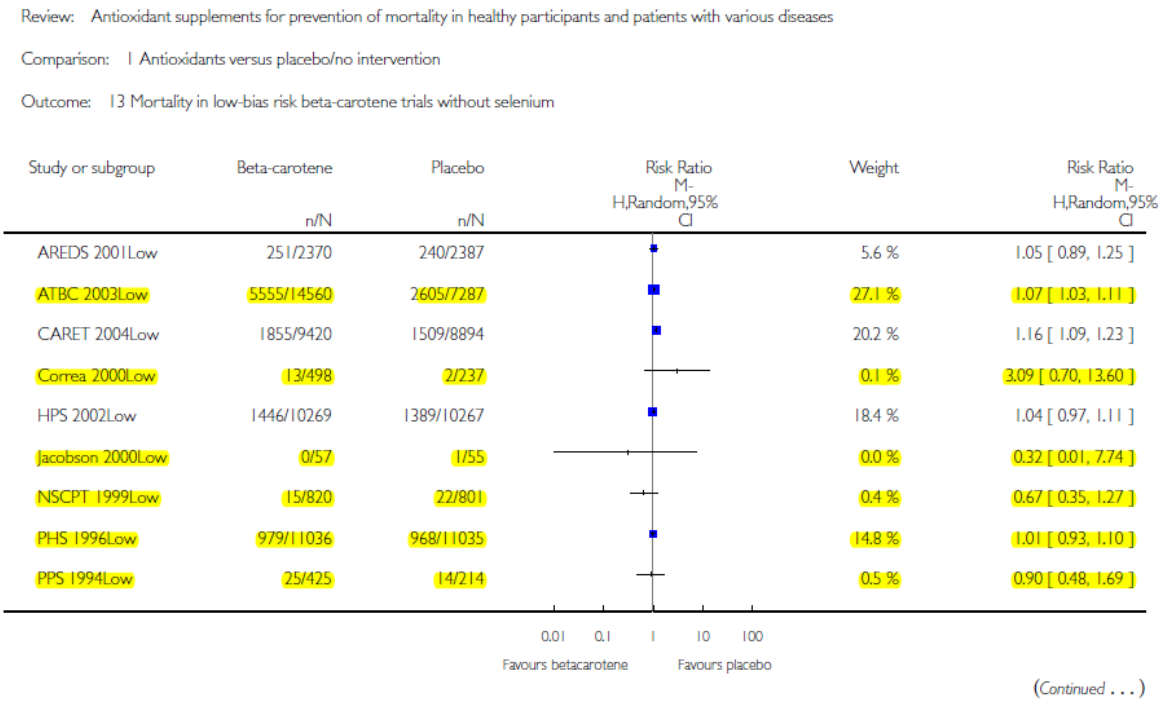


Figure 4.1-2 Risk ratios (95% CI) of all-cause mortality for individuals treated with beta-carotene or control therapy.

Columns 2 and 3 in Figure 4.1-2 show how many died in each arm of the study, and column 5 shows how the size of the study is counted in the final weighing of the analyses.

The five studies with pure beta-carotene arms and more than 1000 participants constitute 55% of the weight in the analyses (column 5 in the figure). Again, it is only the ATBC study from Finland which by itself finds a significant effect on increased mortality risk.

In the trials with a low risk of bias, the antioxidant supplements significantly increased mortality. When the different antioxidants were assessed separately, analyses including trials with a low risk of bias and excluding selenium trials found significantly increased mortality by beta-carotene (RR 1.07, 95% CI 1.02 to 1.11).

Bjelakovic et al. 2012

In 2012, Bjelakovic et al performed an update of their 2008 meta-analysis (Bjelakovic et al., 2012). This time, a part of the report analysed studies where only pure beta-carotene had been given. Compared to the 2008 meta-analysis, no new studies were included, but the authors included two very small studies with less than 1000 participants all together, i.e. Correa 2000 and PPS. However, because of the low number, they are given very little weight, and in column six we see that the PHS and ATBC constitutes 96.3% of the total study weight. Again, the meta-analysis shows an increased risk of all-cause mortality, RR 1.06 (95% CI 1.02, 1.10).

Figure 4.1-3 below has been submitted to the authors of the present report as a personal contribution from Goran Bjelakovic. It includes only RCTs with pure beta-carotene arms.

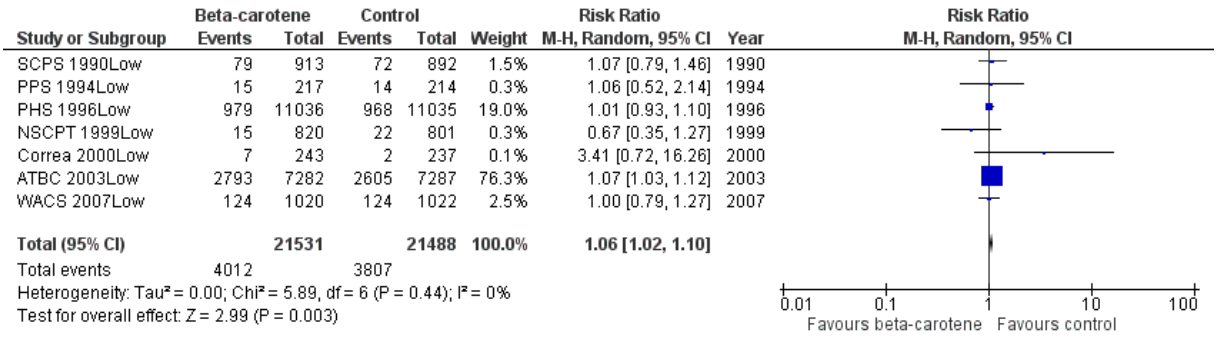


Figure 4.1-3 Risk ratios (95% CI) of all-cause mortality for individuals treated with beta-carotene or control therapy.

Bjelakovic et al. 2013

Bjelakovic et al. (2013) performed another meta-analysis of antioxidants and all-cause mortality in 2013. It was based on the above described Cochrane meta-analysis published in 2012. This time the group investigated the outcome in relation to dosages being below or above the RDA for the different antioxidants.

There is no RDA for beta-carotene, but the US Institute of Medicine (IOM) has proposed that 12 mg of beta-carotene is equivalent to the activity of 1000 µg of all-trans-retinol. Thus

Bjelakovic et al. (2013) used the value of 9.6 mg beta-carotene as an "RDA", equivalent to a dose of vitamin A of 800 µg.

Their meta-analysis then investigated mortality risk according to intervention exposure above and below this value and conclude that beta-carotene in a dose above 9.6 mg significantly increases mortality (RR 1.06, 95% CI 1.02-1.09, I(2) =13%).

However, VKM has chosen to disregard this meta-analysis in the further risk characterisation. The analysis is in reality comparing intervention versus placebo groups; none of the included studies have a supplemental dose of beta-carotene in the range between 0 and 20 mg/day. Thus, it cannot be used to consider dose-response relationships between exposure and negative health effects nor to set a NOAEL. Furthermore, it uses the RDA for retinol as a starting point for deciding a tentative RDA for beta-carotene, which for the purpose of this risk assessment of beta-carotene is found inappropriate.

4.2 Special groups

Smokers: The minimum dose which causes effects in smokers and asbestos workers is not known. As a matter of prudence, these groups should not take supplements containing beta-carotene.

Others: As long as the mechanisms for the putative adverse effects have not been established, there is a possibility that also subjects exposed to increased concentrations of other airway irritants and toxicants than tobacco smoke and asbestos (e.g. diesel exhaust and other fumes and dusts) may be at risk. Subjects with chronic inflammatory conditions, like asthma and COPD, in the lungs may also be at risk.

No data have been found for pregnant women, elderly or children.

4.3 Establishment of a safe upper level or tentative safe upper level

The steps 1 – 4 used in this chapter are the steps suggested to use by EFSA in the document Guidelines for the development of tolerable upper intake levels for vitamins and minerals (SCF, 2000a).

Step 1. Hazard identification, which concludes with a summary of the evidence concerning the capacity of the nutrient to cause one or more types of adverse effects in humans.

Up till two decades ago, beta-carotene was thought to be harmless even in large doses. In the wake of the Finnish ATBC RCT which found an increased risk of CVD and death in male smokers, animal studies have indicated three possible mechanisms for such a detrimental effect. Although conclusive explanations for the negative effects have not yet been agreed upon, there is a scientific rationale for the argument that population groups with vulnerable lungs, like smokers, should not take beta-carotene supplements at all (Aarsetoy et al., 2006). Furthermore, meta-analyses of beta-carotene in relation to all-cause mortality indicate that supplemental beta-carotene increases the risk of death from all causes. However, the latter finding is driven, statistically, by the findings among smokers.

Step 2. Hazard characterisation – the quantitative and qualitative evaluation of the nature of the adverse effects associated with a nutrient; this includes a dose response assessment.

The dose used in the Finnish ATBC Study was 20 mg beta-carotene/day. The effect was only observed during the intervention period; in follow-up studies conducted after the active period was finished, the risk declined and was no longer significant. 20 mg beta-carotene may thus be considered as a LOAEL when referring to a single RCT study.

The Danish prospective study found a dose-dependent increase in lung cancer risk with increased intake of supplemental beta-carotene. Unfortunately, the paper does not allow for setting an NOAEL or LOAEL based on the published data.

In the three meta-analyses studying all-cause mortality, all found a 6-7% increased risk of death. One of the meta-analyses also found an increased risk of CVD in smokers. However, all results were driven, statistically, by the ATBC study. Studies with a more mixed population (both males and females) and with a more normal prevalence of smokers (10 – 20%), found no such increased risk.

Step 2, continued: Based on these evaluations, an UL is derived, taking into account the scientific uncertainties in the data. ULs may be derived for various life-stage groups within the population.

VKM found it extraordinary challenging to decide which uncertainty factor to use for beta-carotene. SCF guidelines for establishment of tolerable upper intake levels do not give clear guidance/advice in deciding the numeric level of the uncertainty factor.

If NOEAL is derived from human data, an uncertainty factor of 10 is recommended as a starting point to encompass inter-individual variation and sensitivity. The SCF guidelines state that a small uncertainty factor is to be used if it is judged that little population variability is expected for the adverse effect, and a larger uncertainty factor (close to 10) may be used if variability is expected to be great.

For beta-carotene, a NOAEL is not available, and an uncertainty factor may be applied to account for the uncertainty in deriving a UL from the LOAEL. The size of the uncertainty factor involves a judgement based on the severity and incidence of the observed effect at the LOAEL and the steepness (slope) of the dose response, if this is possible to make. For beta-carotene, we have not found data to make a dose-response curve.

In addition, the following considerations were discussed before deciding on an uncertainty factor:

The study which found a negative effect of beta-carotene supplementation (ATBC) was very large (n=29 133) which indicates that it encompasses inter-individual variation and sensitivity. Additionally, the most vulnerable groups, in this case smokers, was an inclusion criteria. Both these factors indicate that the uncertainty factor can be in the lower end.

The meta-analyses using the endpoint "increased risk of all-cause mortality" found an increased risk of death in the beta-carotene groups. This is severe and indicates a maximum uncertainty factor. However, as all results in the all-cause mortality meta-analyses are driven, statistically, by one large study on smokers, we choose to use a lower number.

Based on the above considerations, VKM chooses to use 5 as an uncertainty factor.

An UL for beta-carotene cannot be derived, but a tentative upper level (TUL) is set at 4 mg/day, based on a LOAEL of 20 and the uncertainty factor of 5.

Smokers and anyone else in the population with inflammation in their lungs should be discouraged from taking beta-carotene containing supplements all together.

Step 3. Exposure assessment – evaluates the distribution of usual total daily nutrient intakes among members of the general population.

Mean intake of beta-carotene in 2-year-olds is 1.5 mg/day. Mean intake for adults is 2.4 mg/day and in the 95th percentile 6.9 mg/day.

Only about 3% of the adults reported use of beta-carotene supplements. It might be that use of tanning pills have been underreported in the food survey Norkost 3.

Beta-carotene from regular foods such as vegetables and fruits is, however, not considered to be of any health concern. Negative health effects from beta-carotene in natural foods have never been reported. On the contrary, the consumption of vegetables and fruits should be increased, and the recommendation of "5 a day" should be achieved in all age groups of the population.

Step 4. Risk characterisation – analyses of the conclusions from steps 1 through 3 and characterises the risk. The risk will depend on the fraction of the population exceeding the UL and the magnitude and duration of excessive intake.

