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Norwegian Scientific Committee for Food Safety

VKM Report 2015: 05

Risk assessment of folic acid 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).

Acknowledgment

The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of VKM members to answer the request from the Norwegian Food Safety Authority. Project leader from the VKM secretariat has been Bente Mangschou. The members of the working group Margaretha Haugen, Wenche Frølich, Tor A Strand and Grethe S Tell are acknowledged for their valuable work on this opinion.

Competence of VKM experts

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

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Summary

The Norwegian Scientific Committee for Food Safety (VKM) received a request from the Norwegian Food Safety Authority to assess whether the Tolerable Upper Intake Level (UL) of folic acid should be amended in light of new scientific evidence suggesting a possible link between high intake levels of folic acid and risk of cancer. Folic acid obtained from both food supplements and fortified foods should be assessed. Folic acid is a synthetic form of folate and is commonly used in food supplements and in food fortification because of its stability and bioavailability. Folic acid is reduced, methylated and released into the circulation. Natural folates occur in reduced forms, e.g. as tetrahydrofolate (THF), which are unstable and may thus loose biochemical activity during harvesting, storage, processing, and preparation.

Folate is cofactor for enzymes in one-carbon metabolism where it provides one-carbon units for the formation of RNA and DNA. Folate is also essential for the normal functioning of the methionine cycle, which is responsible for both the conversion of homocysteine to methionine and the production of the universal methyl donor S-adenosylmethionine (SAM). SAM donates its methyl group to more than 100 methyltransferases for a wide range of substrates such as DNA, hormones, proteins, neurotransmitters and membrane phospholipids.

While no Tolerable Upper Intake Level (UL) has been derived for natural dietary folates, the Scientific Committee on Food (SCF, 2000) set an UL of 1000 µg folic acid per day in 2000. The UL was set because it was found that high intakes of folic acid could correct megaloblastic anemia, which is the hallmark manifestation of vitamin B_{12} deficiency. In this way folic acid may mask vitamin B_{12} deficiency which again may cause irreversible neurological damage. The ULs for children and adolescents (1-17 years) were adjusted on the basis of body weight and range from 200 to 800 µg/day. The UL for folic acid has been reassessed by other authorities, most recently in 2009 by EFSA (EFSA, 2009), who upheld an UL of 1 mg/day.

In 2009, Ebbing et al. published results from combined analyses of two randomised, placebo-controlled trials with B-vitamin supplementation in patients with ischemic heart disease. They found an increased risk of cancer in those who were randomised to receive folic acid in combination with vitamin B_{12} (Ebbing et al., 2009). Folates are important for cell division. It is therefore possible that tumor growth or growth of premalignant cells may be stimulated by high concentrations of folate in the blood. Another concern with use of folic acid is circulating unmetabolised folic acid (UMFA) which is often found at intakes of more than 200 µg per day (Kelly et al., 1997). It has been argued that UMFA could affect homeostatic regulation of folate (Smith et al., 2008), and that it may reduce natural-killer cell cytotoxicity (Troen et al., 2006). In vitro studies have also demonstrated that folic acid can down-regulate tumor suppressor genes (Lubecka-Pietruszewska et al., 2013).

This opinion addresses the question whether the current UL for folic acid should be amended based on new scientific evidence. Furthermore, VKM has been requested to estimate folic acid intake from food supplements and from foods that are fortified with folic acid, in all age groups in the population above 1 year. In addition, possible consequences of amending the maximum limit for folic acid in food supplements should be discussed.

In the literature search for this opinion (articles published from 2009 to 15 October 2014 were obtained), we found eight meta-analyses and five single studies where the aims were to study the relation between folic acid supplementation and incidence of cancer. Meta-analyses including studies in which folic acid was used in combination with other supplements were not included in the final evaluation, as the effect of folic acid could not be distinguished from the effect of the other substances. Only one meta-analysis was therefore considered relevant for the evaluation of UL for folic acid; a meta-analysis of patients with colorectal adenomas who received 1 mg folic acid per day for 3-6 years (Figueiredo et al., 2011). No increased risk of colorectal adenomas or cancer was found in this meta-analysis. Nor did the included single studies report increased risk of colorectal cancer following folic acid supplementation (Gao et al., 2013; Wu et al., 2009).

Brain tumor and childhood leukemia were investigated in two case-control studies in offspring of women using folic acid supplementation during pregnancy (Amigou et al., 2012; Milne et al., 2012). Both studies indicated no negative effects of folic acid supplementation during pregnancy.

A secondary analysis of one of the single studies on colorectal adenomas found a statistically significant increased risk of prostate cancer following folic acid supplementation (Figueiredo et al., 2009). However, the small number of prostate cancer cases in this single study does not make this result robust.

In six studies circulating UMFA was reported among subjects who used folic acid supplements or who were subjected to folic acid food fortification. Whether UMFA contributes to the development of cancer or other undesirable health effects is not known. These studies do not provide new evidence for amending the existing UL for folic acid.

About 26% of women and 18% of men aged 18-70 years participating in the nationwide dietary survey Norkost 3, reported to take folic acid supplements. The mean intake of folic acid among users was 149 µg/day among women and 172 µg/day among men. Among pregnant women participating in The Norwegian Mother and Child Cohort Study, 62% reported use of folic acid supplements in 2008. Mean folic acid intake was 388 µg/day, and the 95th percentile was 800 µg/day. Information on intake of folic acid from supplements for other age groups is not available.

Intake of folic acid from fortified foods is not available in the national food consumption surveys for any age groups. However, according to a Norwegian model for food fortification from 2006 and later updates (last update in 2013), 53 µg folic acid per 100 kcal can be added to food and drinks without exceeding the UL for folic acid in any age groups. With the

current levels of folic acid in food supplements and current levels in fortified products, the UL for folic acid will not be exceeded.

VKM was also requested to assess the impact of any increase of the current maximum limit of 200 µg for folic acid in supplements. Increasing the maximum limits in food supplements to 400 µg will imply exceedance of UL for children younger than 6 years and an intake close to UL in children 7-10 years. An increase in the maximum limits in food supplements to 600 µg will imply exceedance of UL for children younger than 10 years and an intake close to UL in children 11-14 years. Increasing the maximum limits in food supplements to 400 µg or 600 µg will not imply exceedance of UL among adults as evaluated in the Norwegian food fortification model (VKM, 2013).

No new evidence for increased risk of cancer related to folic acid was found in the reviewed literature. Studies in subjects who had a history of colorectal adenomas, a group considered particularly vulnerable to develop cancer, reported no increased risk of colon cancer or adenomas after 3 -5 years of treatment with 1000 µg of folic acid per day. VKM therefore concludes that studies published after 2009 and until 15 October 2014 examining cancer do not provide support to alter the existing UL for folic acid.

Key words: Folic acid, tolerable upper intake level, UL, risk assessment, VKM, Norwegian Scientific Committee for Food Safety.

Sammendrag på norsk

Vitenskapskomiteen for mattrygghet (VKM) har på oppdrag fra Mattilsynet blitt bedt om å vurdere øvre trygt inntaksnivå (Tolerable Upper Intake Level; UL) for folsyre i lys av ny forskning som har antydet at det kan være en sammenheng mellom høyt inntak av folsyre og kreft. Folsyre er en syntetisk form for folat, og blir brukt i kosttilskudd og i berikning av mat og drikke fordi det er mer stabilt og absorberes bedre enn naturlige folater. I kroppen blir folsyre redusert, metylert og frigjort til sirkulasjonen. Naturlige folater forekommer i redusert form som tetrahydrofolat (THF), de er ustabile og blir lett nedbrutt ved innhøsting, lagring og tilberedning av matvarer.

Folater overfører en-karbon-enheter i sentrale biokjemiske reaksjoner som aminosyrestoffskiftet og syntesen av puriner og pyrimidiner. Folat er viktig for normal funksjon i metioninsyklusen der homocystein omdannes til metionin, og i produksjon av den universelle metyldonoren S-adenosylmethionine (SAM). SAM donerer sin metylgruppe til mer enn 100 metyltransferaser for en lang rekke substrater, herunder DNA, hormoner, proteiner, nevrotransmittere og membranfosfolipider.

Det er ikke fastsatt UL for naturlige folater, men for folsyre fastsatte EUs vitenskapskomite for næringsmidler (Scientific Committee on Food) i 2000 en UL på 1000 µg per dag (SCF, 2000). Denne UL er basert på at et høyt inntak av folsyre kan korrigere megaloblastisk anemi som er et symptom på vitamin B_{12}-mangel. På denne måten kan høye nivåer av folsyre maskere vitamin B_{12}-mangel. Dette kan blant annet medføre at mangelen vedvarer, noe som igjen kan forårsake irreversible nevrologiske skader. UL for barn og ungdom (1-17 år) ble justert på grunnlag av kroppsvekt til 200-800 µg/dag. UL for folsyre er revurdert av ulike andre myndigheter, senest i 2009 av EFSA, som opprettholdt UL på 1000 µg/dag (EFSA, 2009).

I kombinerte analyser av to randomiserte studier på pasienter med iskemisk hjertesykdom ble det rapportert om økt forekomst av kreft hos de som ble randomisert til behandling med folsyretilskudd i kombinasjon med vitamin B_{12} (Ebbing et al., 2009). Folater er viktig for celledeling, og dermed vil tumorvekst og vekst av premaligne celler kunne bli stimulert ved høye folatkonsentrasjoner. Ikke-metabolisert folsyre (unmetabolised folic acid; UMFA) er påvist i blod ved inntak av folsyre fra beriket mat og/eller ved bruk av folsyretilskudd på over 200 µg per dag (Kelly et al., 1997). UMFA kan påvirke den homeostatiske reguleringen av folat (Smith et al., 2008) og kan hemme cytotoksiske lymfocytter (Troen et al., 2006). In vitro-studier har også vist at folsyre kan nedregulere tumor suppressorgener (Lubecka-Pietruszewska et al., 2013).

Denne risikovurderingen er basert på en gjennomgang av vitenskapelig litteratur publisert etter 2009 for å se om det er behov for å endre den eksisterende UL for folsyre. Videre er VKM bedt om å beregne inntak av folsyre fra kosttilskudd og berikede produkter i alle

aldersgrupper i befolkningen over 1 år, og om å vurdere eventuelle helseskadelige konsekvenser av å endre den eksisterende maksimumsgrensen for folsyre i kosttilskudd.

I litteratursøket (fra 2009 til 15 oktober 2014), ble det funnet åtte metaanalyser og fem enkeltstudier, som alle undersøkte sammenhenger mellom folsyretilskudd og forekomst av kreft. Sju av de åtte metaanalysene inkluderte studier som ga tilskudd med flere vitaminer i kombinasjon. Disse metaanalysene er ikke tillagt vekt i den endelige evalueringen av UL ettersom en eventuell effekt fra folsyre ikke kan skilles fra de andre vitaminene. Den ene metaanalysen med bare rene folsyreintervensjoner fant ingen økt risiko for tykktarmsadenomer (godartet epitelcellesvulst) eller tykktarmskreft etter 3-6 år med 1 mg folsyretilskudd blant pasienter allerede diagnostisert med tykktarmsadenomer (Figueiredo et al., 2011). Tilsvarende resultater ble rapporter i to randomiserte studier der intervensjonsgruppene ble gitt opp til 1 mg folsyre per dag uten økt risiko for tykktarmskreft (Gao et al., 2013; Wu et al., 2009).

Forekomst av hjernesvulst og leukemi hos barn av mødre som hadde tatt folsyretilskudd i svangerskapet ble undersøkt i to kasus-kontrollstudier (Amigou et al., 2012; Milne et al., 2012). Det ble ikke rapportert om negative helseeffekter i disse studiene.

I en sekundæranalyse av et randomisert klinisk forsøk på pasienter med tykktarmsadenomer ble det funnet en statistisk signifikant økt risiko for prostatakreft ved bruk av folsyretilskudd (Figueiredo et al., 2009). Imidlertid er ikke resultatet fra denne ene studien robust på grunn av få prostatakrefttilfeller totalt sett.

I seks studier ble det rapportert om sirkulerende UMFA hos individer som brukte folsyretilskudd eller mat beriket med folsyre. I hvilken grad UMFA bidrar til utvikling av kreft eller andre uønskede helseeffekter, er fortsatt uavklart. Disse studiene gir heller ikke grunnlag for å endre eksisterende UL for folsyre.

Ca. 26 % av kvinnene og 18 % av mennene i en landsomfattende kostholdsundersøkelse blant norske menn og kvinner 18-70 år (Norkost 3) rapporterte å ta kosttilskudd med folsyre. Gjennomsnittlig inntak av folsyre fra tilskudd blant de som rapporterte slik bruk var 149 µg per dag hos kvinner og 172 µg per dag hos menn. I Den norske mor og barn-undersøkelsen rapporterte 62 % av gravide kvinner i 2008 at de brukte folsyretilskudd. Gjennomsnittlig folsyreinntak var 388 µg, og 95 % tok under 800 µg/dag. Informasjon om inntak av folsyre fra kosttilskudd er ikke tilgjengelig for andre aldersgrupper.

Informasjon om inntak av folsyre fra berikede matvarer er ikke tilgjengelig. I følge en norsk modell for vurdering av berikning fra 2006 med senere oppdateringer (sist oppdatert i 2013), kan det tilsettes 53 µg folsyre per 100 kcal mat og/eller drikke uten at det vil føre til overskridelse av UL for folsyre i noen aldersgrupper. Med dagens nivåer i kosttilskudd og dagens nivåer i berikede produkter vil ikke UL for folsyre overskrides.