As beta-carotene from food, irrespective of amount, is considered innocuous, it is not appropriate in this specific case to add the intake from food to the possible intake from supplements, as is usually done when deriving an UL.

The question therefore becomes one of dose of added beta-carotene to supplements. VKM concludes that with a LOAL of 20 mg beta-carotene/day and a safety factor of 5, 4 mg beta-carotene/day is an appropriate tentative upper level for supplemental beta-carotene.

5 Uncertainties

5.1 Uncertainty linked to the use of all-cause mortality as an endpoint in meta-analyses

An important aim of systematic reviews and meta-analyses is to assess the extent to which different studies give similar or dissimilar results. In the particular case of meta-analyses of beta-carotene and health outcomes, the present report shows that the meta-analyses confirm the results from single studies, i.e. there are no effects of supplemental beta-carotene on endpoints like cancer, CVD or ARMD. The one important exception is that supplemental beta-carotene increases the risk of lung cancer in smokers, which was observed in the Finnish ATBC study. Because the ATBC study is so large, it is given a lot of weight in meta-analyses, and this explains the results of the meta-analyses on beta-carotene and all-cause-mortality. VKM finds it uncertain whether results from meta-analyses on all-cause mortality are applicable to the population in general when the study which drives the results, statistically speaking, only comprises male smokers.

VKM also finds it uncertain whether results found in one vulnerable population group like smokers are possible to extrapolate to other vulnerable groups, like people with COPD or asthma, when the biological mechanisms for an effect has not been fully elucidated or agreed upon.

5.2 Uncertainty linked to the uncertainty factor

As described in chapter 4.3, the SCF guidelines for establishment of tolerable upper intake levels do not give clear guidance/advice in deciding the numeric level of the uncertainty factor. This seems to leave the decision to scientific judgement.

5.3 Uncertainties linked to study design and execution

The evidence considered in this report has mainly been collected from RCTs; to a limited extent also from prospective cohort studies. Meta-analyses of RCTs provide a substantial amount of the evidence we considered. RCTs and prospective cohort studies rank as number one and two among primary studies on the quality-of-evidence scale, surpassed only by meta-analyses of RCTs regarding evidence quality. Publication bias will tend to result in more 'positive' or 'expected' results being published, 'negative' or 'unexpected' results being under-represented. The possibility of publication bias must be tested for in studies used for meta-analysis, as must study heterogeneity. A large, well conducted RCT will compete favourably with a meta-analysis with regard to quality of evidence.

However, we note that most of the RCTs we refer to in this risk assessment found no significant differences between the active and the placebo groups, yet they were all published, and even in very high ranking journals. We therefore have the impression that publication bias is not a big issue in this particular case.

Ethnicity may theoretically play a role both with regard to health effect outcomes and doses tolerated. The ATBC study was conducted in a presumably predominantly ethnic Finnish population (found an effect) whereas studies in more mixed populations (USA) more easily would loose an ethnicity-dependent effect because of too low statistical power, and in particular if it was linked to a sub-group of Caucasians.

5.4 Putative contaminants in synthetic beta-carotene

In some discussions it has been speculated that the adverse effects of beta-carotene found in some studies may have been caused either by minor differences in synthetic beta-carotene compounds, which may be slightly different from natural beta-carotene, or by trace amounts of some hypothetical, undetected lung carcinogen in synthetic beta-carotene. However, there is no evidence to substantiate such speculations, and VKM finds that there is little uncertainty on this point.

6 Conclusions with answers to the terms of reference

Answers to the questions from the Norwegian Food Safety Authority:

Because of insufficient data, neither SCF (2000b) nor EFSA have been able to establish a tolerable upper intake level (UL) for beta-carotene. Other expert groups have set indicative or temporary upper guidance levels (EVM, 2003; IOM, 2000; Rasmussen et al., 2006).

a) What upper safe level for beta-carotene should be used as basis for establishing a maximum limit in food supplements? A description of the adverse health effects related to this upper safe level shall be included.

VKMs answer to a) There are still not sufficient data to set an upper safe level for beta-carotene in supplements. All RCTs with beta-carotene were stopped after the Finnish ATBC study published results which showed an increased risk of lung cancer of beta-carotene in smokers. The dose in this study was 20 mg beta-carotene per day, thus this can be considered as a LOAEL. There are no supplementation studies to support a NOAEL, i.e. where graded doses of beta-carotene have been given as sole supplement (and compared with placebo) at dosages between zero and 20 mg/day. Several meta-analyses investigating beta-carotene supplementation studies in relation to all-cause mortality found that beta-carotene supplements increase the risk of all-cause mortality. However, one large study including smokers only drove the results of the meta-analyses, statistically speaking. Thus a medium safety margin can be set when establishing a tentative safe upper level, and VKM suggests a safety margin of 5.

- This will result in a tentative upper level of 4 mg/day for supplemental beta-carotene.

VKM is of the opinion that because beta-carotene may increase the risk of lung-cancer in smokers, there is a theoretical risk that beta-carotene may increase the risk of adverse health effects in all people with chronic inflammatory conditions in the lungs. Such groups include asthmatics and COPD-patients who, in addition to smokers, should be discouraged to take beta-carotene supplements.

b) Are there particular circumstances in Norway that should be taken into consideration in this context?

VKMs answer to b) We do not see any particular circumstances for Norway that should be taken into consideration.

c) Are there any circumstances that may result in toxic effects from conversion of carotenoids to retinol?

VKMs answer to c) As far as our literature investigations have allowed us to conclude, there does not seem to be any circumstances which may result in toxic effects from conversion of carotenoids to retinol.

d) It is important to ensure that the total intake of vitamins and minerals from all sources does not exceed the UL. VKM is therefore requested to estimate the intake of beta-carotene from the diet, in all age groups in the population above 1 year.

VKMs answer to d): This has been done, see chapter 3. We have estimated the intake both from the diet and supplements in adults and in two-year-olds. However, beta-carotene from food is considered completely harmless, and VKM supports the official national and international guidelines to consume plenty of fruits and vegetables, the main sources of beta-carotene in the diet.

e) VKM is requested to conduct scenario estimations to illustrate the consequence of a possible maximum limit for beta-carotene in food supplements.

VKMs answer to e) VKM does not find this request relevant given our answers above.

7 Data gaps

Results from a number of large, high-quality, randomised studies on beta-carotene supplementation for prevention of various health outcomes are available in the literature. Most of these included doses between 20 and 50 mg/day given for a duration of 2 to 12 years.

A major data gap was precise guidelines for deriving at an uncertainty factor when meta-analyses is the starting point and all-cause mortality is the outcome and basis for LOAEL.

Certain other limitations may, however, be noted.

- The studies were not designed with the aim of investigating toxicity or adverse health effects of beta-carotene supplementation. On the contrary, they were designed to study preventive effects
- The most influential studies were done in selected population subgroups e.g. healthy male physicians, or male smokers
- Possible adverse effects on patient population who may have reduced lung function for various reasons (e.g. asthma, chronic obstructive pulmonary disease, exposure to asbestos or air pollutants) have not been thoroughly studied
- As most of the antioxidant supplementation studies were designed and carried out after the recognition that free radicals were involved in disease development, many trials provided combination drugs containing several antioxidant. As a consequence, when yielding either harmful or preventive effects, the actions of the single nutrients cannot be disentangled.
- None of the studies with pure beta-carotene arms allowed for a dose-response evaluation.
- In meta-analyses with regard to the effect on mortality, there is not sufficient data available to disentangle the specific causes of death which may be aggravated by beta-carotene supplementation

It follows from the above that a study designed to evaluate harmful or toxic effects of beta-carotene in humans would have to avoid all the mentioned limitations. However, for ethical reasons, such a study will never be conducted, given the knowledge we have today that beta-carotene in doses at 20 mg/day or more increases risk of lung cancer and, and, possibly all-cause mortality. The only realistic data gaps which can be worked on are those to give us better understanding of beta-carotene metabolism in the body

- We need more knowledge about potential mechanisms

Concerning exposure data, a variety of high-dose beta-carotene containing tanning pills marketed through the internet and drugstores are widely used, due to an intentional side effect of carotenodermia. Although these drugs are subject to the regulations of dietary supplements, they are often not marketed as dietary supplements. Therefore, they may not

be considered as such by the user, and may be underreported in dietary surveys. We do not have information about the amount of beta-carotene supplements taken as tanning drugs, and we believe that the consumption of supplemental beta-carotene may be higher than that reported.

8 References

Appendices

Appendix I, Literature search

1. beta Carotene/ or carotenoids/ or (beta carotene or betacarotene or b-carotene* or carotenoid*).tw.

2. (adverse health effect* or adverse effect* or negative effect* or negative health or risk factor* or health risk or health hazard* or harm*).tw.

3. 1 and 2

4. dietary supplement/ or dietary supplements/ or dietary supplementation/ or dietary supplement*.tw. or carotenoid intake*.tw. or carotenoid consumption*.tw. or dietary carotenoid*.tw.

5. 3 and 4

6. animals/ or animal/ or animal experiment/ or animal*.tw.

7. rat/ or rats/ or (rat or rats).tw.

8. mice/ or mouse/ or (mice or mouse).tw.

9. ferrets/ or ferret/ or ferret*.tw.

10. nonhuman/ or nonhuman*.tw.

11. or/6-10

12. humans/ or human/ or human experiment/ or (human or humans).tw.

13. 11 not (11 and 12)

14. 5 not 13

15. limit 14 to (danish or english or norwegian or swedish)

16. limit 15 to yr="2002 -Current"

Appendix II, Excluded papers

Reference	Topic	Reason for exclusion
Aronow M.E., Chew E.Y. (2014) Age-related Eye Disease Study 2: perspectives, recommendations, and unanswered questions. Current Opinion in Ophthalmology 25:186-190.	Age-related macular degeneration progression.	Review of the findings of the AREDS study, no comparison of beta-carotene supplementation vs. placebo.
Bairati I. et al. (2006) Antioxidant vitamins supplementation and mortality: A randomized trial in head and neck cancer patients. International Journal of Cancer 119:2221-2224.	Head and neck cancer-	RCT where beta-carotene was given in combination with alpha-tocopherol (and discontinued early for ethical reasons when results from the CARET and the Physicians' Health Study were published). A potential independent effect of beta-carotene cannot be distinguished from total effect.
Barker M.E. et al. (2005) Serum retinoids and beta-carotene as predictors of hip and other fractures in elderly women. Journal of Bone and Mineral Research 20:913-920.	Fractures	Not an RCT. Biological markers, no exposure data.
Bergstrom T. et al. (2012) Vitamins at physiological levels cause oxidation to the DNA nucleoside deoxyguanosine and to DNA-alone or in synergism with metals. Mutagenesis 27:511-517.	In vitro study.	Short description of the findings in the introduction of the report.
Biesalski H.K. et al. (2010) Reexamination of a Meta-Analysis of the Effect of Antioxidant Supplementation on Mortality and Health in Randomized Trials. Nutrients 2:929-949.		
Bjelakovic G. et al. (2011) Antioxidant supplements for liver diseases. Cochrane Database of Systematic Reviews.	Liver diseases.	Very short intervention periods. Patients with liver diseases.
Boeke C.E. et al. (2014) Adolescent Carotenoid Intake and Benign Breast Disease. Pediatrics 133:E1292-E1298.	Benign breast disease.	Observational study with no specific information on beta-carotene supplements.
Chan S.T. et al. (2012) Quercetin supplementation suppresses the secretion of pro-inflammatory cytokines in the lungs of Mongolian gerbils and in A549 cells exposed to benzo[a]pyrene alone or in combination with beta-carotene: in vivo and ex vivo studies. Journal of Nutritional Biochemistry 23:179-185.		Animal study.
Chatterjee M. et al. (2012) Biological activity of carotenoids: its implications in cancer risk and prevention. Curr Pharm Biotechnol 13:180-90.	Cancer	Review. No original data or analyses.