VKM ble også bedt om å vurdere konsekvensene av en eventuell økning av gjeldende maksimumsgrense på 200 µg for folsyre i kosttilskudd. Dersom maksimumsgrensene økes til

400 µg, vil det kunne medføre en overskridelse av UL for barn under 6 år, og et inntak tett opp til UL for barn i aldersgruppen 7 - 10 år. Dersom maksimumsgrensene for folsyre i kosttilskudd økes til 600 µg, vil det kunne medføre en overskridelse av UL for barn under 10 år og tett opp til UL for barn 11 - 14 år. En økning av maksimumsgrensen for folsyre i kosttilskudd til 400 µg eller 600 µg vil ikke medføre en overskridelse av UL for den voksne befolkningen. Disse beregningene er basert på den norske modellen for vurdering av berikningssaker (VKM, 2013).

Det er altså ikke vist noen sikker sammenheng mellom folsyretilskudd og kreft i den gjennomgåtte litteraturen. Folsyretilskudd er gitt til pasienter som på forhånd var behandlet for tykktarmsadenomer. Hos denne gruppen, som anses som ekstra sårbar for utvikling av kreft, fant man ingen økt risiko for tykktarmskreft eller nye adenomer i tykktarmen etter 3 -5 års behandling med 1000 µg folsyre per dag. Derfor konkluderer VKM med at studier som har undersøkt sammenhengen folsyre og kreft etter 2009 og fram til oktober 2014 ikke gir grunnlag for å endre eksisterende UL for folsyre.

Abbreviations and glossary

5-MTHF – 5-methyl tetra hydrofolate
CI – confidence interval
CNS – central nervous system
CVD – cardiovascular disease
DR – dietary reference value
EAR – estimated average requirement (EAR+2SD=recommended intake)
EFSA – European Food Safety Authority
ESCO – EFSA Scientific Cooperation Working Group
FFQ – food frequency questionnaire
HR – hazard ratio
IOM – Institute of medicine
IQR – interquartile range
LOAEL – lowest observed adverse effect level
MoBa – The Norwegian Mother and Child cohort study
NHANES – National Health and Nutrition Examination Survey
NNR – Nordic nutrition recommendations
NOAEL – no observed adverse effect level
NTD – neural tube defect
OR – odds ratio
RCT – randomised controlled trial
RBC – red blood cell
RR – risk ratio or relative risk
SAM – S-adenosylmethionine
SC – Scientific Committee on Food
SD – standard deviation
THF – tetra hydrofolate
UL – tolerable upper intake level
UMFA – unmetabolised folic acid
VKM – Norwegian Scientific Committee For Food Safety

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.

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.

The Norwegian Food Safety Authority is currently evaluating the national maximum limit for folic acid, considering the criteria listed above.

Pursuant to the Norwegian regulation on food supplements, the minimum and maximum limit for folic acid in food supplements is 25 µg and 200 µg per daily dose, respectively. Exemptions for using 400 µg folic acid per daily dose have been given provided certain labelling requirements are fulfilled. The exemptions are based on a recommendation issued by the Norwegian Directorate of Health, saying that women who might become pregnant should supplement their diet with 400 µg folic acid per day.

Pursuant to the food supplement regulation, only one form of folic acid may be used in food supplements; pteroylmonoglutamic acid. Also calcium-L-methylfolate may be used in food supplements as a folate source. The terms of reference in this opinion addresses only folic acid.

Relevant background documents

1. Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level on Folate (SCF, 2000)
2. Folic acid: An update on scientific developments (EFSA, 2010)
3. ESCO report on Analysis of Risks and Benefits of Fortification of Food with Folic Acid (EFSA, 2009)
4. Cancer incidence and mortality after treatment with folic acid and vitamin B_{12} (Ebbing et al., 2009)
5. Safe Upper Levels for Vitamins and Minerals, UK (EVM, 2003)
6. A safe strategy for addition of vitamins and minerals to foods (Rasmussen et al., 2006)

Terms of reference as provided by the Norwegian Food Safety Authority/ Norwegian Environment Agency

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

1. In 2000, the Scientific Committee on Food (SCF) set a tolerable upper intake level (UL) for folic acid of 1 mg/day for adults. ULs for adolescents and children were adjusted downwards on the basis of body weight. Subsequently, new scientific evidence has emerged suggesting a possible link between high intake levels of folic acid and risk of cancer (Ebbing et al., 2009; EFSA, 2009). In the light of the latest scientific data on folic acid, should the UL be amended?
2. It is important to ensure that the total intake of vitamins and minerals from all sources does not exceed the UL. However, folic acid is the synthetic form of the vitamin which is not found naturally in foods. VKM is therefore requested to estimate the intake of folic acid from food supplements and foods that are added folic acid, in all age groups in the population above 1 year.
3. VKM is requested to elucidate the consequence of amending the maximum limit for folic acid in food supplements for the total intake of folic acid (from food supplements and foods added folic acid).

Assessment

1 Introduction

Folic acid supplementation and fortification of food with folic acid have substantially reduced the incidence of neural tube defects (NTD)(Botto et al., 2005; Williams et al., 2002). However, there are indications that high intake of folic acid may increase the risk of cancer, cause cognitive decline in susceptible populations, and result in epigenetic changes (Cole et al., 2007; Ebbing et al., 2009; Kim et al., 2001).

Folic acid is a synthetic form of folate and is commonly used in food supplements and in food fortification because of its stability and bioavailability. In Norway, food fortification is not mandatory and foods that are fortified with folic acid do not contribute much to the total intake of folic acid. Folic acid supplementation has been recommended before and during early pregnancy from 1998 but despite of this only 19.8% of the women participating in the Norwegian Mother and Child Cohort study used folic acid supplementation one month before becoming pregnant and less than half of the women used folic acid supplements at gestational month three (Nilsen et al., 2006).

Possible negative health effects from high intakes of folic acid have previously been evaluated by the Scientific Committee for Food (SCF, 2000), Institute of Medicine (IOM, 2000), Nordic Nutrition Recommendations (NNR Project Group, 2012) and European Food Safety Authority (EFSA, 2009; EFSA, 2014).

With intakes of more than 200 µg folic acid unmetabolised folic acid (UMFA) has been recovered in the circulation (Kelly et al., 1997). It has been postulated that UMFA could affect homeostatic regulation of folate (Smith et al., 2008), and reduce natural-killer cell cytotoxicity, which raises the question about UMFA and cancer risk (Troen et al., 2006).

Recently, EFSA published Dietary Reference Values (DRVs) for folate (EFSA, 2014). EFSA states that natural food folates are safe and high intakes have not been associated with any adverse effects. The aim of this report is therefore to evaluate the Tolerable Upper Intake Level for supplemental folic acid.

Adverse effect of folic acid supplementation and/or fortification has so far been reported on cancer development. Therefore and in accordance with terms of reference, cancer development is the evaluated outcome in this report.

2 Hazard identification and characterisation

2.1 Chemistry, absorption and metabolism

Chemistry

Folates encompass several forms of the vitamin which are different with regard to stability and bioavailability. We use the term “folic acid” for the most commonly occurring synthetic form and “folate” for the folates naturally found in foods. Folic acid and folates comprise an aromatic pteridine ring linked to a p-aminobenzoic acid and at least one glutamate residue (Figure 2.1-1). About half of the folate in the body is stored in the liver.

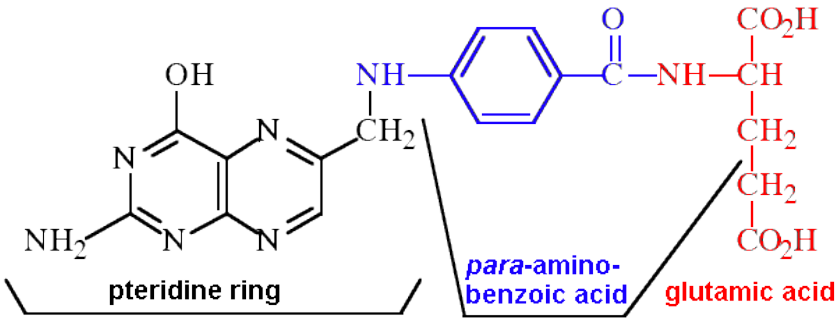

Figure 2.1-1 Structural form folic acid.

Function

Folate is cofactor for enzymes in one-carbon metabolism, where it provides one-carbon units for the formation of RNA and DNA. Folate is also essential for the normal functioning of the methionine cycle, which is responsible for both the conversion of homocysteine to methionine and the production of the universal methyl donor S-adenosylmethionine (SAM). SAM donates its methyl group to more than 100 methyltransferases for a wide range of substrates such as DNA, hormones, proteins, neurotransmitters and membrane phospholipids.

Occurrence and recommendations

Folate is present in most foods. High concentrations are found in liver, green vegetables and legumes. The most important food groups contributing to folate intake are whole grain cereals and vegetables, however, dairy products, fruits and berries are also important sources. Based on losses through urine and the enterohepatic circulation in well-nourished individuals, the estimated average requirement of folate is set to 200 µg/day and recommended intake to 300 µg/day. For children 6 to 9 years the recommended intake is

130 µg/day and for children and adolescents aged 10 to 13 years 200 µg/day. For women in reproductive ages supplementation of 400 µg/day is recommended in addition to foods rich in folate since there is convincing evidence that an adequate supply of folate before and up to 12 weeks after conception reduces the risk of NTD. To ensure folate intake during pregnancy, women are recommended to take a folic acid supplement one month before conception and during the first three months into pregnancy to reduce the risk of NTD (Czeizel and Dudas, 1992; ernæringsråd, 1997; Tell et al., 1998).

Intake and folate status

According to Norwegian dietary surveys the mean dietary intake of folate is within the estimated average requirement (EAR), but does not reach the recommended intake (NORKOST 3; mean intake 279 µg/day (SD 105) for men and 231 µg/day (SD 86) in women). No information about folate intake is available for children and adolescents. However, in 2-year old Norwegian children, the median folate intake from food was 87 µg/day (IQR: 74-106) and the median plasma folate concentration was 19 nmol/L (IQR 14-24) (Hay et al., 2011). The Panel on Dietetic Products, Nutrition and Allergy (NDA) in the European Food Safety Authority (EFSA) considered that the previously defined cut offs for an adequate folate status are still suitable for determining folate requirement (for serum folate 10 nmol/L and for red blood cell folate of 340 nmol/L) (EFSA, 2014). Data from Norway indicate that the mean value of serum folate was below the value considered adequate by EFSA (Dhonukshe-Rutten et al., 2009) and in pregnant women only those using regular folic acid supplementation reached an adequate folate status (Bjorke-Monsen et al., 2013).

Absorption and metabolism

Natural folates rapidly lose activity in foods during harvesting, storage and preparation and up to 75% of folate activity may be lost before ingestion, while folic acid is almost completely stable for months or even years (EFSA, 2014). Folic acid is readily absorbed, but has to be reduced (or "metabolised") before it can enter the metabolic pathway. For naturally occurring folates, one or more glutamate residues have to be removed before absorption can occur. The absorption of folate and the conversion of folic acid have both limited capacity. Excess natural folate may accordingly result in a lower fraction absorbed and excess folic acid may result in unmetabolised folic acid in the circulation. Virtually all folate in plasma is of the reduced and methylated form 5-methyl tetra hydrofolate (5-MHTF). This is also a form that can donate one-carbon units in metabolic processes.

There is evidence that circulating unmetabolised folic acid impairs natural killer cell function and cell-mediated toxicity. Cell-mediated immunity may be important in the protection against the formation of malignant tumors and cancer development. A recent *in vitro* study using breast cancer cell lines also demonstrated a dose dependent relationship between down-regulation of tumor suppressor genes and folic acid concentration, suggesting an alternate mechanism whereby folic acid could contribute to tumor formation (Lubecka-Pietruszewska et al., 2013). Results from large, randomised placebo controlled trials with

folic acid supplementation also indicate that folic acid may increase cancer risk (Bonaa et al., 2006; Cole et al., 2007; Ebbing et al., 2009).

Folate deficiency and high intakes

The predominant feature of folate deficiency is megaloblastic anemia (SCF, 2000). Folates and vitamin B_{12} share metabolic pathways and are important for DNA and protein synthesis and therefore cell growth and differentiation. Poor intake of either folate or vitamin B_{12} often has similar health consequences such as anemia. A high intake of folic acid may mask some of the early symptoms of vitamin B_{12} deficiency. Vitamin B_{12} deficiency may lead to neurological dysfunction and decline in cognitive function, which in some cases are irreversible. Intake of natural folate has so far not been found to mask vitamin B_{12} deficiency. Knowledge built on folic acid supplementation in B_{12} deficient subjects resulted in an Upper Tolerable Intake Level (UL) of 1 mg of folic acid per day, set by the Scientific Committee on Food (SCF) in 2000 (SCF, 2000).

2.2 Mechanisms for cancer development

As mentioned above, unmetabolised folic acid can attenuate natural killer cell cytotoxicity which is an important part of the nonspecific immune response. In vitro studies have also demonstrated that folic acid can down-regulate tumor suppressor genes (Lubecka-Pietruszewska et al., 2013).

Folates are important for cell division. It is therefore possible that tumor growth or growth of premalignant cells may be stimulated by high concentrations of folate in the blood. Many cytostatic drugs target folate metabolism in order to reduce tumor growth. It is possible that folate plays a dual role in carcinogenesis; prevention of early lesions but enhancing growth once preneoplastic lesions have developed.