Reference	Topic	Reason for exclusion
Checkley W. et al. (2010) Maternal vitamin A supplementation and lung function in offspring. N Engl J Med 362:1784-94.	Lung function in offspring.	Too low dose to be relevant of setting an UL.
Chew E.Y. et al. (2014) Secondary Analyses of the Effects of Lutein/Zeaxanthin on Age-Related Macular Degeneration Progression AREDS2 Report No. 3. Jama Ophthalmology 132:142-149.	Age-related macular degeneration progression.	RCT testing the effect of the AREDS multi-supplement where the beta-carotene component is replaced by lutein/zeaxanthin. No comparison of beta-carotene vs. placebo.
Cortes-Jofre M. et al. (2012) Drugs for preventing lung cancer in healthy people. Cochrane Database of Systematic Reviews.	Lung Cancer.	Only analyses of vitamin A.
Costa Mallen 2014	Age-related macular degeneration progression.	RCT testing the effect of the AREDS multi-supplement where the beta-carotene component is replaced by lutein/zeaxanthin. No comparison of beta-carotene vs. placebo.
Cui X. et al. (2011) Antioxidant intake and risk of endometrial cancer: results from the Nurses' Health Study. Int J Cancer 128:1169-78.	Endometrial cancer.	Observational study with no specific information on beta-carotene supplements.
Evans J.R., Lawrenson J.G. (2012) Antioxidant vitamin and mineral supplements for slowing the progression of age-related macular degeneration. Cochrane Database Syst Rev 11.	Age-related macular degeneration, slowing progression.	This meta-analysis included only studies that gave beta-carotene supplementation in a "cocktail drug" in combination with other antioxidant substances. Thus a potential independent effect of beta-carotene cannot be distinguished from the total effect.
Goralczyk R. (2009) Beta-carotene and lung cancer in smokers: review of hypotheses and status of research. Nutr Cancer 61:767-74.	Lung cancer.	Review with a mechanistic focus. No original data or analyses.
Hart C. et al. (2012) The emerging harm of antioxidants in carcinogenesis. Future Oncol 8:535-48.	Cancer	Pedagogic review, no original data or analyses.
Meyer F. et al. (2007) Acute adverse effects of radiation therapy and local recurrence in relation to dietary and plasma bet in head and neck a carotene and alpha tocopherol cancer patients. Nutrition and Cancer-an International Journal 59:29-35.	Head and neck cancer.	Same study as Bairati 2006, analysis of effect of dietary (FFQ) and plasma beta-carotene at baseline on cancer recurrence.
Meyer F. et al. (2008) Interaction between antioxidant vitamin supplementation and cigarette smoking during radiation therapy in relation to long-term effects on recurrence and mortality: A randomized trial among head and neck cancer patients. International Journal of Cancer 122:1679-1683.	Head and neck cancer.	Same study as Bairati 2006, analysis of whether smoking was an effect modifier for effect of supplementation.

Reference	Topic	Reason for exclusion
Moyer V.A., Force U.S.P.S.T. (2014) Vitamin, mineral, and multivitamin supplements for the primary prevention of cardiovascular disease and cancer: U.S. Preventive services Task Force recommendation statement.	Authorative guideline/recommendation paper, no original data, no dose level evaluation.	Authorative guideline/recommendation paper, no original data, no dose level evaluation.
Osganian S.K. et al. (2003) Dietary carotenoids and risk of coronary artery disease in women. American Journal of Clinical Nutrition 77:1390-1399.		Prospective study/dietary data.
Potter J.D. (2014) The failure of cancer chemoprevention. Carcinogenesis 35:974-982.	Cancer	Pedagogic review, no original data or analyses.
Siems W. et al. (2005) Beta-carotene breakdown products may impair mitochondrial functions - potential side effects of high-dose beta-carotene supplementation. Journal of Nutritional Biochemistry 16:385-397.		Review of in vitro studies. Short description of the findings in the introduction of the report.
Sin H.P.Y. et al. (2013) Lifestyle modification, nutritional and vitamins supplements for age-related macular degeneration. Acta Ophthalmologica 91:6-11.	Age-related macular degeneration.	Review, no meta-analysis. No studies providing beta-carotene vs. placebo presented beyond those already included in the Cochrane meta-analysis from 2012 (Evans & Lawrenson).
Valacchi G. et al. (2009) Beta-carotene prevents ozone-induced proinflammatory markers in murine skin. Toxicology and Industrial Health 25:241-247.		Animal study.
Wang L. et al. (2008) Associations of plasma carotenoids with risk factors and biomarkers related to cardiovascular disease in middle-aged and older women. American Journal of Clinical Nutrition 88:747-754.		Cross-sectional study/plasma β-carotene data and risk factors and biomarkers related to CVD.
Wang Y. et al. (2014) Dietary Carotenoids Are Associated with Cardiovascular Disease Risk Biomarkers Mediated by Serum Carotenoid Concentrations. Journal of Nutrition 144:1067-1074.		Cross-sectional study /dietary carotenoid intake and CVD risk biomarkers.
West K.P. et al. (2011) Effects of vitamin A or beta carotene supplementation on pregnancy-related mortality and infant mortality in rural Bangladesh: a cluster randomized trial. JAMA 305:1986-95.		Malnutrition and deficiencies.

Appendix III, Summary Tables

Summary Tables RCT and prospective cohorts

Authors, reference. Title	The Alpha-Tocopherol-Group. (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. N Engl J Med 330:1029-35
Study design and type	Randomised, double-blind placebo controlled intervention study.
Objective	To determine whether supplementation with α-tocopherol, beta-carotene, or both, would reduce the incidence of lung cancer in male smokers.
Number of participants, country and age	29133 (randomised), Finland, 50-69 years old (mean 57.2). Conducted between 1985-1993.
Baseline characteristics of study subjects	Male smokers (>5 cigarettes daily).
Exposure, substance, food, (type and amount)	The study had four arms: i) α-tocopherol (n=7286) ii) beta-carotene (n=7282) iii) α-tocopherol + beta-carotene (n=7278) iv) placebo (n=7287). All got verum or placebo in similar-looking capsules. Dose of beta-carotene 20 mg/day.
Measurement of exposure (biomarker, internal validation)	Pre-intervention serum samples, thrice-yearly home visits with remaining capsule counting, measurements in random serum samples throughout the study, serum measurement after three years of intervention.
Follow-up period, drop-outs	Intervention period 5-8 years, median 6.1 years. No losses to follow-up. Post-intervention follow-up published separately.
Health outcome	Lung cancer incidence (primary outcome), occurrence of other major cancers, overall and cause specific mortality, occurrence of other diseases (secondary outcomes).
Measurement of outcome	Data collected from Finnish Cancer Registry, follow-up visit interviews and questionnaires, Finnish National Hospital Discharge Registry, National Death Registry.
Statistical analysis	2x2 factorial design, intention to treat principle.
Results	Higher incidence of lung cancer among men who received beta-carotene supplementation (change in incidence 18%; 95% CI 3-36; total mortality: beta-carotene associated with increase in mortality of 8%; 95% CI 1-16).
Conclusion	Beta-carotene supplementation 20 mg/day was associated with an increase in lung cancer incidence and total mortality.
Confounders adjusted for	
Relevance for our risk assessment purpose	Highly relevant in relation to safety of beta-carotene supplementation and determination of an UL.

Authors, reference. Title	Virtamo J., Pietinen P., Huttunen J.K., Korhonen P., Malila N., Virtanen M.J., Albanes D., Taylor P.R., Albert P., Group A.S. (2003) Incidence of cancer and mortality following alpha-tocopherol and beta-carotene supplementation: a postintervention follow-up. JAMA 290:476-85
Study design and type	Post-intervention follow-up of RCT study (ATBC Study).
Objective	To analyse post-intervention effects of α-tocopherol and beta-carotene.
Number of participants, country and age	Of the original randomised Finnish ATBC Study cohort (n=29 133), with intervention 5 to 8 years from 1985-1988, 25563 men were still alive at the beginning of the post-trial follow-up in 1993 (average age 63.5 years). 75% were still smokers with no difference between the 4 intervention groups.
Baseline characteristics of study subjects/cells	29 133 male smokers aged 50 to 69 years were randomised for the ATBC study.
Exposure, substance, food, (type and amount)	Post-intervention study. Intervention study: Beta-carotene supplementation 20 mg/day or α-tocopherol (50 mg), or both agents, or placebo. The intervention lasted for 5 to 8 years (median 6.1 year).
Measurement of exposure (biomarker, internal validation)	ATBC study: compliance was assessed by counting remaining capsules at each visit during intervention period, by measurement of α-tocopherol and beta-carotene levels in serum after 3 years of supplementation, and by measurements in random serum samples throughout the study.
Follow-up period, drop-outs	Post-intervention follow-up for cancer incidence and cause-specific mortality was 6 years (until 1999) and for total mortality 8 years (until 2001; longer than cancer and case-specific mortality because of less time needed for verification). Mean total follow-up time was 14.1 years.
Health outcome	Site-specific cancer incidence and total and cause-specific mortality and calendar time-specific risk for lung cancer incidence and total mortality.
Measurement of outcome	Data from Finnish Cancer Registry and the Register of causes of Death. Cancer cases were confirmed through medical record review. Cause-specific data on deaths were based on *ICD-8*, *ICD-9*, and *ICD-10* as detailed in the paper.
Statistical analysis	The follow-up period was divided into 4 intervals to demonstrate temporal changes in the effects of α-tocopherol and beta-carotene. Crude rates per 10 000 person-years were calculated for each of the 4 groups, as were crude relative risk (RR) point estimates and their 95% CI. Calendar time-specific RRs were calculated for lung cancer incidence and total mortality.
Results	No significant overall difference in lung cancer incidence was observed between beta-carotene recipients and non-recipients during the posttrial period (RR1.03; 95% CI 0.91-1.20). The elevated risk of lung cancer in the beta-carotene group observed during the trial period continued during the first post-trial period, although statistically not significant. The RR fell below 1.0 approximately 4 years post-trial. The incidence of other cancers post-intervention was low and the RR estimates therefore imprecise. The risk for colon cancer in the beta-carotene group appeared to be elevated (RR, 1.44; 95% CI 1.09-1.90), but the elevation was observed only during the second post-trial period. The higher mortality rate of beta-carotene recipients compared with non-recipients evident by the end of the intervention period, returned toward null 4 to 6 years later. Most of the excess deaths in the beta-carotene group were from cardiovascular disease.

Authors, reference. Title	Virtamo J., Pietinen P., Huttunen J.K., Korhonen P., Malila N., Virtanen M.J., Albanes D., Taylor P.R., Albert P., Group A.S. (2003) Incidence of cancer and mortality following alpha-tocopherol and beta-carotene supplementation: a postintervention follow-up. JAMA 290:476-85
Conclusion	The effects of α-tocopherol and beta-carotene weaned away during the post-intervention follow-up, with no late adverse or beneficial effects observed for beta-carotene supplementation.
Confounders adjusted for	-
Relevance for our risk assessment purpose	General: absence of late-appearing effects; none for setting a specific dose-level.

Authors, reference. Title	Wright M.E., Groshong S.D., Husgafvel-Pursiainen K., Genova E., Lucia M.S., Wolff H., Virtamo J., Albanes D. (2010) Effects of beta-carotene supplementation on molecular markers of lung carcinogenesis in male smokers. Cancer Prev Res (Phila) 3:745-52
Study design and type	Histopathological study of biomarkers in tumour material from cancer cases in the ATBC study.
Objective	To elucidate molecular mechanisms behind increased lung cancer incidence observed in male smokers receiving beta-carotene supplementation.
Number of participants, country and age	Specimens could be retrieved for 52 men randomised to receive 20 mg of beta-carotene per day and 30 men randomised to the placebo arm. Finland. Male smokers aged 50-69 years at randomisation.
Baseline characteristics of study subjects/cells	Normal-appearing bronchial epithelial tissue and tumour tissue.
Exposure, substance, food, (type and amount)	Beta-carotene 20 mg/day without or with vitamin E (50 mg/day).
Measurement of exposure (biomarker, internal validation)	Compliance was assessed by counting remaining capsules at each visit during the intervention period, by measurement of α-tocopherol and beta-carotene levels in serum after 3 years of supplementation, and by measurements in random serum samples throughout the study.
Follow-up period, drop-outs	Active intervention 5-8 years (median 6.1 years).
Health outcome	Lung cancer (various types).
Measurement of outcome	In normal appearing bronchial epithelium, expression (extent and intensity) of cytochromes P450 (CYP) 1A1, 1A2, and 2E1; retinoic acid receptor-β (RAR-β); activated protein-1 (AP-1); cyclin D1; and Ki67.
Statistical analysis	Wilcoxon rank sum test and Fischer's exact test to examine differences in continuous (extent) and categorical (intensity) staining values between the two intervention groups, and also to compare group characteristics. Stratification of results by a number of case characteristics was also performed.
Results	Men receiving beta-carotene had higher expression of cyclin D1, but not Ki67 in in normal epithelium (based on 3 of 13 versus 0 of 11 cases staining positively, p=0.04) and slightly higher Ki67, but not cyclin D1, in malignant tissue. Beta-carotene supplementation had no significant effect on RAR-β, AP-1, CYP1A1, CYP1A2, or CYP2E1 expression.
Conclusion	Male smokers with beta-carotene may have an increased risk of cancer because of aberrant cell growth as indicated by cyclin D1; however the number of cases is small and observed effects were small.
Confounders adjusted for	-
Relevance for our risk assessment purpose	Marginal.