2.3 Previous reports on UL for folic acid

Possible negative health effects from high intakes of folic acid have previously been evaluated by several international bodies. Main findings from these are presented below. In several publications the terms "folate" and "folic acid" are used inaccurately. In the following sections, we have used the same terminology as those used in the reports that are reviewed.

2.3.1 Scientific Committee on Food, 2000

The Opinion of the Scientific Committee on Food on the Tolerable Upper Intake Level of folic acid was published in 2000 by the Scientific Committee on Food (SCF) under the Health and Consumer Protection Directorate – General of the European Commission (SCF, 2000).

This report discusses the various forms of folate and the potential risks of high intake as well as the risks involved by folic acid supplementation/fortification.

Potential health risks are discussed in light of the estimated folate intake in five countries (Austria, Germany, Ireland, Italy and the Netherlands). The intake in Europe is remarkably similar across different countries, and seemingly adequate except in women with a wish to become pregnant. The recommended intake of folate among women planning for pregnancy is 400 µg per day, however, >90% of women in the childbearing age had an intake lower than this.

The ”nutritional background” of the report focuses on differences between naturally occurring folates in foods versus the synthetic folic acid found in supplements and fortified food. Folic acid, which is an oxidised folate, is more stable and more readily absorbed than naturally occurring folates.

Potential adverse effects of folic acid supplementation:

1. modification of vitamin B_{12} deficiency symptoms due to folic acid supplementation:
    a. masking of hematological symptoms,
    b. exacerbation of neurological symptoms;
2. epileptogenic and neurotoxic effects of folic acid;
3. decreased efficacy of folate antagonists used in chemotherapy;
4. carcinogenicity

Vitamin B_{12} deficiency and neurotoxicity. Folate and vitamin B_{12} share metabolic pathways and are important for DNA and protein synthesis and therefore cell growth and differentiation. Deficiencies of both vitamins result in macrocytic anemia. A high intake of folic acid can delay the onset of macrocytic anemia caused by vitamin B_{12} deficiency and accordingly mask the symptoms of B_{12} deficiency. Vitamin B_{12} is important for brain function and for the developing nervous system and deficiency has been associated with decreased cognitive performance and neurological manifestations. There are indications that folic acid in high doses has a neurotoxic effect particularly among vitamin B_{12} deficient individuals. Results from the studies suggesting that folic acid supplementation mask B_{12} deficiency and/or has a neurotoxic effect are conflicting, small, use high doses of folic acid (5-100 mg daily), and are old (> 50-60 years). More recent reviews have concluded that there is no convincing harmful effect on the central nervous system (CNS) of folic acid, even in individuals who are vitamin B_{12} deficient.

Antifolate drugs, such as methotrexate, are commonly used and folates may potentially attenuate the effect of these. To what extent folic acid supplementation modifies the effects of these drugs is not clear.

Cancer. Intake of folic acid is positively associated with some cancers, but adequate folate status may also protect against other cancers.

The report concludes as follows:“Although no systematic toxicological studies of folic acid or other folates are available, an upper safe level can be set for folic acid on the basis of findings in vitamin B_{12} deficient patients treated with high doses of folic acid. There is no

evidence for risk associated with high intakes of natural, reduced folates, and thus no data to set an upper limit for natural folate. Although there is no conclusive evidence in humans, the Committee concluded that the risk of progression of the neurological symptoms in vitamin B_{12}-deficient patients as a result of folic acid supplementation cannot be excluded and should be considered the most serious adverse effect. In nearly all studies showing neurological relapse, dose levels >5 mg folic acid per day have been applied and data on the effect of dose levels between 1-5 mg is limited to a few cases."

In analogy with the US DRI Committee, it is concluded that the best available estimate for a lowest-observed-adverse-effect level (LOAEL), obtained from a sensitive group, for folic acid is 5 mg, and as dosages up to 1 mg of folic acid are unlikely to cause masking of the hematological signs in patients with pernicious anemia, the UL is set at 1 mg of folic acid." Suggested ULs for folic acid from SCF is given in Table 2.3.1-1.

Table 2.3.1-1 ULs for folic acid in SCF, 2000 in µg/day.

Age	UL (µg)
1-3	200
4-6	300
7-10	400
11-14	600
15-17	800
Adults	1000

The SCF report recommended more research on the effects of high folate on the "symptomatology of vitamin B_{12} deficiency", and the risk of folic acid supplementation in populations where vitamin B_{12} deficiency is prevalent. They also recommended studies on the safety and efficacy of other synthetic folates.

2.3.2 Institute of Medicine, 2000

In 2000, the United States Institute of Medicine (IOM) published an evaluation of UL for folate (IOM, 2000). No observations of adverse effects of high intake of folate (dietary) from traditional foods or fortified foods were found, and the evaluation is limited to evidence concerning intake of supplemented folic acid. In the hazard identification chapter, the IOM describes the association between high doses of folic acid that may correct megaloblastic anemia. At the same time it may mask vitamin B_{12} deficiency and the neurological damage may not be diagnosed until these neurological consequences have become irreversible. High doses of folic acid could precipitate or exacerbate the anemia and cognitive symptoms associated with vitamin B_{12} deficiency, perhaps by increasing homocysteine or methylmalonic acid concentrations. However, the high homocysteine and methylmalonic acid concentrations in people with low vitamin B_{12} and high folate concentrations could be due to severe malabsorptive conditions or pernicious anemia rather than high folic acid intakes. The high folate concentrations in vitamin B_{12} deficient individuals are probably due to the folate trap

mechanism. Neurological effects are mostly seen in B_{12} deficient subjects, such as older persons and those adhering to a vegan diet.

Concerns were raised that high folic acid supplementation might accelerate the progression of preneoplastic lesions, increasing the risk of colorectal and possibly other forms of cancer in certain individuals.

Based on the metabolic interactions between folate and vitamin B_{12}, IOM established a UL for adults, pregnant and lactating women for the synthetic forms of folate (i.e., folic acid) available in dietary supplements and fortified foods. The UL for infants and children were adjusted from the adult value on the basis of relative body weight. The ULs do not apply to individuals taking high doses of folic acid under medical supervision. Suggested ULs for folic acid from the IOM is provided in Table 2.3.2-1.

Table 2.3.2-1 ULs for folic acid in IOM (2000) in µg/day.

Age	Men	Women	Pregnancy	Lactation
Birth to 6 months	Not possible to establish*	Not possible to establish*		
7-12 months	Not possible to establish*	Not possible to establish*		
1-3 years	300	300		
4-8 years	400	400		
9-13 years	600	600		
14-18 years	800	800	800	800
19+ years	1000	1000	1000	1000

*Breastmilk, formula, and food should be the only sources of folate for infants.

2.3.3 ESCO Working Group (EFSA), 2009

The ESCO Report from Analysis of Risks and Benefits of Fortification of Food with Folic Acid (2009) was prepared by the EFSA Scientific Cooperation Working Group on Analysis of Risks and Benefits of Fortification of Food with Folic Acid (EFSA, 2009).

The EFSA Scientific Cooperation Working Group (ESCO WG) on “Analysis of risks and benefits of fortification of food with folic acid” was established by EFSA in 2008. The group was asked to i) review current practice in the Member States regarding the level of voluntary fortification of foods with folic acid and ii) consider new evidence regarding the risk of high intakes of folic acid and the need to review current guidance on safe upper levels of folic acid for all population groups.

The ESCO report concluded that the beneficial effect of folic acid in reducing the risk of NTDs was well established.

Prevalence of NTD-affected pregnancies ranges from 4.1 to 19.7 per 10 000 births. Parts of this variation can be explained by different methods of data collection and reporting.

Although suggestions for additional beneficial effects of folic acid supplementation including reduction in cardiovascular disease (CVD), cancer and cognitive decline have been made, evidence for such benefits has so far not been supported by randomised controlled trials (RCTs).

National recommendations/reference values for folate intake varied across the European countries from 200 µg to 400 µg folate per day for adults. A recommendation of an additional 400 µg per day was recommended for women of childbearing age/planning a pregnancy.

Available data indicate that across European countries, the average dietary folate intake ranges from 151 to 345 µg/day for men and from 122 to 339 µg/day for women. Average intake of folate from diet and supplements ranges from 338 to 385 µg/day for men and from 220 to 478 µg/day for women. No European country had introduced mandatory fortification.

The report also underlined the importance of distinguishing between different sources of folic acid (from supplements and fortified foods) as pharmacokinetics may vary depending on form and dosage.

Available animal studies suggested a possible association between high intakes of folic acid and promotion of cancer development and progression. A study from USA and Canada suggested that colorectal cancer incidence increased at around the same time that mandatory fortification with folic acid was introduced.

The totality of evidence from RCTs up to the date of the ESCO report did not suggest that folic acid intakes were associated with increased cancer risk. The report stressed however that the interpretation of the data from the available studies were limited by a number of issues including the duration of the trials and limited power of the meta-analyses.

In the ESCO report it was concluded that intakes of folic acid should not exceed the established UL of 1 mg/day (SCF, 2000). The UL was based on limited data. If further data become available, it was underlined that UL needed to be discussed further.

2.3.4 EFSA meeting summary report

EFSA and the Swedish National Food Administration organised a scientific meeting on “Folic Acid: An update on Scientific Developments” in Uppsala, Sweden on 21-22 January 2009 (EFSA, 2010). The aim of the meeting was to consider the evidence regarding folic acid and cancer risks, including cancer of the colon, breast and prostate.

Scientific evidence concerning folate metabolism, animal and mechanistic studies and human studies was reviewed and discussed. The experts should consider if it was possible to

identify an association of folic acid intake with risk of cancer and whether the available data were sufficient to allow quantitative risk assessment. The conclusions from the meeting were:

• There was a disagreement between experts regarding the interpretation of the trial evidence. The UL of 1 mg/day, which is based on limited data, may need to be revised when further data become available.

• There are currently insufficient data to allow a full quantitative risk assessment of folic acid and cancer or to determine whether there is a dose-response relationship or a threshold level of folic acid intake associated with potential colorectal cancer risk.

• The current evidence does not show an association between high folic acid intakes and cancer risk, but neither do they confidently exclude a risk.

• It is important to distinguish between different sources of folate, i.e. natural food folate and folic acid from fortified foods and from supplements.

• Setting maximum safe levels for the amount of folic acid that can be added to foods voluntarily fortified with folic acid and supplements will be important in ensuring that consumption of foods fortified with folic acid and folic acid supplements does not lead to intakes above the UL.

2.3.5 Nordic Nutrition Recommendations 5th edition, 2012

In the Nordic Nutrition Recommendations (NNR) from 2012 it is stated that there is no evidence for adverse health effects associated with high intakes of folate from natural sources, but high intake of folic acid can mask hematological symptoms caused by deficiency of vitamin B_{12} (NNR Project Group, 2012). Suggested ULs for folic acid from NNR is given in Table 2.3.5-1.

Table 2.3.5-1 ULs for folic acid from NNR (2012) in µg/day.

Age	Men/Women	Pregnancy	Lactation
1-3 years	200		
4-6 years	300		
7-10 years	400		
11-14 years	600		
15-17 years	800		
18 + years	1000	1000	1000

2.3.6 Dietary reference value (DRV) EFSA, 2014

Recently, EFSA published Dietary Reference Values (DRVs) for folate (EFSA, 2014). In this opinion, EFSA states that natural folates are safe and that high intakes have not been associated with any adverse effects (SCF, 2000). The Tolerable Upper Intake Level (UL) was

set by SCF based on safety concerns for high intake of the synthetic form of the vitamin, i.e. folic acid and its ability to mask vitamin B_{12} deficiency by reversing megaloblastic anemia. In this way high folic acid intake may allow the neurological dysfunction of B_{12} deficiency to progress to irreversible subacute degeneration of the spinal cord. To set the UL it is referred to the SCF report of 2000 i.e. UL to 1000 µg per day for adults and ranging from 200 µg/day (1-3 years) to 800 µg/day (15-17 years).

The importance of folic acid as protective or as enhancing in cancer development was further discussed referring to observational studies which have suggested that folic acid supplement use was inversely associated with cancer incidence (Ericson et al., 2007; Giovannucci et al., 1998). However, safety concerns were expressed with the publication of two studies suggesting that chronic ingestion of folic acid at doses of 1 mg/day or above might increase the risk of colorectal neoplasia in individuals with a recent history of colorectal adenomas (Cole et al., 2007) or increase the risk of development of prostate cancer, stimulating proliferation of already established neoplastic foci in the colorectal mucosa (Kim, 2004). The EFSA Panel further refers to the meta-analyses of Vollset et al. (2013) and to Mackerras et al. (2014) showing that folic acid supplementation at a median dose of 2 mg/day and administered with or without other B-vitamins for an average duration of 5.2 and 7.3 years did not significantly increase the overall or site-specific cancer incidence compared with placebo. The EFSA Panel notes that the follow-up period of the trials included in these meta-analyses were rather short considering the development of cancer. Thus, the question of the relationship between folic acid and cancer requires to be clarified by studies designed with sufficiently long follow-up periods addressing the biological hypothesis for the dual effect of folic acid on cancer development (EFSA, 2009).

Concerns were also raised regarding the potential adverse effects associated with the presence of unmetabolised folic acid in the circulation. Three studies from Europe (Boilson et al., 2012; Obeid et al., 2010; Sweeney et al., 2009) and a nationally representative study from the US (Bailey et al., 2010) reported that a considerable proportion (40-90%) of the investigated populations exposed to fortified foods and involving both supplement and non-supplement users had a detectable concentration of unmetabolised folic acid in the blood even in fasting conditions. The metabolic and biological consequences of the presence of unmetabolised folic acid in the circulation are as yet uncertain (Morris et al., 2010; Troen et al., 2006).

2.3.7 Summary previous reports on folic acid

The main adverse effects of folic acid discussed in the above mentioned reports are masking of vitamin B_{12} deficiency and potential adverse effects on cancer development.

All the reports agree with the Tolerable Upper Intake Level (UL) for folic acid established by SCF in 2000, for adults at 1 mg/day. High intake of folic acid may correct megaloblastic anemia, which is the hallmark symptom of vitamin B_{12} deficiency. Folic acid masks vitamin B_{12} deficiency by reversing megaloblastic anemia and in this way allows the neurological

dysfunction of vitamin B_{12} deficiency to progress. Neurological damage may not be diagnosed until the neurological consequences have become irreversible.

A lowest-observed-adverse-effect level (LOAEL) was set at 5 mg per day. As dosages up to 1 mg of folic acid are unlikely to cause masking of the hematological signs in patients with pernicious anemia, the UL was set at 1 mg of folic acid.

The ULs for children and adolescents (1-17 years) are adjusted on the basis of body weight and range from 200 to 800 µg/day.

2.4 Literature search

A literature search was conducted in order to assess updated knowledge about cancer development related to high intakes of supplemental folic acid. The search aimed to retrieve studies addressing high intakes of folic acid in food supplements.

2.4.1 Search strategy

In order to retrieve relevant publications addressing high intakes of supplementary folic acid and cancer, literature searches in MEDLINE and EMBASE were conducted (3 September 2014). Both databases were used to ensure comprehensive study retrieval. The strategy for the searches was discussed within the project group.

The search included the terms folic acid supplement and cancer. Only human studies were included. The search terms used are presented in Appendix I.

The search period was limited to publications from 2009 to today as older relevant literature was expected to be covered in the comprehensive ESCO-report from 2009. The search was further limited to include publications written in English, Danish, Swedish or Norwegian.

An additional search was conducted in MEDLINE 15 October 2014 to identify specifically RCTs published after 2009. The search included the term folic acid (in title) and was limited to only RCTs in addition to language limitations. For view of the search terms used, see to Appendix I.

2.4.2 Publication selection

The study types for inclusion in this opinion were systematic reviews and meta-analyses of human studies, RCTs and prospective studies with data on folic acid supplementation in at least one group. The criteria for inclusion were:

- Folic acid in relation to health outcome was the main issue (or one of the main issues) in the article
- Results for folic acid could be separated from results from other interventions e.g. supplements from other B-vitamins
- The results for folic acid supplementation could be compared to a placebo group

In vitro studies were excluded from the results in this opinion. Position papers, conference abstracts/summaries, editorial comments and various dietary guidelines were excluded. Additionally, studies were excluded if the result could be affected by other vitamins.

The literature searches in September 2014 identified 88 articles in MEDLINE and 44 in EMBASE (duplicates not excluded). The additional MEDLINE search in October 2014 identified 100 articles.

Study titles and abstracts were reviewed by two persons of the project group according to the above mentioned inclusion criteria. Titles were selected if chosen by one of the experts, and resulted in 35 full text articles which were distributed in the project group for full text examination. This resulted in the inclusion of 17 studies. Additionally, two studies from hand searching/retrieval of relevant literature cited in the full-text papers have been identified and are included.

A final total of 19 publications were identified and included in the results in this report (see Figure 2.3.2-1).

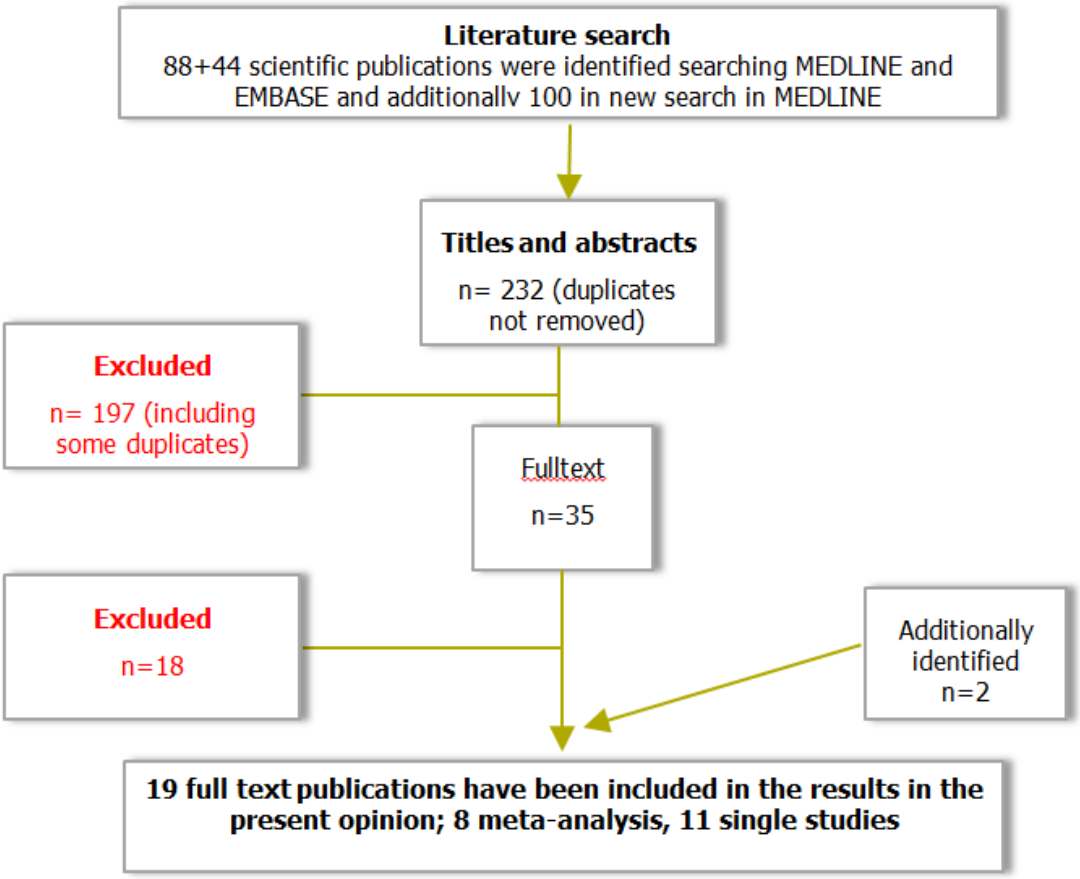

Figure 2.3.2-1 Flowchart for the literature search for supplementary folic acid and associated health outcomes and the subsequent selection of publications.

2.4.3 Data extraction, relevance and quality assessment

Several of the studies retrieved in the literature search were studies investigating possible beneficial effects of folic acid on cardiovascular diseases or prevention of other conditions. The study design of these "effect"-studies might not be suitable for detecting increased incidence of cancer, 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.

2.5 Human studies published after 2009 investigating supplementary folic acid in relation to cancer

This chapter includes a brief description of the studies included in this opinion. Only studies investigating outcomes related to cancer have been included.

2.5.1 RCTs and case-control studies

A list of excluded papers can be found in Appendix II. An overview of RCTs and case control studies included in this report is given in Table 2.5.1-1.

Table 2.5.1-1 Overview of RCTs and case-control studies with folic acid reporting cancer.

Reference RCT	Study design	Participant characteristics	Country	Dose	Outcome	Intervention duration/ exposure	Results
Gao et al. (2013)	RCT. 384 in treatment group and 407 in control group	Healthy above 50y, both sexes.	China	1 mg	Adenoma	3y	Unadjusted RR 0.49 (0.37,0.63)
Wu et al. (2009)	RCT, nested within Nurses Health Study. 338 in treatment group and 334 placebo group	Patients with a history of adenoma	USA	1 mg	Adenoma	3-6.5y	RR 0.82 (0.59,1.13)
Figueiredo et al. (2009)	Secondary analysis of RCT. 237 treatment group and 235 placebo group	Patients with a history of adenoma	USA	1 mg	Prostate cancer	Median 7 y (up to 10 y)	Age adjusted HR 2.63 (1.23,5.65)
Reference case control studies	**Study design**	**Participant characteristics**	**Country (study start)**	**Dose**	**Outcome**	**Intervention duration/exposure**	**Results**
Milne et al. (2012)	Case-control study	Pregnant women	Australia	Not known	Brain tumor in children (offspring) after 0-14 y	Prepregnancy and pregnancy supplementation use of folic acid	Reduced risk among folic acid using mothers OR 0.68 (0.46–1.00)
Amigou et al. (2012)	Frequency matched case-control study	Pregnant women	France	Not known	Acute leukemia in children (offspring) < 15 years	Prepregnancy and pregnancy folic acid supplementation	Prepregnant use of folic acid OR =0.4 (0.3–0.6]). not significant for in pregnancy

Gao et al., 2013 (adenoma)

In an RCT by Gao et al. (2013), 791 healthy Chinese older than 50 years were randomised to receive 1 mg folic acid or a multivitamin tablet not containing folic acid. The participants were followed for three years. The folic acid group was requested to visit their doctor every three months while the control group was contacted by mail or phone. All had a colonoscopy on the final day of the study.

The occurrence of colorectal adenomas was compared between the two groups. Colorectal adenomas occurred in 64 (14.88%) in the folic acid group and 132 (30.70%) in the control group (unadjusted RR 0.49; 95% CI, 0.37-0.63). The risk of more advanced adenomas was also significantly and substantially reduced in the group receiving folic acid.

This study indicate that primary prevention with 1 mg/day folic acid supplementation could reduce the incidence of colorectal adenomas, especially left-sided and advanced disease in those with no previous adenomas.

However, this study suffers from somewhat poor design such as being unblinded, different follow-up procedures in the two groups, and it did not include a placebo but compared folic acid supplementation to a multivitamin tablet.

Wu et al., 2009 (adenoma)

A cost-effective randomised placebo controlled trial was nested within two ongoing cohort studies (the Health Professionals Follow-Up study and the Nurses' Health study) by Wu et al. (2009). Participants who had a history of colorectal adenoma, were cancer free, and who planned to have another endoscopy within the next four years, were found eligible for enrollment. The participants were randomised to receive 1 mg of folic acid or placebo for 3 to 6.5 years (n=672).

Incidence of at least one recurrent adenoma was not significantly associated with folic acid supplementation. Among participants with low plasma folate concentrations at baseline (<7.5 ng/mL), those randomly assigned to receive folic acid experienced a significant decrease in adenoma recurrence (RR: 0.61; 95% CI: 0.42, 0.90), whereas for subjects with high folate concentrations at baseline (7.5 ng/mL), supplemental folic acid had no significant effect (RR 1.28; 95% CI: 0.82, 1.99, P-interaction=0.01).

There was no evidence for an increased risk of advanced or multiple adenomas. On the contrary, in individuals with poor folate status the risk of cancer was reduced.

Figueiredo et al., 2009 (prostate cancer)

Figueiredo et al. (2009) analysed to what extent supplementation of 1 mg of folic acid increased the risk of prostate cancer using data from a previously published RCT (Cole et al., 2007). The trial was carried out between 1994 and 2006 and the analyses included 643 men who had been randomised to receive folic acid or placebo. The participants were followed up

for a median of 7 years, maximum 10 years. The outcome, prostate cancer, was ascertained from medical reports and included reports of histopathology.

Cox proportional hazards models were used to estimate the association between baseline vitamin B status and prostate cancer and the effect of folic acid supplementation on prostate cancer.

Intake of folate and folic acid from the diet were similar in the two groups, as were plasma concentration of folate and unmetabolised folic acid (UMFA). However, the folic acid supplemented group had a lower vitamin B_{12} status at inclusion.

Folic acid supplementation was associated with an increased risk of prostate cancer (hazard ratio (HR) 2.63; 95% CI 1.23-5.65). Plasma folate concentration in the non-supplemented group was inversely associated with the risk of prostate cancer (borderline significant).

This secondary analysis demonstrated that folic acid supplementation and poor folate status was associated with an increased risk of prostate cancer. A small number of prostate cancer cases made the result not robust.

Milne et al., 2012 (brain tumor)

In a case control study by Milne et al. (2012) the aim was to measure to what extent pre-pregnancy or pregnancy folic acid supplementation alone or with other supplements was associated with childhood brain tumors. The study included 327 cases of brain tumors from 10 Australian pediatric oncology centers between 2005 and 2010. The 867 controls were selected randomly. The cases and controls were between 0 and 14 years. The parents/guardians were mailed questionnaires with questions on folic acid supplements or intake of other vitamins before and/or during pregnancy, specified by trimester. Intake of folic acid was categorised in three groups; 0.1-300 µg, 300-450 µg and >450 µg folic acid. Non-compliers were contacted by phone.

The association between folic acid and brain tumors was estimated in multiple, unconditional regression analyses. The exposure variables were whether or not folic acid was taken in pre-pregnancy, during the first trimester or during the last two trimesters of pregnancy. A dose response relation between folic acid and risk of cancer was also estimated.