Authors, reference. Title	Teikari J.M., Laatikainen L., Virtamo J., Haukka J., Rautalahti M., Liesto K., Albanes D., Taylor P., Heinonen O.P. (1998) Six-year supplementation with alpha-tocopherol and beta-carotene and age-related maculopathy. Acta Ophthalmol Scand 76:224-9
Study design and type	Randomised controlled trial: With end-of-trial examination in a random subsample.
Objective	To investigate a potential protective effect of antioxidant vitamins (beta-carotene and/or alpha-tocopherol) in the development of age-related maculopathy (ARM).
Participants, country and age	29,000 male smokers aged 50-69 years at baseline originally participated in this 2-factorial primary prevention trial of lung cancer, main results published in NEJM 1994, 330:1029-103. 7282 received pure beta-carotene supplementation and 7287 received placebo, while the remaining received beta-carotene alone in combination with alpha-tocopherol, or alpha-tocopherol alone. The current analysis includes a random subsample of 941 men aged 65 years and older at study end from two of the 14 recruitment areas, who received an end-of-study ophthalmologic examination.
Baseline characteristics of study subjects	Finnish men aged 65+, who had been smoking five or more cigarettes per day at baseline, and who had completed a 5-8 years supplementation intervention.
Exposure (type and amount)	20 mg/day of beta-carotene for 5-8 years (median 6.1 years) compared with placebo, through a single capsule (blinded).
Health outcome	The main outcome of the trial was lung cancer prevention. In the current analysis, ARM was the outcome. 269 cases were identified.
Measurement of outcome	Age-related maculopathy (yes/no) at eye examination at final follow-up trial visit. A person was considered to have ARM if he had a class I or higher change in either eye, and severity was classified according to the worst eye. ARM was treated as a dichotomous response in the main analyses; zero in the classification meant no ARM while scores I-IV indicated ARM.
Statistical analysis	Logistic regression analysis of prevalence of ARM in each supplementation group at the end of follow-up, with the general estimation equations approach. This method accounts for the within-person relatedness of left and right eye observations. Adjustment was made for diabetes, high blood pressure, outdoor ultraviolet exposure, amount of smoking, use of alcohol, total serum cholesterol, length of education, body mass index, myopia in adolescence and nuclear cataract.
Results	269 cases with ARM were identified, of whom 68 in the beta-carotene group (prevalence 29.1%) and 53 in the placebo group (prevalence 24.9%). There was no statistically significant effect of supplementation on ARM: OR 1.01 (95% CI 0.77-1.03) for beta-carotene vs. placebo.
Conclusion	Authors' conclusion: No beneficial effect of long-term supplementation with alpha-tocopherol or beta-carotene on the occurrence of ARM was detected among smoking males.
Relevance for our risk assessment purpose	No harmful effects on eye changes were reported in this study of male smokers who had taken 20 mg/day beta-carotene supplements for 5-8 years. The authors cite other studies that have reported changes in scotopic B-wave in electroretinography and crystal formation in the macula with prolonged beta-carotene supplementation. They also refer to their main results on possibly higher mortality due to ischemic heart disease and lung cancer in the beta-carotene group in the current trial, published in 1994. They thus question the safety of prolonged use of beta-carotene supplementation.

Authors, reference. Title	Greenberg E.R., Baron J.A., Stukel T.A., Stevens M.M., Mandel J.S., Spencer S.K., Elias P.M., Lowe N., Nierenberg D.W., Bayrd G., et al. (1990) A clinical trial of beta carotene to prevent basal-cell and squamous-cell cancers of the skin. The Skin Cancer Prevention Study Group. N Engl J Med 323:789-95
Study design and type	Randomised double blind study.
Objective	To test whether beta-carotene reduced risk of new cancers.
Number of participants, country and age	1805 patients living in four different US states.
Baseline characteristics of study subjects	All patients were recently diagnosed with a non-melanoma skin cancer. 70% were males, 30% females. Approximately half were over and half were under 65 years of age.
Exposure, substance, food, (type and amount)	Participants received either 50 mg beta-carotene or placebo per day.
Measurement of exposure (biomarker, internal validation)	Quarterly questionnaires, annual examinations, annual plasma beta-carotene analyses.
Follow-up period, drop-outs	5 years.
Health outcome	Skin cancer.
Measurement of outcome	New skin basal-cell or squamous-cell skin cancer. Annual examinations, biopsy of all skin lesions.
Statistical analysis	Cox proportional-hazards model.
Results	No difference between the groups in the rate of occurrence of the first new non-melanoma skin cancer. No differences in sub-group analyses.
Conclusion	In patients with previous non-melanoma skin cancer, treatment with beta-carotene does not reduce the occurrence of new skin cancers.
Confounders adjusted for	Sex, age, study centre, number of previous cancers, age of diagnosis of first skin cancer, skin type, smoking, plasma retinol levels at study entry.
Relevance for our risk assessment purpose	Yes.

Authors, reference. Title	Green A., Williams G., Neale R., Hart V., Leslie D., Parsons P., Marks G.C., Gaffney P., Battistutta D., Frost C., Lang C., Russell A. (1999) Daily sunscreen application and betacarotene supplementation in prevention of basal-cell and squamous-cell carcinomas of the skin: a randomised controlled trial. Lancet 354:723-9
Study design and type	Randomised controlled trial, 2x2 factorial design.
Objective	Investigate if daily use of sunscreen or beta-carotene supplements prevents skin cancer in health adults.
Number of participants, country and age	A total of 1,621 participants completed a skin cancer survey in 1986, had completed a second survey in 1992, and had a complete skin examination by a dermatologist with removal of all diagnosed tumors of the skin. Australian study conducted in Nambour.
Baseline characteristics of study subjects	Mean age 49 years, 56% of whom were women.25 – 30 % of the participants had a previous cancer.
Exposure, substance, food, (type and amount)	Participants were allocated to 1 of 4 groups: daily use of sun protection factor 15 plus a broad-spectrum sunscreen (to cover exposed sites on head, neck, arms, and hands) and beta-carotene, one 30-mg tablet per day (n = 404); daily sunscreen use and placebo tablets (n = 408); beta-carotene use alone (n = 416); and placebo alone (n = 393). Participants in the groups receiving beta-carotene alone or placebo alone were told they could continue using sunscreen at their usual discretionary rate (74% of these adults used sunscreen 2 days per week).
Measurement of exposure (biomarker, internal validation)	Full skin examination by a dermatologist.
Follow-up period, drop-outs	4.5 years follow-up, 15% drop-outs.
Health outcome	New skin cancers.
Measurement of outcome	Person-time–based incidence and total tumor incidence of basal cell carcinoma or squamous cell carcinoma that was diagnosed 1or more years after the intervention began.
Statistical analysis	Poisson regression and proportional-hazards model.
Results	No significant difference between beta-carotene and placebo groups.
Conclusion	Neither beneficial nor harmful effect on the rates of skin cancer as a result of beta-carotene supplementation.
Confounders adjusted for	Smoking.
Relevance for our risk assessment purpose	Yes.

Authors, reference. Title	Hughes M.C., Williams G.M., Baker P., Green A.C. (2013) Sunscreen and prevention of skin aging: a randomized trial. Ann Intern Med 158:781-90
Study design and type	RCT, four intervention arms.
Objective	To determine whether regular use of sunscreen compared with discretionary use or beta-carotene supplements compared with placebo retard skin aging, measured by degree of photo-aging.
Number of participants, country and age	903 adults younger than 55 years out of 1621 adults randomly selected from a community register in Nambour, Australia.
Baseline characteristics of study subjects	Both sexes, 25-55 years old, 58% with fair skin, 36% with medium skin, 5% with dark skin, 86% with no history of skin cancer, 18.6% worked mainly outdoors.
Exposure, substance, food, (type and amount)	Random assignment into 4 groups: daily use of broad-spectrum sunscreen and 30 mg of beta-carotene, daily use of sunscreen and placebo, discretionary use of sunscreen and 30 mg of beta-carotene, and discretionary use of sunscreen and placebo.
Measurement of exposure (biomarker, internal validation)	Dermal beta-carotene level.
Follow-up period, drop-outs	4.5 years.
Health outcome	Skin aging.
Measurement of outcome	Change in microtopography between 1992 and 1996 in the sunscreen and beta-carotene groups compared with controls, graded by assessors blinded to treatment allocation.
Statistical analysis	
Results	The daily sunscreen group showed no detectable increase in skin aging after 4.5 years. Skin aging from baseline to the end of the trial was 24% less in the daily sunscreen group than in the discretionary sunscreen group (relative odds, 0.76 [95% CI, 0.59 to 0.98]). Beta-carotene supplementation had no overall effect on skin aging, although contrasting associations were seen in subgroups with different severity of aging at baseline.
Conclusion	Regular sunscreen use retards skin aging in healthy, middle-aged men and women. No overall effect of beta-carotene on skin aging was identified, and further study is required to definitively exclude potential benefit or potential harm.
Confounders adjusted for	Sunscreen intervention, number of sunburns, photoaging of the neck.
Relevance for our risk assessment purpose	Yes. Apparently no side effects of consuming 30 mg beta-carotene daily for 4.5 years.

Authors, reference. Title	Hennekens C.H., Buring J.E., Manson J.E., Stampfer M., Rosner B., Cook N.R., Belanger C., LaMotte F., Gaziano J.M., Ridker P.M., Willett W., Peto R. (1996) Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 334:1145-9
Study design and type	Randomised, double-blind, placebo-controlled trial with two-by-two factorial design.
Objective	To provide either a definitive positive result or an informative null finding regarding a putative protective effect of beta-carotene and/or acetyl salicylic acid (aspirin) against cancer and cardiovascular disease.
Number of participants, country and age	22071 male physicians, United States of America, 40-84 years of age at the beginning of the study (1982).
Baseline characteristics of study subjects	No history of cancer (except non-melanoma skin cancer), myocardial infarction, stroke, or transient cerebral ischemia. At baseline, 11% were current smokers and 39% former smokers.
Exposure, substance, food, (type and amount)	Four exposure groups: beta-carotene plus aspirin placebo, beta-carotene plus aspirin, aspirin plus beta-carotene placebo, beta-carotene placebo plus aspirin placebo. n (beta-carotene) = 11036¸n (beta-carotene placebo) = 11035. Beta-carotene was from BASF Corporation.
Measurement of exposure (biomarker, internal validation)	Serum beta-carotene levels were measured in blood obtained at un-announced visits to study participants' offices in 3 districts. Those assigned to beta-carotene had 4 times higher concentrations than those given placebo (1.2 vs 0.3 mg/l), and there was a highly significant correlation between participants' reports of compliance and their plasma beta-carotene levels. At the end of 11 years of follow-up (last year completed for all participants), 80 % of study participants were still taking study pills, with an average compliance of over 97%.
Follow-up period, drop-outs	Intervention period 1982-1995, intervention duration 12 years, range 11.6 to 14. 2 years. After an average of 5 years, in 1988, participants could request that their white pills be active aspirin, because of a demonstrated significant protective effect against cardiovascular disease; beta-carotene remained randomised until study end. At the end of 11 years of follow-up (last year completed for all participants), 99.2 % of participants still provided information on morbidity, follow-up for mortality was 99.99 % complete.
Health outcome	Malignant neoplasms, cardiovascular diseases, death from the specific conditions studied and from all causes.
Measurement of outcome	Yearly questionnaires (or telephone contact in case of no response), confirmation by expert committee review of medical records (including pathology report for malignant neoplasms) from hospital and/or treating physician. Malignancies diagnosed within two years of randomisation were analysed separately.
Statistical analysis	Cox proportional-hazards model. Intention-to-treat analysis.
Results	No differences between groups regarding early or late incidence of malignant neoplasms overall, cardio-vascular disease or overall mortality. No differences in lung cancer, number of deaths from lung cancer, deaths from cardiovascular disease, or deaths from any cause. No differences in myocardial infarction or stroke. Among current and former smokers, no significant differences in any of the end points listed.
Conclusion	Among healthy men, 12 years of supplementation with beta-carotene produced neither benefit nor harm regarding malignant neoplasms, cardiovascular disease and death from all causes.