Pre-pregnancy supplementation of folic acid was associated with a significant reduction in the odds of childhood brain tumor (OR 0.65; 95% CI 0.43-0.98). The effect of folic acid during pregnancy was lower and did not reach statistical significance. The effect of providing other vitamins or minerals was much less clear and did not attain statistical significance. This study shows that folic acid before or during early pregnancy reduces the risk of childhood tumors. It should be noted that these tumors are very rare.

Amigou et al., 2012 (leukemia)

In a case-control study by Amigou et al. (2012) the authors measured the association between maternal folic acid supplementation before and during pregnancy and the risk of childhood acute leukemia. They also measured the association between MTHFR and MTRR genetic polymorphisms and acute leukemia.

Cases and controls were 14 years or younger. The 764 cases were identified from the French National registry of Childhood Hematopoietic Malignancies between January 2003 and December 2003. These cases were frequency matched with 1681 randomly selected controls.

Folic acid supplementation before and during pregnancy was associated with a substantial and significant reduction in the odds ratios of childhood acute leukemia. The association was the same in different subtypes of leukemia. The results also suggest that the genotype homozygous for variants of the genes required for folate metabolism may be a risk factor for acute leukemia.

2.5.2 Systematic reviews and meta-analyses

An overview of systematic reviews and meta-analyses included in this report is presented in Table 2.5.2-1.

Table 2.5.2-1 Overview of meta-analyses with folic acid reporting cancer incidence.

Reference	Number of studies with pure folic acid arm	Folic acid doses	Main endpoints	Results
Mackerras et al. (2014)	7 of 26	0.4-20 mg/day	Cancer, various and total cancer	Total cancer 1.04 (0.97-1.11)
Qin et al. (2013)	3 of 15	0.5-40 mg/day	Cancer, various and total cancer	Total cancer (1.05 (0.99-1.11)
Vollset et al. (2013)	3 of 13	0.5-1 mg/day	Total cancer	Total cancer 1.06, 95% CI 0.99-1.13
Baggott et al. (2012)	2 of 6	0.5-1 mg/day	Total cancer	Total cancer 1.21; 1.05-1.39
Wien et al. (2012)	5 of 10	0.5-5 mg/day	Cancer, various and total cancer	Total cancer 1.07 (1.00-1.14). Six RCTs reported prostate cancer incidence, with a combined RR=1.24 (1.03-1.49)
Figueiredo et al. (2011)	3 of 3	0.5-1 mg/day	Colorectal adenomas	RR was 0.98 (95% CI 0.82,1.17) for all adenomas and 1.06 (0.81,1.39) for advanced lesions
Fife et al. (2011)	2 of 3	0.5-1 mg/day	Colorectal adenomas	After 3 years; OR was 1.09 (0.93-1.28). Above 3 years; OR was 1.35 (1.06-1.70)

Reference	Number of studies with pure folic acid arm	Folic acid doses	Main endpoints	Results
Ibrahim and Zekri (2010)	3 of 6	0.5-5 mg/day	Colorectal adenomas	OR was 1.08 (0.87-1.33)

Mackerras et al., 2014

The meta-analysis "Folic acid, selected cancers, and all-cause mortality" was published in 2014 (Mackerras et al., 2014). The purpose of this meta-analysis was to conduct a systematic review of trials of folic acid supplementation on cancer incidence (total and site specific [colorectal, lung, breast, prostate]). In addition, the relation between folic acid and recurrence of colorectal adenoma, and total mortality, was examined. The search did not place any restrictions on health or disease outcome in the trials, as long as they reported any cancer outcome. The investigators included 26 RCTs. The search was conducted in May 2013. MEDLINE was searched from January 1, 2001 to 16 May 2013. Cochrane CENTRAL was also searched on May 16 2013.

The folic acid dosed in the trials varied from 0.4-20 mg/day. Trials were excluded if folic acid was administered in a broad multivitamin/mineral supplement, but studies with multiple B-vitamins were included. Trials had to administer folic acid and placebos for one year or longer and have a follow-up period of at least one year. Any placebos for folic acid had to be a blank or minimal dose of folic acid; factorial designs testing other substances were permitted.

Overall relative risks (RR) were calculated using counts from each study. An overall weighted (unadjusted) RR for each outcome was calculated de novo from the numbers randomised and event numbers using the DerSimonian-Laird inverse variance random effects method. Its 95% CI was calculated using the Greenland-Robins formula.

No significant associations between folic acid supplementation and overall or site- specific cancer, or all-cause mortality, were found. RR for total incident cancer (13 studies) was 1.04 (95% CI: 0.97-1.11).

In conclusion, no significant relation was found between folic acid supplementation, up to 5 mg per day, and risk of cancer or mortality.

In this meta-analysis seven studies with single folic acid supplementation reporting cancer were included and among these, only three studies had cancer (colorectal) as primary outcome (Cole et al., 2007; Logan et al., 2008; Wu et al., 2009).

Qin et al., 2013

In 2013, Qin et al published the meta-analysis "Folic acid supplementation and cancer risk" (Qin et al., 2013). The purpose of this meta-analysis was to systematically evaluate the effect of folic acid supplementation on cancer risk in RCTs. Post-treatment trials and observational follow-up studies were not included. The investigators searched the MEDLINE database of studies published from January 1966 to October 2012. 15 trials were included in the final analyses. Of the 15 trials, 13 reported total cancer incidence, and fewer reported site specific cancers or cancer mortality. Folic acid dose ranged from 0.5–40 mg/day (the highest dose in a study on patients with renal disease), with varying inclusion of other B-vitamins. Duration of the intervention ranged from 24 to 88 months.

Studies were assessed for quality of randomisation, blinding, reporting of withdrawals, generation of random numbers and concealment of allocation (possible score 0-5). Quality of the 15 trials ranged from 3-5.

Relative risk (RR) was used to measure the effect of folic acid supplementation on risk of cancer using a random-effects model. Possible effect modifications of several variables were examined.

Overall, folic acid supplementation had no significant effect on total cancer incidence (RR=1.05, 95% CI 0.99-1.11), colorectal cancer, other gastrointestinal cancer, prostate cancer, other genitourinary cancer, lung cancer, breast cancer, hematological malignancy and total cancer mortality. A significantly reduced risk was found for melanoma (RR=0.47; 95% CI 0.23-0.94). A higher total cancer incidence risk from folic acid supplementation was found in trials with the highest proportion of participants on lipid lowering drugs (RR=1.10; 1.00-1.20) or with lower percent baseline hypertension (RR=1.08; 1.00-1.16).

The authors concluded that folic acid supplementation had no significant effect on the incidence of total cancer or those mentioned above, except for a reduced risk of melanoma.

In this meta-analysis only three of the included studies were interventions with folic acid supplementation only, and with cancer as an endpoint (Cole et al., 2007; Logan et al., 2008; Wu et al., 2009).

Vollset et al., 2013

The meta-analysis "Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: Meta-analyses of data on 50 000 individuals" was also published in 2013 (Vollset et al., 2013). The purpose of this meta-analysis was to measure any effects on overall and site-specific cancer rates in randomised trials of folic acid supplementation at doses higher than those obtained from fortification. Thirteen randomised trials were included, 10 on prevention of cardiovascular disease, three in patients with colorectal adenoma. All trials were completed before 2011.

Doses in the trials ranged from 0.5-2.5 mg folic acid per day. One trial used 40 mg/day (renal disease patients). Treatment duration in each trial was of at least one year, with average treatment duration of 5.2 years.

Unique to this meta-analysis is that the investigators obtained individual-level data from each trial, totaling 49 621 participants. Log-rank analyses were used to calculate cancer incidence rate ratio (RR). Folic acid supplementation had no significant effect on overall cancer incidence during the first five years of treatment in the trials of cardiovascular disease prevention (RR=1.06, 95% CI 0.99-1.13). No trend towards greater effect with longer treatment was seen. There were no significant effects of folic acid supplementation on the incidence of cancer of the large intestine, prostate, lung, breast or any other specific site. In the three trials among patients with colorectal adenoma, the meta-analysis found a non-significant increases risk RR= 1.33 (95% CI 0.98-1.80).

The authors concluded that folic acid supplementation had no significant effect on cancer incidence.

In this meta-analysis only three of the included studies were interventions with folic acid supplementation only, and with cancer as an endpoint (Cole et al., 2007; Logan et al., 2008; Wu et al., 2009).

Baggott et al., 2012

The "Meta-analysis of cancer risk in folic acid supplementation trials" by Baggott et al. was published in 2012 (Baggott et al., 2012). The purpose of this meta-analysis was to evaluate the risk of cancer secondary to folic acid supplementation. Six randomised placebo-controlled trials of folic acid supplementation were included in the final analysis. A search of PubMed was conducted on June 11, 2009. Subsequently, PubMed was searched on a nearly daily basis using the key word ‘folate’ to update the search – it is not clear which was the last search date.

Included studies used 0.5 – 2.5 mg folic acid per day. Supplements included other nutrients. The duration of intervention was 12 months or more, with an average duration of seven years.

The authors report a weighted analysis of trial-specific effects, expressed as relative risks (RR). One of the six trials found a significantly increased risk of cancer, the others found no significantly increased risk. Overall, the total cancer incidence were higher in the folic acid-supplemented groups than in the placebo groups (RR=1.21; 1.05-1.39).

The authors concluded that total incidence of various cancers were higher in the folic acid-supplemented groups than in the non-folic acid- supplemented groups.

The article is very short and does not provide detailed enough information on the various studies included (or excluded) in the meta-analysis.

In this meta-analysis only two of the included studies were interventions with folic acid supplementation only, and with cancer as an endpoint (Cole et al., 2007; Logan et al., 2008).

Wien et al., 2012

In 2012, Wien et al. published the systematic review and meta-analysis "Cancer risk with folic acid supplements" (Wien et al., 2012). The purpose of this meta-analysis was to explore if there is an increased cancer risk associated with folic acid supplements. Nineteen studies, including 12 RCTs were included in the overall analysis, 10 of the 12 trials reported overall cancer incidence.

The search was conducted during March-May 2010, using MeSH and free-text search terms for folate, folic acid, cancer and neoplasm. The following electronic databases were searched: EMBASE, Ovid MEDLINE, Cochrane Library, Centre for Reviews and Dissemination, NHS evidence, Clincial evidence, and others.

Doses of folic acid in the studies were at least 0.4 mg/day, and up to 40 mg/day in one study of renal disease patients. Folic acid supplements given were with or without other B-vitamins.

The quality of the included studies was assessed using the Cochrane Collaboration's tool for Risk of Bias assessment for RCTs and checklists for cohort and case-control studies.

When feasible, the authors pooled data by meta-analyses with Cochrane Collaboration software and used random-effects model calculating RRs. The PRISMA statement for reporting systematic reviews and meta-analyses were used and RRs were reported.

In the 10 RCTs reporting overall cancer incidence, the RR associated with folic acid supplements was 1.07 (1.00-1.14). Six RCTs reported prostate cancer incidence, with a combined RR=1.24 (1.03-1.49). No significant increased risk for any other cancer type was found. Six trials reporting overall cancer mortality found no significant differences between folic acid supplementation versus not (RR=1.09, 95% CI 0.90-1.30).

The authors concluded that meta-analysis of 10 RCTs showed a borderline significant increase in incidence of overall cancer in the folic acid group. Prostate cancer was the only site-specific cancer where folic acid significantly increased the risk.

In this meta-analysis five of the included studies were interventions with folic acid supplementation only, and with cancer as the endpoint (Charles et al., 2004; Cole et al., 2007; Figueiredo et al., 2009; Logan et al., 2008; Wu et al., 2009).

Figueiredo et al., 2011

The meta-analysis "Folic acid for the prevention of colorectal adenomas: Combined analysis of randomized clinical trials" was published in 2011 (Figueiredo et al., 2011). The aim of this meta-analysis was to evaluate the recurrence of colorectal adenomas in patients treated for

the disease. Treatment was 0.5 mg or 1 mg per day of folic acid for mean 30.6 months. The authors used random-effects meta-analysis to estimate risk ratio (RR). In this meta-analysis all participants in the study of Cole et al. and Logan et al. are included, not taking aspirin use into consideration.

No significant increase or decrease in recurrence of adenomas was seen in the folic acid group compared to placebo. In the group of participants with low folic acid status (<11 nmol/L) at the beginning of the study and in the group of participants with high alcohol intake there was a non-significant decrease in adenomas recurrence with folic acid supplementation.

The authors concluded that after 3.5 years of folic acid use there was no clear decrease or increase in occurrence of new adenomas in patients with a history of adenoma.

Fife et al., 2011

The meta-analysis "Folic acid supplementation and colorectal cancer risk" was also published in 2011 (Fife et al., 2011). The purpose of this meta-analysis was to determine the effect of folic acid supplementation on colorectal cancer risk. A structured search of the MEDLINE, EMBASE, Cochrane and CINAHL databases was undertaken in July 2008. All published full text English language articles were searched for that included randomised or pseudo-randomised comparison of subjects who received folate vs subjects who did not in relation to their risk of adenoma or advanced adenomatous lesions, including colorectal cancer. Subgroup analysis were used to investigate possible differences in the occurrence of adenoma and advanced adenomatous lesions and early (up to three years) vs late more than three years) follow-up.

Three trials met the inclusion criteria and were included in the review. Two studies were based either in North America (1021 patients, aged 21-80 years), or in the UK (945 patients, aged <75 years), while the third included patients from both North America and Europe (5522 patients, aged >55 years). In the third and largest included study, folic acid was given in combination with other B-vitamins (B_6 and B_{12}).