Authors, reference. Title	Hennekens C.H., Buring J.E., Manson J.E., Stampfer M., Rosner B., Cook N.R., Belanger C., LaMotte F., Gaziano J.M., Ridker P.M., Willett W., Peto R. (1996) Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 334:1145-9
Confounders adjusted for	Controlled for effect of assignment to aspirin, age at baseline.
Relevance for our risk assessment purpose	Highly relevant in relation to safety of beta-carotene supplementation and determination of an UL.

Authors, reference. Title	Liu C., Wang X.D., Mucci L., Gaziano J.M., Zhang S.M. (2009) Modulation of lung molecular biomarkers by beta-carotene in the Physicians' Health Study. Cancer 115:1049-58
Study design and type	Histopathological analysis of material collected in RCT.
Objective	Evaluation of the effect of long-term beta-carotene supplementation on molecular markers of lung carcinogenesis in primary prevention study of cancer and cardiovascular disease.
Number of participants, country and age	39 cases of available formalin-fixed, paraffin-embedded lung tissue blocks out of 194 surgery and lung biopsy cases of lung cancer diagnosed among 22071 physicians ages 40-84 years at randomisation.
Baseline characteristics of study subjects/cells	Lung cancer cells. Study subjects had no history of cancer or cardiovascular disease at randomisation. Study subject characteristics reported in Hennekens CH et al. (1996) NEJM 334, 1145-1149.
Exposure, substance, food, (type and amount)	Randomised trial of 2 x 2 factorial design supplementation of beta-carotene (50 mg) and aspirin (350 mg) on alternate days. Aspirin part stopped January 1988.
Measurement of exposure (biomarker, internal validation)	Beta-carotene (50 mg) and/or aspirin (350 mg). Compliance checked by plasma level analysis in subgroups. Blood beta-carotene levels in verum group 4 x higher than in placebo group. Placebo group much higher levels than in comparable studies (CARET, ATBC).
Follow-up period, drop-outs	13 years. Trial ended 31 December 1995 (aspirin part January 1988). One case out of a total of 40 cases with available tissue blocks excluded because of prostate cancer before beta-carotene randomisation.
Health outcome	Lung cancer.
Measurement of outcome	Positivity for total p53, RARβ, cyclin D1, PCNA, and CYP1A1 determined by histopathological evaluation by 2 independent investigators blinded to treatment groups. Positivity for 2000 cells determined for the various markers, positivity of sections set according to specified percentages of cells positive.
Statistical analysis	Baseline distribution of cancer risk factors by Wilcoxon rank-sum test (continuous variables) and Mantel-Haenszel test (categorical variables). Outcomes: ORs and CIs by unconditional logistic regression with adjustments for age and smoking status. Multivariate models.
Results	CYP1A1 in lung cancer cases assigned to receive beta-carotene tended to be reduced (95% CI; 0.1-1.1; p=0.06), otherwise there was no significant difference in the molecular markers examined between cancer cases assigned to beta-carotene and placebo.
Conclusion	Beta-carotene supplementation had no significant effect on tumor positivity for total p53, RARβ, cyclin D1, PCNA, or CYP1A1. This is in agreement with the study's main finding that beta-carotene supplementation confers no benefit or harm regarding the risk of lung cancer.
Confounders adjusted for	Baseline characteristics, aspirin treatment.
Relevance for our risk assessment purpose	Can be interpreted as mechanistic support for the finding in the main RCT. However, it is unclear how relevant levels of the selected markers in tumor cells are for the risk of development of cancer.

Authors, reference. Title	Christen W.G., Manson J.E., Glynn R.J., Gaziano J.M., Chew E.Y., Buring J.E., Hennekens C.H. (2007) Beta carotene supplementation and age-related maculopathy in a randomized trial of US physicians. Arch Ophthalmol 125:333-9
Study design and type	Randomised, double-blind, placebo-controlled trial.
Objective	To study whether beta-carotene supplementation was preventive against age-related maculopathy (ARM).
Participants, country and age	Male physicians aged 40-84 at baseline in 1982, USA. N=21 142 participants followed for at least 7 years who provided information on ARM on the 7-year questionnaire (those who died during the first 7 years of follow-up were excluded). Of these, n=10 585 were in the beta-carotene group and 10 557 were in the placebo group.
Exposure (type and amount)	One red capsule containing 50 mg beta-carotene (Lurotin, BASF AG) or placebo, taken every other day.
Biomarker, internal validation	To assess the validity of reported compliance with the assigned treatment, plasma beta-carotene concentrations were measured in blood obtained at unannounced visits in a subsample. Those assigned to beta-carotene had significantly higher concentrations than those given placebo (1.2 vs. 0.3 mg/L [2.24 vs. 0.56 mmol/l], p<0.001). There was a high correlation between subjects' reported compliance and plasma beta-carotene concentrations (r=0.69, p<0.001).
Follow-up period, drop-outs	The study was initiated in 1982 and terminated at the end of 1995. At end of follow up, average duration of supplementation was 12 years (range 11.6-14.2). At the end of 11 years of follow-up (the last year completed for all participants), 99.2%were still providing information on morbidity; follow-up for mortality was 99.99 percent complete. In both groups, 80% were still taking the study pills, with an average compliance of 97%. The remaining 20% were not taking any study pills.
Health outcome	Age-related maculopathy (ARM), an early stage of age-related macular degeneration; 549 cases identified during follow-up.
Measurement of outcome	Information on diagnoses of ARM made during the first 7 years of the trial was requested on the 84-month questionnaire: "Have you ever had macular degeneration diagnosed in your right (left) eye?" If yes, month and year of diagnosis was reported. Subsequent annual questionnaires requested information on diagnoses during the preceding year. Medical records were examined for 92% of participants reporting ARM. Ophthalmologists and optometrists were contacted and supplied additional information concerning the diagnosis and severity. *ARM* (main outcome) was defined as a self-report confirmed by medical record evidence of an initial diagnosis during follow-up, with a reduction in best-corrected visual acuity to 20/30 or worse attributable to ARM. ARM with or without vision loss was defined as all incident cases. Avanced ARM was defined as visually significant ARM with pathologic findings of disciform scar, retinal pigment epithelium detachment, geographic atrophy, or subretinal neovascular membrane.
Statistical analysis	Cox proportional hazards regression with adjustment for age at baseline and, during the first five years, random assignment to the aspirin or placebo group in the aspirin component of the trial (the latter did not affect the estimates).
Results	Relative risk for ARM was 0.96 (95% CI 0.78-1.20) in the beta-carotene vs. placebo group. Corresponding relative risks were 1.01 (95% CI 0.86-1.20) for ARM with or without vision loss, and 0.97 (95% CI 0.69-1.37) for advanced ARM.
Conclusion	12 years supplementation with beta-carotene had no marked beneficial or harmful effect on risk of ARM.
Relevance for our risk assessment purpose	No evidence of a harmful effect on age-related maculopathy of an average daily dose of 25 mg beta-carotene taken for 12 years in healthy middle-aged and older men.

Authors, reference. Title	Lee I.M., Cook N.R., Manson J.E., Buring J.E., Hennekens C.H. (1999) Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. J Natl Cancer Inst 91:2102-6
Study design and type	Randomised, double-blind, placebo-controlled trial. Women's Health Study.
Objective	Originally testing aspirin, vitamin E, and b-carotene in the prevention of cancer and cardiovascular disease. The b-carotene component was terminated early after a median treatment duration of 2.1 years (range =0.00–2.72 years).
Number of participants, country and age	39 876 women aged 45 years or older.
Baseline characteristics of study subjects	Female health professionals, aged 45 years or older and without a history of cancer (except nonmelanoma skin cancer), coronary heart disease, or cerebrovascular disease.
Exposure, substance, food, (type and amount)	Randomly assigned to one of the following eight treatment groups. The active agents were 100 mg of aspirin, given on alternate days; 600 IU of vitamin E, given on alternate days; and 50 mg of beta-carotene, given on alternate days. group 1: aspirin 100 mg, beta-carotene 50 mg, vitamin E 600 IU; group 2: aspirin 100 mg, beta-carotene 50 mg, vitamin E placebo; group 3: aspirin 100 mg, beta-carotene 50 mg placebo, vitamin E 600 IU; group 4: aspirin 100 mg, beta-carotene placebo, vitamin E placebo; group 5: aspirin placebo, beta-carotene 50 mg, vitamin E 600 IU; group 6: aspirin placebo, beta-carotene 50mg, vitamin E placebo; group 7: aspirin placebo, beta-carotene placebo, vitamin E 600 IU; group 8: aspirin placebo, beta-carotene placebo, vitamin E placebo;
Measurement of exposure (biomarker, internal validation)	
Follow-up period, drop-outs	Median of 4.1 years (2.1 years' treatment plus another 2.0 years' follow-up).
Health outcome	Incidence of cancer, cardiovascular disease, or total mortality.
Measurement of outcome	Women reported the occurrence of relevant end points either on their follow-up questionnaires or through letters or telephone calls. Deaths usually were reported by family members or postal authorities. Written consent to review relevant medical records was then requested. Medical records were obtained from hospitals and treating physicians. Reports of cancer, cardiovascular disease, or death were considered confirmed or refuted only after review of all relevant information by an end-points committee of physicians who were blinded to treatment assignment.
Statistical analysis	Cox proportional hazards regression was used to estimate the relative risk (RR) of an end point among those assigned to receive b-carotene compared with those assigned to receive placebo. The data were analysed according to intention to treat, regardless of actual compliance. Adjustment for age and randomised treatment assignments to aspirin and vitamin E was done, since randomisation was stratified according to these three variables.
Results	There were no statistically significant differences in incidence of cancer, cardiovascular disease, or total mortality. There were 378 cancers in the b-carotene group and 369 cancers in the placebo group (relative risk [RR] = 1.03; 95%

Authors, reference. Title	Lee I.M., Cook N.R., Manson J.E., Buring J.E., Hennekens C.H. (1999) Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. J Natl Cancer Inst 91:2102-6
	confidence interval [CI] = 0.89–1.18). There were no statistically significant differences for any site-specific cancer or during years 1 and 2 combined and years 3 and up combined. For cardiovascular disease, there were no statistically significant differences for myocardial infarction (42 in the b-carotene group versus 50 in the placebo group), stroke (61 versus 43), deaths from cardiovascular causes (14 versus 12), or the combined end point of these three events (116 versus 102; among women with more than one event, only the first was counted). Deaths from any cause were similar in the two groups (59 versus 55). Among smokers at baseline (13% of all women), there were no statistically significant differences in overall incidence of cancer (RR = 1.11; 95% CI = 0.78–1.58) or cardiovascular disease (RR = 1.01; 95% CI = 0.62–1.63).
Conclusion	Among apparently healthy women, there was no benefit or harm from b-carotene supplementation for a limited period on the incidence of cancer and of cardiovascular disease.
Confounders adjusted for	
Relevance for our risk assessment purpose	More women in the b-carotene group than in the placebo group reported yellowing of the skin (2131 versus 1944; p=0.003). The prevalence of other minor side effects, such as symptoms suggestive of gastric upset or peptic ulcer, nausea, constipation, or diarrhea, did not differ between women in the two groups.

Authors, reference. Title	Lin J., Cook N.R., Albert C., Zaharris E., Gaziano J.M., Van Denburgh M., Buring J.E., Manson J.E. (2009) Vitamins C and E and beta carotene supplementation and cancer risk: a randomized controlled trial. J Natl Cancer Inst 101:14-23
Study design and type	RCT, 2x2x2 factorial trial including one arm with only beta-carotene. A sub-group within the Woman's Health Study called The Women's Antioxidant Cardiovascular Study (WACS). A secondary prevention trial.
Objective	To study morbidity and mortality after supplementation with anti-oxidants in women with initially no signs of cancer.
Number of participants, country and age	7627 women who were free of cancer before random assignment. USA.
Baseline characteristics of study subjects	Above 40 years old, postmenopausal or not intending to become pregnant and had a known CVD or at least three cardiac risk factors: hypertension, high cholesterol levels, diabetes, parental history of myocardial infarction or obesity.
Exposure, substance, food, (type and amount)	In the pure beta-carotene arm: 50 mg beta-carotene every second day.
Measurement of exposure (biomarker, internal validation)	Validation through questionnaires.
Follow-up period, drop-outs	Average 9.4 years of treatment.
Health outcome	
Measurement of outcome	Cancer or death as reported in registries.
Statistical analysis	
Results	624 women developed incident invasive cancer and 176 women died from cancer.
Conclusion	Supplementation with vitamin C, vitamin E, or beta-carotene offers no overall benefits in the primary prevention of total cancer incidence or cancer mortality.
Confounders adjusted for	
Relevance for our risk assessment purpose	Yes

Authors, reference. Title	Song Y., Cook N.R., Albert C.M., Van Denburgh M., Manson J.E. (2009) Effects of vitamins C and E and beta-carotene on the risk of type 2 diabetes in women at high risk of cardiovascular disease: a randomized controlled trial. Am J Clin Nutr 90:429-37
Study design and type	Randomised controlled trial. The Women`s Antioxidant Cardiovascular Study.
Objective	To investigate the long-term effects of supplementation with vitamin C, vitamin E and beta-carotene for primary prevention of type 2 diabetes.
Number of participants, country and age	8171 female health professionals' ≥ 40 years with a history of CVD or ≥ 3 CVD risk factors (hypertension, high cholesterol, diabetes Mellitus, parental history of premature MI before 60 years, obesity and current cigarette smoking).1597 subjects reported prevalent diabetes at baseline and was excluded. 6574 women who were free of diabetes at baseline.
Baseline characteristics of study subjects/cells	6574 women who were free of diabetes at baseline and at high risk of CVD.
Exposure, substance, food, (type and amount)	2 x 2 x 2 factorial design. 1. Vitamin C (Ascorbic acid; 500 mg/d) 2. Vitamin E (RRR-α-Tocopherol acetate. 600 IU/every other day) 3. Beta-carotene (50 mg/every other day) 4. Placebo
Measurement of exposure (biomarker, internal validation)	Blood concentration of respective intervention item. 1. Blood levels of ascorbic acid vs placebo; 1.9 vs 1.3 mg/dl 2. Blood levels of vitamin E vs placebo (20.2 vs 12.2 µg/ml) 3. Blood levels of beta-carotene vs placebo (54.4 vs 19.5 µg/ml Compliance (self-reporting of at least 2/3 of study pills) was ~ 73% for all groups.
Follow-up period, drop-outs	Median follow-up of 9.2 years.
Health outcome	Incident of Type 2 diabetes. 895 women were diagnosed with type 2 diabetes in the follow-up period.
Measurement of outcome	Annually report on incidence of type 2 diabetes. The self-reported diagnosis was confirmed by using a supplementary diabetes questionnaire on diabetes symptoms, screening test and hypoglycaemic medication.
Statistical analysis	Intention-to-treat principle for analysis. 2-sample t test for continuous variables and chi-square tests for categorical values. Kaplan Meier survival curves. Cox proportional hazards models to calculate estimates of relative risks (RRs) and 95%Cis for each antioxidant. Specific subgroup analysis to examine effects of vitamins on major risk factors for Type 2 diabetes at baseline such as age, BMI, smoking, alcohol use, family history of diabetes, physical activity menopause status and hormone therapy, history of hypertension, history of hypercholesterolemia and baseline dietary intake of vitamin C, E or total carotenoids.
Results	There was no effect of beta-carotene treatment (RR:0.97; 95% CI: 0.85, 1.11; p=0.68) on incidence of type 2 diabetes.

Authors, reference. Title	Song Y., Cook N.R., Albert C.M., Van Denburgh M., Manson J.E. (2009) Effects of vitamins C and E and beta-carotene on the risk of type 2 diabetes in women at high risk of cardiovascular disease: a randomized controlled trial. Am J Clin Nutr 90:429-37
Conclusion	No significant effect of beta-carotene on risk of developing type 2 diabetes in women at high risk of CVD.
Confounders adjusted for	Adjustment for age and other randomised treatment.
Relevance for our risk assessment purpose	No difference in reports of adverse effect (e.g. bleeding, gastrointestinal symptoms, fatigue or drowsiness) between placebo group and beta-carotene group.

Prospective studies

Authors, reference. Title	Asgari M.M., Maruti S.S., Kushi L.H., White E. (2009) Antioxidant supplementation and risk of incident melanomas: results of a large prospective cohort study. Arch Dermatol 145:879-82
Study design and type	Prospective cohort study.
Objective	To investigate the association between long-term antioxidant supplement use and risk of melanoma.
Participants, country and age	77 719 (37 382 men and 40 337 women) were enrolled, of whom 69 671 were included in the analysis. Age 50-76 years at baseline, Western Washington State, USA.
Exposure	Use (duration and dose) of multivitamin antioxidant dietary supplements, including specific effects of individual beta-carotene supplements and selenium supplements.
Measurement of exposure	A baseline 24-page self-administered postal questionnaire about lifestyle factors, health history, dietary intake (FFQ), supplement use, personal characteristics, and cancer risk factors completed in 2000-2002.
Health outcome	Incident melanoma up to 31.12.2006, including both cases of melanoma in situ and cases of invasive melanoma.
Measurement of outcome	n=461 cases identified through linkage to the Surveillance, Epidemiology, and End Results (SEER) cancer registry.
Statistical analysis	Cox proportional hazards regression.
Results	Association between calculated average daily intake of supplemental beta-carotene across 10 years and incident melanoma: RR 0.87 (95% CI 0.48-1.56) for 3 mg/day or higher compared with none. No dose-response trend (p=0.38).
Confounders adjusted for	Age at baseline (years), sex (female or male), education (high school or less, some college, or advanced degree), first-degree family history of melanoma (no or yes), personal history of nonmelanoma skin cancer (no or yes), ever had moles removed (no or yes), freckles between ages 10 and 20 years (no or yes), had 3 or more severe sunburns between ages 10 and 20 years (no or yes), natural red or blond hair between ages 10 and 20 years (no or yes), and reaction to 1 hour in strong sunlight (tan or no sunburn, mild sunburn, painful sunburn, severe sunburn with blistering).
Conclusion	No association between self-reported use of antioxidants and risk of melanoma.
Relevance for our risk assessment purpose	No indication of a harmful effect on melanoma risk of an average daily supply of more than 3 mg beta-carotene from supplements. The researchers defined the highest category of beta-carotene starting at 3000 µg (6000 µg/day used 7 days/week for 5 years – corresponding to the dose given in a previous supplementation study which reported a 4-fold higher melanoma risk in women randomised to receive antioxidant supplements). There is no information on the distribution or upper dose in the ">=3000 µg" category. As this was a population-based cohort study, it was not designed to provide information concerning any other adverse effect of long-term daily beta-carotene supplementation.

Authors, reference. Title	Asgari M.M., Brasky T.M., White E. (2012) Association of vitamin A and carotenoid intake with melanoma risk in a large prospective cohort. J Invest Dermatol 132:1573-82
Study design and type	Prospective cohort study.
Objective	To investigate the association between dietary, supplemental, and total intake of retinol and carotenoids and risk of melanoma.
Participants, country and age	77 719 (37 382 men and 40 337 women) were enrolled, of whom 69 635 were included in the analysis. Age 50-76 years at baseline, Western Washington State, USA.
Exposure	Dietary, supplemental, and total intake of retinol and carotenoids (including beta-carotene, lutein, and lycopene). Beta-carotene was analysed as individual supplement use categorised as current/former/never; supplement dose none, 6-600 and >600 µg/day; dietary intake (µg/day), and total intake from diet and supplement (µg/day). Both individual beta-carotene supplements and multivitamin supplements contributed to the calculated supplemental beta-carotene dose.
Measurement of exposure	A baseline 24-page self-administered postal questionnaire about lifestyle factors, health history, dietary intake (FFQ), supplement use, personal characteristics, and cancer risk factors completed in 2000-2002.
Health outcome	Incident melanoma up to 31.12.2007, including both cases of melanoma in situ and cases of invasive melanoma.
Measurement of outcome	n=566 cases identified through linkage to the Surveillance, Epidemiology, and End Results (SEER) cancer registry.
Statistical analysis	Cox proportional hazards regression.
Results	Beta-carotene supplementation and risk of melanoma: HR 0.95 (95% CI 0.64-1.40) for current use compared with never use. HR 1.08 (95% CI 0.86-1.36) for supplemental dose higher than 600 µg/day compared with non-users, no dose-response trend (p=0.36). Total beta-carotene supply from diet and supplements and risk of melanoma: HR 1.13 (0.86-1.49) for intake higher than 9.3 mg/day compared with 3.5 mg or lower, no dose-response trend (p=0.47).
Confounders adjusted for	Age, education, body mass index, alcohol, freckles between ages 10-20 years, >=3 severe sunburns between ages 10-20 years, red or blond hair between ages 10-20 years, reaction to 1 hour in strong sunlight, family history of melanoma, history of non-melanoma skin cancer, mole removed, macular degeneration.
Conclusion	No association was found between beta-carotene supplementation and risk of melanoma, nor between total beta-carotene supply from diet and supplements and risk of melanoma.
Relevance for our risk assessment purpose	No indication of a harmful effect on melanoma risk of an average daily supply of more than 600 µg beta-carotene from supplements or an average daily total supply of more than 9 mg beta-carotene from diet and supplements. As this was a population-based cohort study, it was not designed to provide information concerning any other adverse effect of long-term daily beta-carotene supplementation.

Authors, reference **Title**	Roswall N., Olsen A., Christensen J., Dragsted L.O., Overvad K., Tjonneland A. (2010) Source-specific effects of micronutrients in lung cancer prevention. Lung Cancer 67:275-81
Study design and type	Prospective cohort study.
Objective	Evaluation of the association between intake of beta-carotene, folate, vitamin C, vitamin E and risk of lung cancer, focusing on source-specific effects of dietary and supplemental intake. Further, whether an association differs by sub-types of lung cancer, and modification of an effect by smoking.
Number of participants, country and age	Out of 160725 individuals invited to participate from 1993 to 1997, 57053 accepted the invitation. Participants were Danes living in the greater Copenhagen and Aarhus areas, 50-64 years of age.
Baseline characteristics of study subjects/cells	Inclusion criteria were, in addition to age and area of residence, no previous cancer diagnosis in the Danish Cancer Registry.
Exposure, substance, food, (type and amount)	Dietary intake and food supplements.
Measurement of exposure (biomarker, internal validation)	A 192-item validated food frequency questionnaire by mail at baseline, and information about supplements in open-ended questions on brands, doses and consumption frequency and duration. Supplement brand contents were checked with producers/distributors. Further, at a visit to the study clinic, lifestyle information including active and passive smoking, and work exposure to potentially carcinogenic substances (further definitions of risk occupations in the paper). No serum/plasma level measurements. No analysis of data from individuals taking beta-carotene supplements only; only 1408 individuals (2.5%) took beta-carotene supplements. Dietary intake ranged from 36 - 94 667 µg/day (median 3213 µg), supplemental intake from 1-40 500 µg/day (median 2255 µg).
Follow-up period, drop-outs	Follow-up for lung cancer started on the day of visit to the study centre and continued until date of diagnosis of any cancer (by linkage to the Danish Cancer Registry), date of death, date of emigration (Central Population Registry) or December 31st 2006, whichever came first. Patients were excluded if they had a cancer diagnosis before baseline (n=571), or if information on one or more potential confounders or exposure variables (n=925) was lacking. This left 55 557 participants for the analyses.
Health outcome	Lung cancer.
Measurement of outcome	Linkage to Danish Cancer Registry.
Statistical analysis	Cox proportional hazard models, detailed in the paper. Due to the low number of participants taking beta-carotene supplements, the analyses are imprecise; however statistically significant associations were observed.
Results	A significantly higher risk for lung cancer was observed with supplemental beta-carotene (IRR 1.64; 95% CI 1.20-2.23). A dose-response effect was observed. No effect was observed from dietary beta-carotene intake. Regarding sub-types of lung cancer, no statistical significance was achieved for any individual sub-type. No statistically significant interaction between any micronutrient and smoking status was observed.
Conclusion	A harmful effect of beta-carotene supplementation but not dietary intake was observed, in line with results from some RCT studies.

Authors, reference **Title**	Roswall N., Olsen A., Christensen J., Dragsted L.O., Overvad K., Tjonneland A. (2010) Source-specific effects of micronutrients in lung cancer prevention. Lung Cancer 67:275-81
Confounders adjusted for	Details in the paper.
Relevance for our risk assessment purpose	Some: observation of a source-specific adverse effect from supplemental beta-carotene; dose-response effect.