Folate supplementation varied between 1 mg /day for three years, follow-up six or eight years, 0.5 mg/day for three years, follow-up for three years, and 2.5 mg/day for five years, follow-up for five years (+50 mg B_6 and 1 mg B_{12}).

All three trials used computer-generated randomisation and adequate completeness of follow-up, blinding and assessments of baseline comparisons between the study groups. Weighted treatment effect (using fixed effects) was calculated across trials using RevMan.

Overall, the risk of adenomatous lesions was not increased among patients who received folate supplementation for up to three years. However the risk for adenomatous lesions was increased for those receiving folate over three years.

In conclusion: this meta-analysis challenges the results from epidemiological studies that folate status is inversely related to the risk of developing colorectal cancer.

In this meta-analysis two of the included studies were interventions with folic acid supplementation only, and with cancer as endpoint (Figueiredo et al., 2009; Logan et al., 2008).

Ibrahim and Zekri, 2010

The last included meta-analysis, "Folic acid supplementation for the prevention of recurrence of colorectal adenomas" is by Ibrahim and Zekri (2010). The purpose with this meta-analysis was to determine the relation between folic acid and the risk of recurrence of colorectal adenomas, as conflicting data have emerged from preclinical and clinical studies examining this relation.

A comprehensive search of citations from PubMed, EMBASE, Cochrane databases and abstracts of relevant proceedings was made. The aim was to identify interventional randomised, placebo-controlled studies where folic acid in specific dose and for specific duration was administered to evaluate the effect on recurrence of adenomatous colorectal polyps.

Five eligible trials were identified, with 805 patients in the folic acid groups and 775 patients in the placebo group. Data were analysed using RevMan.

The intake of folic acid varied from 0.5 mg/day for one year in one study, 1 mg /day for one year in another study, for two years in a third study and for three years in a fourth study, and 5 mg/day for one year in the fifth study.

Examination of folic acid dose effect showed that the two studies that had used 1 mg/day favoured folic acid over placebo with an odds ratio (OR) of 0.62 (95% CI; 0.48-080). However, the overall effect for all the included studies was not significant. It was significant heterogeneity in methodological quality.

The present meta-analysis failed to show potential benefit for folate supplementation.

In this meta-analysis three of the included studies were interventions with folic acid supplementation only, and with cancer as the endpoint (Figueiredo et al., 2011; Logan et al., 2008).

2.6 Human studies investigating intake of supplementary folic acid and concentration of unmetabolised folic acid

Unmetabolised folic acid (UMFA) has been recovered in the circulation in connection with folic acid supplementation and/or fortification, indicating that the conversion of folic acid to 5-MTHF is not complete. Folic acid is not naturally occurring in the human body and the

consequences of UMFA in the circulation are unknown. It has been postulated that UMFA could affect the homeostatic regulation of folate and reduce natural-killer cell cytotoxicity (Troen et al., 2006). Articles regarding UMFA that were identified during our literature search are presented here.

An overview of studies reporting UMFA that are included in this report is given in Table 2.6-1.

Table 2.6-1 Overview of studies reporting unmetabolised folic acid.

Reference	Participant characteristics	Country (study start)	Design	Intake	Main endpoint	Results
Boilson et al. (2012)	137 elderly subjects 60-86 years of age.	Ireland 2001-2003	Cross sectional study	High use of food with folic acid fortification	Unmetabolised folic acid (UMFA)	UMFA was detected in 94.1% of the subjects accounting for 1.3% of total plasma folate.
Bailey et al. (2010)	1121 elderly >=60 years from NHANES 2001-2002.	USA 2001-2002	Cross sectional study	Mandatory folic acid fortification of grain products since 1998	UMFA in subjects fasted for mean 10 h.	38% of the older population had UMFA but this could not be explained by intake of folic acid alone. Smaller dose consumed more frequently gave higher UMFA than larger dose consumed less frequently.
Morris et al. (2010)	1858 senior participants in NHANES 1999-2002	USA 1999-2002	Cross sectional study	Mandatory folic acid fortification of grain products since 1998	Anemia, macrocytosis and cognitive test performance	UMFA was detected in 33%. Serum B_{12} values <148pmol/L or MMA>210 nmol/L UMFA was related to lower cognitive test scores and lower cell volume. The authors address the finding that low B_{12} and high folic acid intake needs further investigations.
Obeid et al. (2010)	87 pregnant women and 29 cord blood including 24 of the mothers.	Germany 2004 and 2007	Cross sectional	Women using 400 µg folic acid supplementation during preganacy	Folate and folic acid status in serum and cord blood.	Women who used supplementation had higher folate and 5-MTHF and formyl-THF concentrations than did non supplemented women. UMFA did not accumulate in cord blood while 5-MTHF and THF did.
Nguyen et al. (2009)	40 nonpregnant women (age 18-45y)	Canada	Randomised, 2-arm, open-label, intervention study	1.1-5 mg/day		UMFA, plasma and red blood cell (RBC) folate.

Reference	Participant characteristics	Country (study start)	Design	Intake	Main endpoint	Results
Sweeney et al. (2009)	50 adult blood donors (age 27-60) not fasted. 20 mothers just delivered with caesarean section (fasted)	Ireland in 2006	Cross sectional	Voluntary fortification and food supplements	Unmetabolised folic acid	Unmetabolised folic acid was present in 49 of 50 blood donors, comprising 2.25% of total plasma folate; and in 18 of 20 fasted women undergoing caesarean section.

Boilson et al., 2012

The aim of this cross sectional study by Boilson et al. (2012) was to measure concentration of UMFA in plasma in an elderly population. The subjects were fasted and exposed to voluntary folic acid fortification and participated in a Lifeways Cross-Generation Cohort study in Ireland. One hundred and thirty seven subjects were included, aged 60 to 86 years, mean age was 67.4 years, and dietary intake was calculated with use of a Food Frequency Questionnaire (FFQ) to evaluate habitual folic acid intake. Two of the included subjects did not complete the FFQ. Total plasma folate, red cell folate, plasma UMFA and homocysteine concentration were analysed.

Median folic acid intake through food was 126 µg per day and median folic acid from supplements was 200 µg per day but with a huge range. Mean intake of folate from food was 351 µg per day among the women and 384 µg among the men. Only eleven subjects (8%) had used folic acid supplementation the last 24 hours before blood was drawn, while all except 13 (9%) reported use of foods fortified with folic acid. UMFA was detected in 94% of the participants accounting for 1.3% of the total plasma folate. UMFA correlated with plasma folate, red cell folate and use of folic acid supplementation and intake of folic acid fortified foods.

The authors concluded that UMFA was found in most subjects of this elderly population even after an overnight fast, and they argued for careful consideration of legislating mandatory folic acid fortification.

Bailey et al., 2010

Bailey et al. (2010) conducted a cross sectional study with the aim to examine the concentrations of UMFA in relation to intake of folic acid from the diet and from supplements. The study is from USA with mandatory folic fortification of bread, flour, corn meal, rice, noodles, macaroni, and other grain products since 1998. In this study, surplus serum from the National Health and Nutrition Examination Survey (NHANES) 2001-2002 was used and 1121 subjects aged over 60 years were included. Dietary data was collected with a 24-hour dietary recall.

UMFA was detected in 38% of the subjects and in this group UMFA constituted 6% of the serum folate. Folic acid intake through diet was calculated to 178 µg per day and through

food supplements to 175 µg per day. UMFA was higher in the group with the highest concentration of 5-MTHF. Forty percent of those with UMFA in serum were in the upper quartile of total folic acid intake, but total folic acid intake was only moderately correlated to UMFA concentration. Serum folate, 5-MTHF and vitamin B_{12} concentration were higher in the group with UMFA, but not in red blood cell folate or homocysteine concentration.

The authors conclude that the UMFA concentration should be monitored since UMFA might be related to cancer development.

Morris et al., 2010

Morris et al. (2010) conducted a cross sectional study from USA with the aim to evaluate the associations between circulating UMFA or 5-methyl tetra hydrofolate (5-MTHF) and cognitive performance. This study included 1858 subjects participating in NHANES from 1999 to 2002 who had normal renal function and no history of stroke, anemia therapy or disease of the liver, thyroid or coronary arteries. The subjects went through a program with blood drawing and cognitive and dietary assessments. Cognitive status was tested with a Digit-Symbol Substitution test and dietary information was collected with use of 24-h dietary recall interview and supplement use was registered for the last 30 days. Blood was drawn after an overnight.

Circulating UMFA was detected in 33% of the subjects and was related to increased odds for anemia and alcohol use. In the subjects with low B_{12} status and who had UMFA in their circulation had lower cognitive test scores and lower mean cell volume compared to those not having UMFA in their circulation. In those with adequate B_{12} status a high serum concentration of 5-MTHF was related to higher cognitive score.

The authors conclude that more studies are needed to clarify the hematologic and neuropsychiatric risks of low B_{12} status and high folic acid intake.

Obeid et al., 2010

In a cross sectional study from Germany, Obeid et al. (2010) analysed blood drawn at delivery from 87 women and also in 24 samples of cord blood from those mothers and in five cord bloods not connected with the included women. Peripheral venous blood was obtained 1-2 hours before delivery. Serum concentrations of UMFA (> 0.29 nmol/L) were found 44% of the women and in 55% of the cord blood samples. Pregnant women who had been taking 400 µg folic acid a day during pregnancy had a non-significant higher concentration of UMFA than women not using folic acid supplement. Concentrations of total folates and 5-MTHF and formyl-THF were also significantly higher in the former group. The concentration in cord blood was not significantly different because of folic acid supplementation.

The authors concluded that UMFA concentration in cord blood was not higher in supplemented than non-supplemented mothers, and that it is not likely that UMFA accumulate in the fetus like 5-MTHF and THF.

Nguyen et al, 2009

Nguyen et al. (2009) compared steady state folate concentrations in women of childbearing age who were randomised to take 5 or 1.1 mg folic acid daily for 30 weeks. The study was a randomised, 2-arm, open-label, intervention study. 40 nonpregnant women between 18-45 years, who did not take folic acid supplements, were included. The study took place in Ontario, Canada. The women were randomised to take either 5 or 1.1 mg folic acid daily for 30 weeks, as part of a multivitamin pill. The outcome was plasma and red blood cell (RBC) folate, measured at baseline and at weeks 2, 4, 6, 12 and 30. During the 30 weeks, one woman in each arm dropped out.

There were no significant differences in plasma or RBC folate at baseline. Plasma folate concentrations increased to 165.3 ± 109.9 nmol/L for the 5 mg folic acid group and to 96.8 ± 41.1 nmol/L for the 1.1 mg folic acid group by week 30 (differences were statistically significant). Significant differences were also found in RBC folate concentrations between the two groups at weeks 4, 6, 12 and 30. Concentrations at week 30 were 2339 ± 782 and 1625 ± 339 nmol/L for the 5 and 1.1 mg folic acid groups, respectively. At baseline, UMFA was detected in 70% and 65% of subjects in the 5 and 1.1 mg folic acid groups, respectively. By week 30, UMFA was detected in 58% of both groups.

The authors concluded that the use of 5 mg folic acid per day produced higher blood folate concentrations, with a faster rate of folate accumulation, compared with 1.1 mg folic acid.

Sweeney et al., 2009

In a small cross sectional study from Ireland, Sweeney et al. (2009) analysed UMFA concentration in blood from 50 non-fasting blood donors, among 20 pregnant women and in cord blood immediately after caesarean section. The mothers had been fasted at least 8 hours before the caesarean section. All subjects were interviewed with regard to use of folic acid fortified foods and supplements. In 18 (90%) of the mothers and in 17 of the cord bloods UMFA was present as well as in 49 (98%) of the blood donors. In the fasted mothers UMFA comprised 1.31% of total folate and in the blood donors 2.25% of the total folate was UMFA.

The authors concluded that although low concentrations of UMFA was found it is persistently present, also in fasted women which indicate that there would be an constantly exposure to UMFA to the fetus and they warn against mandatory fortification of folic acid as long as the impact of UMFA in cancer genesis is not known.

2.6.1 Summary of human studies

In our literature search (from 2009 to 15 October 2014), we found eight meta-analyses and five single studies, where the aims were to study the relation between folic acid supplementation and incidence of cancer. Most of the studies investigated adenomas and colorectal cancer, but also prostate cancer, breast cancer and total cancer have been

evaluated. Brain tumor and childhood leukemia were investigated in two of the included single studies. To evaluate the impact of folic acid supplementation, studies conducted with multiple B-vitamins were regarded as not relevant setting an UL. Only one of the included studies was with healthy subjects. All the other studies (clinical and meta-analyses) were performed in patients with adenomas, CVD, gastritis or follow-up of pregnancy interventions. Three large clinical studies were performed in patients with diagnosed adenoma with the main outcome recurrence of new adenomas. All meta-analyses used two or all three of these clinical studies in their analyses. Additionally, a few smaller clinical studies were included in some of the meta-analyses.

Only secondary analysis from one single study (Cole et al., 2007; Figueiredo et al., 2009) found a statistically significant increased risk of prostate cancer following folic acid supplementation.

For UMFA in humans we found six studies from countries with liberal voluntary folic acid fortification (Ireland) and from countries with mandatory folic acid fortification in addition to food supplements. Main results indicate that most subjects exposed to fortification and food supplementation with folic acid have circulating UMFA.

3 Exposure to folic acid

In the terms of reference from the Norwegian Food Safety Authority it is stated that is important to ensure that the total intake of vitamins and minerals from all sources does not exceed the UL. However, folic acid is the synthetic form of the vitamin which is not found naturally in foods. VKM is therefore requested to estimate the intake of folic acid from food supplements and foods with added folic acid, in all age groups in the population above 1 year.