Authors, reference. Title	Roswall N., Olsen A., Christensen J., Hansen L., Dragsted L.O., Overvad K., Tjonneland A. (2012) Micronutrient intake in relation to all-cause mortality in a prospective Danish cohort. Food Nutr Res 56
Study design and type	Prospective cohort study.
Objective	To evaluate the association between intake of vitamin C, E, folate, beta-carotene from diet and supplements, and overall mortality. Furthermore, to examine effect modification by smoking, alcohol intake, and BMI and to investigate if the effect of supplement use differs with dietary micronutrient intake.
Number of participants, country and age	55453 middle-aged Danes. Information regarding diet, supplement use, and lifestyle was collected through questionnaires. During follow-up, 6767 deaths were identified and incidence rate ratios (IRRs) of mortality related to micronutrient intake were calculated using Cox proportional hazards models.
Baseline characteristics of study subjects/cells	60% males, 40% females. 82% were current or former smokers among the cases, 64% among the non-cases.
Exposure, substance, food, (type and amount)	Dietary supplements. Only 1.8% of cases and 2.6% of non-cases used beta-carotene supplements, average from supplements: 2.25 mg/day.
Measurement of exposure (biomarker, internal validation)	Food frequency questionnaire, intake the last 12 months
Follow-up period, drop-outs	Median follow-up period: 13.8 years
Health outcome	All cause mortality, 6767 died in the study period.
Measurement of outcome	
Statistical analysis	Stratified into "never smokers" "former smokers" and "current smokers".
Results	No difference between those who died and the living as to intake of beta-carotene supplements, also when stratified and after adjustment.
Conclusion	Supplemental beta-carotene intake was not associated with mortality.
Confounders adjusted for	
Relevance for our risk assessment purpose	The supplemental intake of beta-carotene was very low. Because of this, not useful for setting upper levels.

Summary Tables meta-analysis and systematic reviews

Authors, reference. Title	Evans J.R., Lawrenson J.G. (2012) Antioxidant vitamin and mineral supplements for preventing age-related macular degeneration. Cochrane Database Syst Rev 6:CD000253
Study types included	Randomised controlled trials.
Aim of review	To examine the evidence as to whether or not taking antioxidant vitamin or mineral supplements prevents the development of AMD.
Timespan literature search	MEDLINE January 1950 to January 2012, EMBASE January 1980 to January 2012.
Category of exposure	Antioxidant supplements including several antioxidant vitamins (carotenoids, vitamins C and E) or minerals (selenium and zinc) are included. The individual effect of beta-carotene supplementation vs. placebo is investigated (two studies only).
Population in included studies	Middle-aged and older men, Finland and the USA.
Dose range in included studies	50 mg every other day (i.e. average 25 mg/day) and 20 mg/day, respectively.
Timespan follow-up	12 years and 6 years, respectively.
Evaluated for methodological quality	Yes.
Grading of evidence	High quality ("Further research is very unlikely to change our confidence in the estimate of effect").
Results	A total of 21589 men were randomised into ATBC and PHS comparing beta-carotene with placebo. There were 343 cases of AMD in the beta-carotene groups and 327 in the control groups. The results of the trials were consistent (I^2 = 0%) and did not indicate any benefit of supplementation (RR 1.03, 95% CI 0.89 to 1.19). There were 65 cases of advanced AMD in the beta-carotene groups and 67 cases of advanced AMD in the control. Again the results of the trials were consistent (I^2 = 0%) and indicated no effect of supplementation (RR 0.97, 95% CI 0.69 to 1.36).
Conclusion	Long-term supplementation with 20-25 mg/day beta-carotene did not affect risk of AMD in men.
Relevance for our risk assessment purpose	Beta-carotene supplementation did not produce a harmful effect on risk of AMD in men, and the authors state that none of the trials included in this review reported eye-related adverse effects. A limitation is that only two studies were included that had studied the effect of beta-carotene supplementation per se, and that these included middle-aged and older men only.

Authors, reference. Title	Fortmann S.P., Burda B.U., Senger C.A., Lin J.S., Whitlock E.P. (2013) Vitamin and mineral supplements in the primary prevention of cardiovascular disease and cancer: An updated systematic evidence review for the U.S. Preventive Services Task Force. Ann Intern Med 159:824-34
Study types included	103 articles (26 studies) were included. Six studies of beta-carotene examined the benefits and harms of individual and paired supplementation.
Aim of review	The aim of the study was to systematically review evidence for the benefit and harm of vitamin and mineral supplements in community-dwelling nutrient sufficient adults for the primary prevention of CVD and cancer.
Timespan literature search	MEDLINE, Embase, Cochrane Central Register of Controlled Trials, Cochrane Database of systematic reviews of effects were searched from January 2005 to January 2013.
Category of exposure	
Dose range in included studies	Doses were under the set upper tolerable limit.
Timespan follow-up in included studies	Duration of most studies was less than 10 years.
Comperators (placebo)	
Evaluated for methodological quality	Two investigators independently reviewed fair- and good-quality trials for benefit and fair- and good-quality trials and observational trials for harms.
Grading of evidence	Limitations: The analysis included only primary prevention studies in adults without known nutritional deficiencies. Studies were conducted in older individuals and included various supplements and doses under the set upper tolerable limit.
Statistical analysis	Meta-analysis was performed to estimate the effect size of supplementation on CVD incidence, cancer incidence and all-cause mortality. For all cases, unadjusted relative risks based on the number of events and non-events. Fixed-effects Mantel-Haenszel methods was used because few trials could be combined and to help avoid bias associated with rare events.
Results	Consistent null results were found for CVD incidence across 6 trials. For beta-carotene, a probable increase in lung cancer incidence in high-risk subgroups (smokers and asbestos workers).
Conclusion	The review confirms the established harm of beta-carotene supplementation on lung cancer incidence and deaths in individuals at high risk for lung cancer.
Relevance for our risk assessment purpose	Confirm previous observation of harmful effects of beta-carotene supplementation on lung cancer incidence and deaths in individuals at high risk for lung cancer.

Authors, reference. Title	Bjelakovic G., Nikolova D., Simonetti R.G., Gluud C. (2008) Systematic review: primary and secondary prevention of gastrointestinal cancers with antioxidant supplements. Aliment Pharmacol Ther 28:689-703
Study types included	Randomised trials. 12 trials used beta-carotene either singly (n=2) or in combinations (n=10).
Aim of review	To assess the effects of antioxidant supplements in preventing gastrointestinal cancer, both primary prevention and secondary prevention. The health outcomes were gastrointestinal cancers, overall mortality, adverse effects (primary prevention n=8, secondary prevention n=4).
Timespan literature search	Until October 2007.
Category of exposure	The antioxidants beta-carotene, vitamin A, vitamin C, vitamin E, and selenium.
Dose range in included studies	Doses of beta-carotene ranged from 6-30 mg/day; one study reduced to 30 mg x2/week after 1 year.
Comperators (placebo)	
Timespan follow-up in included studies	Not given.
Evaluated for methodological quality	10 studies were of high quality/low risk of bias, 2 studies had high risk of bias. Of two studies that used beta-carotene singly, one large trial (n=22 071) had low risk of bias, one small study (n= 216) had high risk of bias. - Two studies recruited women only, two studies men only.
Grading of evidence	
Statistical analysis	Cochrane methodology. Random-effects model meta-analysis as well as fixed-effects model meta-analysis; the latter reported only if the results of the two models differed significantly. Results presented as relative risk (RR) with 95% confidence intervals (CI).
Results	Antioxidant supplements had no significant influence on gastrointestinal cancer occurrence (RR 0.94; 95% CI 0.83-1.06), nor did beta-carotene given singly influence the occurrence of gastrointestinal cancers (RR 1.04; 95% CI 0.93-1.34). Various combinations of beta-carotene with other antioxidants did not influence GI cancer occurrence. However, analysing low- and high bias-risk trials separately, beta-carotene potentially increased cancer risk. Eta-regression analysis showed that dose of beta-carotene was significantly associated with the estimated intervention effect of antioxidants (RR 1.01; 95% CI 1.002-1.02). Beta-carotene given singly did not significantly influence mortality (RR 1.05; 95 % CI 0.99-1.11)(random effects model); however in the fixed-effect model there was a significant increase (RR 1.06; 95 % CI 1.02-1.10). Also in participants supplemented with beta-carotene and vitamin A (RR 1.16; 95% CI 1.09-1.23) or vitamin E (RR 1.06; 95% CI 1.02-1.11) an increased risk was observed. The dose of beta-carotene in meta-regression analysis was significantly associated with the estimated intervention effect on mortality (RR 1.007; 95% CI 1.002-1.012; p=0.003). – Transient yellowing of the skin was not significantly influenced by beta-carotene supplementation.
Conclusion	Authors did not find evidence that beta-carotene prevented gastrointestinal cancers, on the contrary, there were indications of increased risk for cancer and increased mortality with the dose range studied (6 – 30 mg/day).
Relevance for our risk assessment	Supports increased gastrointestinal cancer occurrence and increased mortality with beta-carotene supplementation.

Authors, reference. Title	Bjelakovic G., Nikolova D., Simonetti R.G., Gluud C. (2008) Systematic review: primary and secondary prevention of gastrointestinal cancers with antioxidant supplements. Aliment Pharmacol Ther 28:689-703
purposeFollow-up period, drop-outs	Supports dose-response effect. Relevant data on gastrointestinal cancers could be extracted from 18 of the 20 trials examining gastrointestinal cancers. 14 studies had data on overall mortality.

Authors, reference. Title	Bjelakovic G., Nikolova D., Simonetti R.G., Gluud C. (2008) Antioxidant supplements for preventing gastrointestinal cancers. Cochrane Database Syst Rev:CD004183
Study types included	Meta-analysis; material and results virtually identical with Bjelakovic G et al 2008 paper in Aliment Pharm Ther, almost word-by-word, except for Adverse effects.
Aim of review	To assess the effects of antioxidant supplements in preventing gastrointestinal cancer, both primary prevention (8 trials) and secondary prevention (4 trials).
Timespan literature search	From 1945 or later for different data searches, all databases until October 2007.
Category of exposure	Supplements.
Dose range in included studies	6 mg to 30 mg (one study after 1 year changed to 30 mg x2/week) per os.
Timespan follow-up in included studies	5 - 14.1 years.
Comperators (placebo)	Placebo or no treatment.
Evaluated for methodological quality	10 studies were according to set criteria (Cochrane) of high quality/low risk of bias, 2 studies had high risk of bias. Of two studies that used beta-carotene singly, one large trial (n=22071) had low risk of bias, one small study (n= 216) had high risk of bias.
Grading of evidence	-
Statistical analysis	Cochrane methodology. Random-effects model meta-analysis as well as fixed-effects model meta-analysis; the latter reported only if the results of the two models differed significantly. Results presented as relative risk (RR) with 95% confidence intervals (CI). Heterogeneity assessed with I^2. Adjusted rank correlation and regression asymmetry test were used for detection of bias. The funnel plot was statistically significantly asymmetric for cancer indicating publication bias (Egger's test p=0.009; Begg's test p=0.096), but not for mortality.
Results	Antioxidant supplements had no significant influence on gastrointestinal cancer occurrence (RR 0.94; 95% CI 0.83-1.06), nor did beta-carotene given singly influence the occurrence of gastrointestinal cancers (RR 1.04; 95% CI 0.93-1.34). Various combinations of beta-carotene with other antioxidants did not influence GI cancer occurrence. However, analysing low- and high bias-risk trials separately, beta-carotene potentially increased cancer risk. MeEta-regression analysis showed that dose of beta-carotene was significantly associated with the estimated intervention effect of antioxidants (RR 1.01; 95% CI 1.002-1.02). Beta-carotene given singly did not significantly influence mortality (RR 1.05; 95 % CI 0.99-1.11)(random effects model); however in the fixed-effect model there was a significant increase (RR 1.06; 95 % CI 1.02-1.10). Also in participants supplemented with beta-carotene and vitamin A (RR 1.16; 95% CI 1.09-1.23) or vitamin E (RR 1.06; 95% CI 1.02-1.11) an increased risk was observed. The dose of beta-carotene in meta-regression analysis was significantly associated with the estimated intervention effect on mortality (RR 1.007; 95% CI 1.002-1.012; p=0.003). Adverse effects beta-carotene: (Overall mortality was a primary outcome) Transient yellowing of the skin RR 1.85 [0.74, 4.67], persistent yellowing of the skin RR 29.14 [21.60, 39.32], belching 2.22 [1.80, 2.74], gastrointestinal upset RR1.03 [1.00 – 1.06]

Authors, reference. Title	Bjelakovic G., Nikolova D., Simonetti R.G., Gluud C. (2008) Antioxidant supplements for preventing gastrointestinal cancers. Cochrane Database Syst Rev:CD004183
Conclusion	Beta-carotene supplements given singly did not significantly influence gastrointestinal cancers (RR 1.04; 95% CI 0.80-1.35), nor did they influence mortality in the random-effect model (RR1.007; 95% CI 0.77-1.23). However, in a fixed-effect model, beta-carotene supplements given singly significantly increased mortality (RR1.06; 95% CI 1.02-1.10). Dose of beta-carotene supplements was significantly associated with gastrointestinal cancers as well as mortality.
Relevance for our risk assessment purpose	High.

Authors, reference. Title	Bjelakovic G., Nagorni A., Nikolova D., Simonetti R.G., Bjelakovic M., Gluud C. (2006) Meta-analysis: antioxidant supplements for primary and secondary prevention of colorectal adenoma. Aliment Pharmacol Ther 24:281-91
Study design and type	Meta-analysis of all relevant randomised clinical trials comparing antioxidant supplements with placebo.
Aim of review	To assess antioxidant supplementation in primary and secondary prevention of colorectal adenoma.
Timespan literature search	Until October 2005.
Category of exposure	Beta-carotene, vitamin A, vitamin C, vitamin E, and selenium.
Dose range in included studies	Beta-carotene doses ranged from 15-25 mg/day.
Comperators (placebo)	Placebo tablets or no intervention.
Timespan follow-up in included studies	Supplementation lasted 1–6 years.
Evaluated for methodological quality	Three of eight trials (37.5%) had low risk of bias, having adequate generation of allocation sequence, allocation concealment, blinding and follow- up. The remaining trials had high risk of bias.
Grading of evidence	No
Statistical analysis	Cochrane Collaboration software.
Results	Neither the fixed effects (RR 0.93; 95% CI0.81-1.1) nor the random effect model meta-analyses (RR 0.82, 95% CI 0.60-1.1) showed statistically significant effects of supplementation with beta-carotene, alone or in combination with other antioxidants.
Conclusion	No evidence was found that antioxidant supplements have beneficial or negative effects in the primary or secondary prevention of colorectal adenoma.
Relevance for our risk assessment purpose	Supports the notion that beta-carotene supplementation has no beneficial effect in the prevention of colorectal adenoma.

Authors, reference. Title	Jeon Y.J., Myung S.K., Lee E.H., Kim Y., Chang Y.J., Ju W., Cho H.J., Seo H.G., Huh B.Y. (2011) Effects of beta-carotene supplements on cancer prevention: meta-analysis of randomized controlled trials. Nutr Cancer 63:1196-207
Study types included	Meta-analysis of randomised controlled trials.
Aim of review	To investigate the effect of beta-carotene supplements given alone on cancer prevention as reported by randomised controlled trials (primary prevention, 3 trials; secondary prevention, 3 trials).The main outcome measures were cancer incidence and mortality, with subgroup analyses of types of cancer.
Timespan literature search	1984/1985 – March 2009.
Category of exposure	Supplement with beta-carotene alone given as only type of supplementation.
Dose range in included studies	30 mg (n=1) – 50 mg (n=4). One study 75 mg/day in interrupted sequences.
Timespan follow-up in included studies	4.3 – 12.9 years.
Comperators (placebo)	Placebo (n=5) or no treatment (n=1).
Evaluated for methodological quality	Jadad score – high quality studies n=4, low methodological quality n=2, all included.
Grading of evidence	-
Statistical analysis	Relative risk (RR) and 95% CI by both fixed and random-effects model; heterogeneity by Higgins I^2 statistic; publication bias by Begg's funnel plot and Egger's test. When substantial heterogeneity was not found, fixed-effects model results are presented, in case of substantial heterogeneity, random-effects model results are given.
Results	Beta-carotene supplementation had no significant effect on the overall incidence of cancer compared to the control group (RR = 1.08; 95 % CI = 0.99 – 1.18; I^2 = 54%; n=6). Also, beta-carotene had no significant effect on total cancer mortality (RR=1.00; 95% CI = 0.87-1.15; I^2=0.0%; n=3). No significant effect on the risk of cancer incidence was found in studies of either high or low methodological quality. Analysed by type of cancer, beta-carotene supplements had no preventive effect on 5 types of cancer: lung cancer, colorectal cancer, head and neck cancer, skin cancer, and prostate cancer. However, beta-carotene significantly increased the incidence of urothelial cancer such as bladder cancer, renal pelvis cancer, and ureter cancer (RR=1.35; 95% CI 1.01-1.81; I^2=0%; n=3). No significant associations were found with smoking status, dose or duration of administration in the cancer subgroup (type) analyses.
Conclusion	No evidence of a preventive effect of beta-carotene supplementation on cancer incidence and mortality (primary and secondary prevention) was found in the meta-analysis of the 6 randomised controlled trials with beta-carotene alone published 1990-2003. Instead, beta-carotene tended to marginally increase total cancer incidence, and significantly increased the risk of urothelial cancer.
Relevance for our risk assessment purpose	High.

Authors, reference. Title	Bjelakovic G., Nikolova D., Gluud C. (2013) Meta-regression analyses, meta-analyses, and trial sequential analyses of the effects of supplementation with beta-carotene, vitamin A, and vitamin E singly or in different combinations on all-cause mortality: do we have evidence for lack of harm? PLoS One 8:e74558
Study types included	53 randomised trials with low risk of bias (24 1883 participants, aged 18-103 years, 44.6% women) assessing beta-carotene, vitamin A, and vitamin E. Beta-carotene was used singly versus placebo in 7 trials, 43 019 participants.
Aim of review	To assess whether different doses of beta-carotene, vitamin A, and vitamin E affect mortality in primary and secondary prevention randomised clinical trials with low risk of bias.
Timespan literature search	No information – probably no restrictions.
Category of exposure	E.g foods or supplement, mixed antioxidant/ single beta-carotene.
Dose range in included studies	25-50 mg per day.
Timespan follow-up in included studies	4 – 12 years.
Comperators (placebo)	Yes.
Evaluated for methodological quality	Yes.
Grading of evidence	No, not relevant with the method used.
Statistical analysis	Meta-regression analyses, meta-analyses, and trial sequential analyses.
Results	Beta-carotene in a dose above 9.6 mg significantly increased mortality (relative risk (RR) 1.06, 95% confidence interval (CI) 1.02 to 1.09, I(2) = 13%). Doses below the RDAs did not affect mortality, but data were sparse.
Conclusion	Beta-carotene and vitamin E in doses higher than the RDA seem to significantly increase mortality.
Relevance for our risk assessment purpose	Yes, of high importance.

Authors, reference. Title	Bjelakovic G., Nikolova D., Gluud L.L., Simonetti R.G., Gluud C. (2012) Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev 3:CD007176
Study types included	Primary and secondary prevention randomised clinical trials on antioxidant supplements, in total 78 randomised clinical trials with 296 707 participants.
Aim of review	To assess the beneficial and harmful effects of antioxidant supplements for prevention of mortality in adults.
Timespan literature search	
Category of exposure	Beta-carotene, vitamin A, vitamin C, vitamin E, and selenium) versus placebo or no intervention.
Dose range in included studies	
Timespan follow-up in included studies	The duration of supplementation varied from 28 days to 12 years (mean duration 3 years; median duration 2 years).
Comparators (placebo)	Placebo tablets or no intervention.
Evaluated for methodological quality	Studies graded as having high or low risk of bias.
Grading of evidence	No
Statistical analysis	Cochrane Collaboration software.
Results	Overall, the antioxidant supplements had no significant effect on mortality in a random-effects model meta-analysis (21,484 dead/183,749 (11.7%) versus 11,479 dead/112,958 (10.2%); 78 trials, relative risk (RR) 1.02, 95%confidence interval (CI) 0.98 to 1.05) but significantly increased mortality in a fixed-effect model (RR 1.03, 95%CI 1.01 to 1.05).
Conclusion	No evidence to support antioxidant supplements for primary or secondary prevention. Beta-carotene and vitamin E seem to increase mortality.
Relevance for our risk assessment purpose	High.

Authors, reference. Title	Bjelakovic G., Nikolova D., Gluud L.L., Simonetti R.G., Gluud C. (2008) Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev:CD007176
Study types included	Primary and secondary prevention randomised clinical trials on antioxidant supplements. Included participants were either healthy (primary prevention trials) or had any disease (secondary prevention trials). Beta-carotene was tested singly in 6 trials.
Aim of review	To assess the effect of antioxidant supplements on mortality in primary or secondary prevention randomised clinical trials.
Timespan literature search	1985 to October 2005
Category of exposure	Beta-carotene, vitamin A, vitamin C, vitamin E, and selenium versus placebo or no intervention.
Dose range in included studies	In the studies with beta-carotene given singly in one study arm, doses were 20-50 mg per day.
Timespan follow-up in included studies	The mean duration of follow-up in all trials was 3.4 years (range, 28 days to 14.1 years).
Comparators (placebo)	Placebo or no intervention.
Evaluated for methodological quality	Studies were divided according to a set criteria for high or low risk of bias.
Grading of evidence	No.
Statistical analysis	Cochrane Collaboration Software.
Results	When the different antioxidants were assessed separately, analyses including trials with a low risk of bias found significantly increased mortality by beta-carotene (RR 1.07, 95%) CI 1.02 to 1.11).
Conclusion	Beta-carotene increases mortality.
Relevance for our risk assessment purpose	High.

Authors, reference. Title	Bjelakovic G., Nikolova D., Gluud L.L., Simonetti R.G., Gluud C. (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 297:842-57
Study types included	Randomised controlled trials involving adults.
Aim of review	To assess the effect of antioxidant supplements on mortality in randomised primary and secondary prevention trials.
Timespan literature search	Up till October 2005.
Category of exposure	Beta-carotene, vitamin A, vitamin C (ascorbic acid), vitamin E, and selenium either singly or combined.
Dose range in included studies	Beta-carotene 1.2 to 50.0 mg (mean, 17.8 mg).
Timespan follow-up in included studies	The mean duration of follow-up in all trials was 3.3 years (range, 28 days-14.1 years).
Comparators (placebo)	Placebo tablets or no intervention.
Evaluated for methodological quality	Studies were divided according to a set criteria for high or low risk of bias.
Grading of evidence	No.
Statistical analysis	Random-effects meta-analyses and reported as relative risk (RR) with 95% confidence intervals (CIs). Meta-regression was used to assess the effect of covariates across the trials.
Results	In low-bias risk trials, after exclusion of selenium trials, beta-carotene (RR, 1.07; 95% CI, 1.02-1.11), singly or combined, significantly increased mortality.
Conclusion	Treatment with beta-carotene, vitamin A, and vitamin E may increase mortality.
Relevance for our risk assessment purpose	High.

Authors, reference. Title	Vivekananthan D.P., Penn M.S., Sapp S.K., Hsu A., Topol E.J. (2003) Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. Lancet 361:2017-23
Study types included	A meta-analysis to assess the effect of antioxidants on long-term cardiovascular mortality and morbidity.
Aim of review	Assess the effect of vitamin E, beta-carotene or both, on CVD mortality and morbidity.
Timespan literature search	Not given, but probably up till 2002/2003.
Category of exposure	E.g. foods or supplement, mixed antioxidant/single beta-carotene.
Dose range in included studies	15-50 mg beta-carotene.
Timespan follow-up in included studies	1.4 to 12 years.
Comparators (placebo)	Placebo tablets.
Evaluated for methodological quality	Only studies with more than 1000 participants were included. Furthermore, analysis was limited to studies in populations from developed countries without overt evidence of vitamin deficiencies, all to limit bias.
Grading of evidence	No.
Statistical analysis	Cochran-Mantel Haenszel tests were used to investigate the effect of vitamin therapy on each endpoint across the beta-carotene and, separately, across vitamin E trials, and pooled odds ratios and 95% CI were calculated.
Results	Beta-carotene led to a small but significant increase in all-cause mortality (7.4 vs 7.0%, 1.07 [1.02-1.11] p=0.003) and with a slight increase in cardiovascular death (3.4 vs 3.1%, 1.1 [1.03-1.17] p=0.003).
Conclusion	Beta-carotene increases the risk of CVD and death.
Relevance for our risk assessment purpose	Four of the eight RCTs included smokers and/or asbestos workers. Emphasis should be put on the four studies without.

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