Intakes of folic acid from food supplements have only been calculated for adults 18-70 year of age. No information about intakes of folic acid supplements is available for 2-year-olds, as the food supplements listed in the questionnaire for the 2-year-olds do not contain folic acid (Småbarnskost 2006) (Kristiansen et al., 2009). However, in an article from 2011 among seven 2-year-old children the median intake was 20 µg (range 7.5-71.4) of folic acid/day from folic acid containing supplements in the supplement users (Hay et al., 2011).

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 to be too old to be used in this opinion.

Some estimates for intake of folic acid from fortified foods have been conducted in the scenarios below.

Intakes of folate from foods are available for all age groups, but are not relevant for risk assessment of folic acid.

3.1 Description of food consumption surveys

The estimated intakes of folic acid presented in this opinion are based on data from the national food consumption surveys for adults aged 18-70-years (Norkost 3) and information for pregnant women who answered the Norwegian Mother and Child Cohort Study (MoBa2) food frequency questionnaire in 2008.

Norkost 3 is based on two 24-hour telephone recall surveys 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 18-70 years participated (participation rate 37%).

The Norwegian Mother and Child Cohort Study (MoBa) is a prospective population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health (Magnus et al., 2006). Participants were recruited from all over Norway from 1999 to 2008. The women consented to participation in 40.6% of the pregnancies. The cohort now includes 114 500 children, 95 200 mothers and 75 200 fathers.

3.2 Intake of folic acid from food supplements in adults

The intakes of folic acid from food supplements are presented in Table 3.1.2-1. Intakes for men and women are presented separately, as women are advised to take folic acid supplement before and during the first trimester of pregnancy. Only 18% of the men and 26% of the women in Norkost 3 reported to take folic acid supplements, and the presented figures are for those who have reported to use folic acid supplements. Mean intakes of folic acid from supplements in those who actually took supplements are 149 µg/day in women and 172 µg/day in men.

Table 3.1.2-1 Intakes of folic acid from food supplements in users only, men (n=152) and women (n=240) mean, median and 95-percentile intakes.

	Mean (SD), µg/day	Median, µg/day	95th perc, µg/day
Men, users only	172 (228)	100	500
Women, users only	149 (121)	100	400

3.2.1 Intake of folic acid in pregnant women

Before and during the three first month of pregnancy women are recommended to take 400 µg of folic acid as supplements. In 2008, 62% of the women participating in MoBa reported use of folic acid supplements with a mean intake of 388 µg/day and 800 µg/day in the 95th percentile. Use of several folic acids containing food supplements and use of food supplements bought through internet or abroad accounts for the high intake in the 95th percentile among the pregnant women (Haugen et al., 2008).

Table 3.1.2.1-1 Intakes of folic acid from food supplements in pregnant women; all participants and users only, mean, median and 95-percentile intakes.

	Mean (SD), µg/day	Median, µg/day	95th perc, µg/day
All participants (n=11 633)	240 (292)	171	735
Users only (n= 7227)	388 (284)	400	800

3.3 Scenarios

VKM is requested to estimate the intake of folic acid from both food supplements and foods with added folic acid, in all age groups in the population above 1 year. Intakes of folic acid from fortified foods are not available in the national food consumption surveys for any age groups. However, folic acid fortified products available on the Norwegian market indicates that the present intake of folic acid from fortified foods in the general Norwegian population can be expected to be low as most of the fortified products on the Norwegian marked not are expected to be consumed on a daily basis. Furthermore, the Norwegian fortification model (VKM, 2013) will ensure that consumption of folic acid from fortified foods will not exceed UL in any age groups.

According to a model for fortification from 2006 and later updates (last in 2013), 53 µg folic acid per 100 kcal can be added to foods and drinks without exceeding the UL for folic acid in any age group http://vkm.no/dav/88ac29e491.pdf.

In the following scenarios, 50% of all wheat flour has been fortified with 53 µg folic acid per 100 kcal. Intakes of folic acid from presumably fortified wheat flour have been calculated separately for men and women, and in different age groups with use of consumption data from Norkost 3. The results are presented in Tables 3.1.3-1 and 3.1.3-2. Young men would be the most exposed group if 50% of wheat flour were fortified, whereas the exposure in women in child-bearing age would be somewhat lower. However, none of the adult population groups would exceed the present UL of 1 mg/day if the maximum addition according to the fortification model is implemented.

Table 3.1.3-1 Scenario: Intake of folic acid for men (aged 18-70) in µg/day if 50% of all wheat flour1 were added 53 µg folic acid per 100 kcal.

	N	Mean	Median	95th percentile
Men, all	862	148	136	289
Men <30 years	138	182	169	379
Men >30 years	724	141	131	274
Men <45 years	453	163	148	310
Men >45 years	409	132	124	247

[1]Scenario addition of 53 µg folic acid per 100 kcalr wheat flour in accordance with the updated model for fortification: http://vkm.no/dav/88ac29e491.pdf.

Table 3.1.3-2 Scenario: Intake of folic acid for women (aged 18-70) in µg/day if 50% of wheat flour[1] were added 53 µg folic acid per 100 kcal.

	N	Mean	Median	95th percentile
Women, all	925	98	90	195
Women <30 years	143	107	101	210
Women >30 years	782	96	87	195
Women <45 years	568	103	97	204
Women >45 years	357	89	80	177

[1]Scenario addition of 53 µg folic acid per 100 kcal wheat flour in accordance with the updated model for fortification: http://vkm.no/dav/88ac29e491.pdf.

VKM is also requested to elucidate the consequence of amending the maximum limit for folic acid in food supplements for the total intake of folic acid (from food supplements and foods added folic acid). VKM has been requested to make calculations for how amendment of the maximum limit in food supplement (200, 400 or 600 µg/day) will affect the maximum amount of nutrients that can be added per 100 kcal of foodstuff according to the Norwegian fortification model (VKM, 2013). The results are presented in Table 3.1.3-2. In adults, an increase in maximum limits to 400 or 600 µg folic acid from food supplements will not affect the previous conclusions in the fortification model. However, an increase in maximum limits will imply a reduction in fortification for other age groups or an exceedance of UL. The 2-year olds are the most vulnerable age groups with the lowest UL for folic acid. The empty

fields in the table, indicate that the change in maximum limit will result in intakes of folic acid from supplements and fortified food/drinks at or above UL in these age groups.

Table 3.1.3-2 Scenarios with amended maximum content of folic acid in food supplements - calculations made in the Norwegian fortification model (VKM, 2013), µg folic acid/100 kcal food or drink.

	200 µg food supplement	400 µg food supplement	600 µg food supplement
2-year olds	-	-	-
4-year olds	34 µg/100 kcal	-	-
9-year olds	48 µg/100 kcal	-	-
13-year olds	53 µg/100 kcal*	39 µg/100 kcal	-
Men, 18-70 years	53 µg/100 kcal*	53 µg/100 kcal*	53 µg/100 kcal*
Women, 18-70 years	53 µg/100 kcal*	53 µg/100 kcal*	53 µg/100 kcal*

*The 2-year olds are the most vulnerable age group due to the lowest UL.

3.4 Summary exposure

Eighteen per cent of men and 26% of women in Norkost 3 reported to take folic acid food supplements. Mean intake of folic acid from supplements in those who actually take supplements are 149 µg/day in women and 172 µg/day in men. Information on intake of folic acid supplements for other age groups is not available.

Among women participating in MoBa in 2008, 62% reported use of folic acid. Mean intake was 388 µg/day and 800 µg/day in the 95th percentile. Use of several folic acids containing food supplements and use of food supplements bought through internet or abroad accounts for the high intake in the 95th percentile among the pregnant women.

Intakes of folic acid from fortified foods are not available in the national food consumption surveys for any age groups. However, according to a model for fortification from 2006 and updates (last in 2013), 53 µg folic acid per 100 kcal can be added to foods and drinks without leading to exceedance of UL for folic acid in any age groups.

In scenarios where 50% of wheat-flour presumably have been fortified with 53 µg folic acid per 100 kcal, young men would have the highest intakes, whereas the exposure in women at child bearing ages, would be somewhat lower.

If maximum limits in food supplements are increased, this will imply a reduction in fortification for the younger age groups or an exceedance of UL

4 Risk characterisation

In 2000, SCF concluded that the best available estimate for a lowest-observed-adverse-effect level (LOAEL) for folic acid was 5 mg, and that dosages up to 1 mg of folic acid were unlikely to cause masking of hematological signs in patients with pernicious anemia, and the UL for folic acid was set at 1 mg.

The totality of evidence from randomised control trials up to the date of the ESCO report (2009) did not conclude that folic acid increased cancer risk. However, based on results from the reviewed studies, increased cancer risk from folic acid supplementation could not be excluded. The report (from 2009) stressed that interpretation of results from available studies were limited due to the short duration of the trials and power of the meta-analysis.

The ESCO report (2009) concluded that intake of folic acid should not exceed the established UL of 1 mg/day (SCF, 2000). The UL was based on limited data, and ESCO underlined that if further data become available, this UL needs to be discussed further.

It was also stated that the relationship between folic acid and cancer requires to be clarified by studies designed with sufficiently long follow-up periods addressing the biological hypothesis for the dual effect of folic acid on cancer development (EFSA, 2009).

In light of this, the results relevant for setting an UL from published studies from 2009 up to 15th of October 2014 are discussed below.

4.1 Folic acid and cancer

Five single studies and eight meta-analyses are included for evaluation of the relation between folic acid supplementation and incidence of cancer. The majority of the studies are related to adenomas and colorectal cancer, but also prostate cancer, breast cancer and total cancer have been evaluated as secondary outcomes. Brain tumor development in children born to mothers using folic acid supplementation during pregnancy was investigated in one study, and another studied childhood acute leukemia following perinatal folic acid supplementation among the mothers.

Since 2009, one RCT of 791 healthy subjects older than 50 years has been published (Gao et al., 2013). The outcome was development of colorectal adenomas, and an unadjusted reduced risk of advanced colorectal adenomas after three years was found in the group randomised to receive 1 mg folic acid compared with a control group receiving a multivitamin tablet not containing folic acid. In this study the treatment group was not blinded and the follow-up procedures in the two groups differed. The study was not designed to look at adverse effects and does not add information useful with regard to establishing an UL for folic acid. Conclusion: An intake of 1 mg folic acid per day for 3 years seems safe for healthy subjects older than 50 years.

A nested case-control study investigated the recurrence of colorectal adenoma among 338 participants who received 1 mg of folic acid and 334 patients who received placebo for 3 to 6.5 years (Wu et al., 2009). No significant association was found in participants with an adequate folate status, but reduced risk of adenomas was observed in subjects with inadequate folate status. Conclusion: One milligram folic acid supplementation seems safe for subjects treated for colorectal adenomas.

Figueiredo et al. (2009) analysed occurrence of prostate cancer among men (n=643) with colorectal adenomas treated with 1 mg folic acid for a median of 7 years. An age-adjusted hazard ratio for prostate cancer was 2.63 (1.23, 5.65) for the men randomised to treatment compared with placebo. This is a secondary finding of the Cole et al. (2007) study originally looking at recurrence of colorectal adenomas and cancer. The study was poorly powered (few prostate cancer cases). Conclusion: Further clinical studies with prostate cancers as primary outcome are needed to confirm these findings.

Two case-control studies have examined the development of cancer in offspring of mothers using folic acid during the perinatal period; one studied leukemia (Amigou et al., 2012) and the other brain cancer (Milne et al., 2012). The dosage of folic acid was reported, but the use of other multivitamins was not detailed. Conclusion: These two studies reported on a protective effect of folic acid supplementation and therefore not useful for evaluating an UL.

Only one meta-analysis published after 2009 included studies with single folic acid arms (Figueiredo et al., 2011). This meta-analysis included the three studies by Cole et al. (2007), Logan et al. (2008) and Wu et al. (2009), and concluded that there was no clear decrease or increase in recurrence of new adenomas in patients after 3.5 years with the use of 0.5 to 1 mg folic acid per day. Conclusion: Folic acid supplementation of 0.5 to 1 mg seems safe in adenoma treated subjects with respect to new adenoma development.

Conclusion: None of the studies regarding cancer published after 2009 provide significant support to alter the existing UL for folic acid supplementation.

4.2 Unmetabolised folic acid

Concerns have been raised regarding potential adverse effects associated with the presence of unmetabolised folic acid (UMFA) in the circulation. Six of the studies identified during the literature search report on UMFA in subjects taking supplements or consuming fortified foods. In the study by Sweeney et al. (2009), 2.25% of the total plasma folate was UMFA, compared to 1.3% in the study of Boilson et al. (2012). Both fasting and non-fasting subjects were included in these studies. A study from Germany among pregnant women reported that women who took 400 µg of folic acid supplements had higher concentrations of UMFA in the blood compared to women not using folic acid supplements, but no differences were found in umbilical cord blood (Obeid et al., 2010). Two studies from USA, both analysing data from NHANES 1999 to 2003, found that among persons older than 60 years almost 40% of the participants had UMFA in the circulation (Bailey et al., 2010; Morris

et al., 2010). USA implemented mandatory folic acid fortification of grain products in 1998 and hence more or less all subjects in these studies had intakes of folic acid. It was reported that those who used folic acid supplements had significantly higher concentrations of UMFA in the circulation than those who did not (Bailey et al., 2010), but that UMFA was only moderately correlated with folic acid intake. In the study of Morris et al. (2010), the authors also looked at the relation between UMFA and anemia, macrocytosis and cognitive performance. They found that in subjects with low vitamin B_{12} status, the presence of UMFA was related to lower cognitive test scores and lower mean cell volume. In the group with low vitamin B_{12} status, a high serum folate concentration was associated with increased odds of anemia and decreased odds for macrocytosis.

Conclusion: UMFA is found in subjects who use folic acid supplements or who are subjected to folic acid food fortification. The impact of UMFA in the circulation and the extent to which UMFA contributes to the development of cancer or other undesirable health effects is still of concern. The reviewed studies do not contribute with evidence useful in terms of a potential modification of the current UL for folic acid.

4.3 Evaluation of ULs for folic acid

It has previously been established that folic acid has the ability to reverse megaloblastic anemia due to vitamin B_{12} deficiency and thus delay appropriate treatment with B_{12}. Longstanding B_{12} deficiency can have serious irreversible neurological consequences. A Tolerable Upper Intake Level (UL) for folic acid was set in 2000 by The Scientific Committee on Food (SCF) and was based on studies among vitamin B_{12} deficient patients who were treated with doses of folic acid up to 30 mg per day. Doses >5mg per day were reported to give neurological relapses in these patients and the LOAEL of 5 mg folic acid per day was set. An uncertainty factor of 5 was applied and UL for folic acid was calculated to 1 mg per day (SCF, 2000).

The latest main concerns of folic acid supplementation and adverse effects have been related to cancer development(Ebbing et al., 2009; EFSA, 2009; EFSA, 2010). While adequate folate status seems to be protective for cancer development, it has been suggested that folic acid supplementation might enhance cancer progression. In our literature search meta-analyses and clinical studies were related to colorectal adenoma patients and it seems that intake of up to 1 mg of folic acid for 3 years was neither protective nor increased the rate of new adenomas. No further studies were found to clarify the relationship between folic acid intake and cancer designed with sufficiently long follow-up periods (EFSA, 2009).

Conclusion: At this time there are no new arguments for increasing or decreasing the UL for folic acid in relation to cancer. In elderly low cognitive function among those with low vitamin B_{12} status were associated with UMFA in the circulation (Morris et al., 2010). If future studies confirm the finding it might call for a reevaluation of UL for folic acid.

4.3.1 General population, different age groups

Vitamin B_{12} deficient: In individuals with poor or suboptimal vitamin B_{12} status, folic acid intake may mask otherwise easily recognisable consequences of vitamin B_{12} deficiency and accordingly delay treatment with vitamin B_{12}. There are also indications that supplementation with folic acid may accelerate cognitive decline in elderly and that this effect is aggravated in B_{12} deficiency.

The prevalence of vitamin B_{12} deficiency in most populations is not known but there are indications that as many as 4% of elderly are deficient even in affluent countries. This number is probably higher in poor countries. Many young children (<5 years) also have poor vitamin B_{12} status (Allen, 2004; TaneJa et al., 2007). The extent to which folic acid has negative health consequences in these is not known. However, in a recent RCT in a population of young children with poor vitamin B_{12} status in North India, supplementation with 2 RDA of folic acid for six months doubled the risk of persistent diarrhea (Taneja et al., 2013). Concomitant vitamin B_{12} supplementation did not modify this effect. However, the doses of vitamin B_{12} given may have been too low to sufficiently replenish their vitamin B_{12} stores.

4.3.2 Special groups

The most important source of vitamin B_{12} is animal source foods such as meat, egg and milk. Vegetarians and vegans are accordingly at risk for deficiency and at a higher risk of adverse effects from high doses of folic acid.

Dihydrofolate reductase (DHFR) activity: Folic acid has to be reduced by the enzyme DHFR before it can enter normal metabolism. Many have a mutation in the DHFR gene which leads to higher levels of UMFA in the circulation (Kalmbach et al., 2008).

Antifolate therapy usually target human and microbial DHFR activity. Intake of folic acid may interfere with these drugs and result in high levels of UMFA in the circulation. There is also a possibility that high intake of folic acid can attenuate the effect of drugs with antifolate activity.

5 Uncertainties

5.1 Uncertainties linked to study design

Evidence considered in this report on risk of cancer development following folic acid supplementation is collected from three RCTs among patients with adenomas (Cole et al., 2007; Logan et al., 2008; Wu et al., 2009). One meta-analysis of the three RCTs was also used. RCTs rank as number one among primary studies on the quality-of-evidence scale, surpassed only by meta-analyses of RCTs regarding evidence quality.

Compliance with the study protocol was controlled through questionnaires by mail every 4 months in the study of Cole et al. (2007). In the study of Logan et al. (2008) all participants were interviewed by phone every 4 months by a nurse to check for compliance. Tablet count was performed once a year. In the study of Wu et al. (2009), participants were asked for a blood sample to assess adherence with the study protocol. Compliance with the study protocol was evaluated as acceptable in all three studies. However, low compliance in these studies could lead to an underestimation of a negative effect. The generalisability of results from the three studies to the general public or to cancers other than colorectal cancer is limited, since all three studies were performed in patients with colorectal adenomas. However, since no increased risk of colorectal cancers was found in this patient group, which is known to be more disposed for colorectal cancer, it seems unlikely that healthy subjects would be at a higher risk.

Ethnicity may theoretically play a role both with regard to health effect outcomes and doses tolerated. Most studies referred to are performed in Caucasian dominated populations but whether the effects differed by ethnicity was not addressed.

Most trials combine folic acid with other vitamins, and only the three trials mentioned above included an intervention arm of folic acid alone. However, the dosage used in these studies is at or below the current UL for folic acid. This UL was set because of adverse effects of folic acid supplementation in subjects with low B_{12} status. In the study by Cole et al. (2007), B_{12} status was checked before inclusion and patients were not included if B_{12} status was poor. Adverse effects were reported in all three studies with no significant differences between folic acid and placebo treated patients.

With regard to UMFA, no toxicological data exist.

5.2 Dietary assessment and concentration of folic acid in foods and supplements

Folic acid is a synthetic stable chemical form of folate which is not found naturally in food. Folic acid is the most common form of folate used in food fortification and in food

supplements. Most European countries, as well as Norway, have not implemented mandatory fortification of folic acid, and so far Norway has very few products voluntarily fortified with folic acid on the market which would contribute to intake of folic acid in the general population. When evaluating folic acid intake, the information given in the labelling of food supplements should therefore give a good estimation of actual intake of this vitamin. Bioavailability of folic acid is about 85%. Possible source of error is the content of folic acid in the food supplements. Validation studies with folic acid have shown a significant increase in blood folate with folic acid supplementation, but folate comes also from the diet which complicates the interpretation of the results (Brantsaeter et al., 2007; Mikkelsen et al., 2006).

6 Conclusions with answers to the terms of reference

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

In 2000, the Scientific Committee on Food (SCF) set a tolerable upper intake level (UL) for folic acid of 1 mg/day for adults. ULs for adolescents and children were adjusted downwards on the basis of body weight. Subsequently, new scientific evidence has emerged suggesting a possible link between high intake levels of folic acid and risk of cancer. In the light of the latest scientific data on folic acid, should the UL be amended?

> VKM concludes that no new scientific data have been published on the issue of folic acid supplementation and cancer risk that would support amending current UL. Doses up to UL for folic acid have been investigated in patients with colorectal adenomas but no significant increase or decrease in colorectal cancer risk was reported.
>
> Unmetabolised folic acid was found in subjects who use folic acid supplements or who are subjected to folic acid food fortification. The impact of unmetabolised folic acid in the circulation and the extent to which this contributes to the development of cancer or other undesirable health effects is still of concern. The reviewed studies do not contribute with evidence useful in terms of a potential modification of the current UL for folic acid based on cancer risk.

It is important to ensure that the total intake of vitamins and minerals from all sources does not exceed the UL. However, folic acid is the synthetic form of the vitamin which is not found naturally in foods. VKM is therefore requested to estimate the intake of folic acid from food supplements and foods that are added folic acid, in all age groups in the population above 1 year.

> No information about intake of folic acid supplements is available for 2-year-olds, because food supplements containing folic acid was not asked for in the survey of 2012. 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 to be too old to be used in this opinion.
>
> The maximum dose of folic acid allowed in food supplements in Norway is 200 µg, and mean folic acid intake from food supplements among adults in Norway assessed in Norkost 3 was 172 µg per day for men and 149 µg per day for women (among users). The 95% percentile was 500 µg for men and 400 µg for women. Supplements for pregnant women contain 400 µg folic acid and the mean intake of folic acid was

388 µg per day and 800 µg per day in the 95th percentile in this group according to MoBa.

Information about folic acid intake from fortified foods in Norway cannot be calculated because folate and folic acid are not distinguished in the Norwegian food tables. However, folic acid fortification is regulated through the Norwegian food fortification model ensuring a safe intake provided food supplement intake is 200 µg per day or below. This was illustrated in the scenario where 50% of all wheat flour was fortified with 53 µg folic acid per 100 kcal. The estimated intake in the 95th percentile was 379 µg per day for men and 210 µg per day for women.

VKM is requested to elucidate the consequence of amending the current maximum limit of 200 µg for folic acid in food supplements for the total intake of folic acid (from food supplements and foods added folic acid).

Increasing the maximum limits in food supplements to 400 µg will imply exceedance of UL for children younger than 6 years and an intake close to UL in children 7-10 years. An increase in the maximum limits in food supplements to 600 µg will imply exceedance of UL for children younger than 10 years and an intake close to UL in children 11-14 years. Increasing the maximum limits in food supplements to 400 µg or 600 µg will not imply exceedance of UL among adults as evaluated in the current food fortification model.

7 Data gaps

In reviewing the literature, three major issues limiting the possibilities to draw firm conclusions regarding a possible harmful effect of folic acid supplementation appear:

- Most randomised controlled clinical trials of folic acid supplementation have used other vitamins in addition to folic acid. Thus, it is not possible to distinguish a possible adverse effect of folic acid from that of the other vitamins.
- Most studies have been of relatively short duration, cancer is a condition which usually develops over several years.
- Few studies of folic acid supplementation have had cancer as the primary endpoint.

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Appendices

Appendix I, Search strategy

Database: Embase <1974 to 2014 September 03>
Search Strategy:
1 cancer*.mp. (2229418)
2 folic acid supplement*.mp. (2351)
3 1 and 2 (206)
4 limit 3 to (human and (danish or english or norwegian or swedish) and yr="2009 - Current") (88)

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>
Search Strategy:
1 folic acid supplement*.mp. (1870)
2 cancer*.mp. or Neoplasms/ (1306631)
3 1 and 2 (139)
4 limit 3 to (humans and yr="2009 -Current" and (danish or english or norwegian or swedish)) (44)

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily, Ovid MEDLINE(R) and Ovid OLDMEDLINE(R) <1946 to Present>
Search Strategy:
1 "folic acid*".m_titl. (6853)
2 limit 1 to (humans and yr="2009 -Current" and (danish or english or norwegian or swedish) and randomized controlled trial) (100)

Appendix II; Excluded papers

Reference	Topic	Reason for exclusion
Burdge G.C., Lillycrop K.A. (2012) Folic acid supplementation in pregnancy: Are there devils in the detail? British Journal of Nutrition 108:1924-1.	Folic acid in pregnancy.	A discussion article. No original data.
Clarke R. et al. (2010) Effects of lowering homocysteine levels with B vitamins on cardiovascular disease, cancer, and cause-specific mortality: Meta-analysis of 8 randomized trials involving 37 485 individuals. Archives of Internal Medicine 170:1622-16.	Folic acid supplementation for 5 years and Hcy.	Main outcome CVD. Only multiple vitamin supplementations.
Gargari B.P. et al. (2011) Effect of folic acid supplementation on biochemical indices in overweight and obese men with type 2 diabetes. Diabetes Research and Clinical Practice 94:33-38.	Effect of folic acid supplementation in overweight and obese with type 2 diabetes.	No information about cancer risk.
Hubner R.A., Houlston R.S. (2009) Folate and colorectal cancer prevention. British Journal of Cancer 100:233-239.	Colorectal cancer prevention.	A discussion article. Not a systematic review or meta-analyses.
Kotsopoulos J. et al. (2012) Folate and breast cancer: What about high-risk women? Cancer Causes and Control 23:1405-1420.	Folate and women with high risk for breast cancer.	Discussion paper.
Levine A.J. et al. (2010) Baseline plasma total homocysteine and adenoma recurrence: Results from a double blind randomized clinical trial of aspirin and folate supplementation. Cancer Epidemiology Biomarkers and Prevention 19:2541-2548.	Aspirin and folate supplementation and plasma homocysteine and adenoma recurrence.	Concludes on plasma tHcy, not folic acid, and risk of colorectal adenomas.
Levine A.J. et al. (2010) A candidate gene study of folate-associated one carbon metabolism genes and colorectal cancer risk. Cancer Epidemiology Biomarkers and Prevention 19:1812-1821.		Discussion if geneotype had anything to say about folic acid supplementation on colorectal cancer.
Levine A.J. et al. (2008) MTHFR genotype and colorectal adenoma recurrence: Data from a double-blind placebo-controlled clinical trial. Cancer Epidemiology Biomarkers & Prevention 17:2409-2415.	Genotype of MTHFAR and colorectal cancer.	Discussion if geneotype had anything to say about folic acid supplementation on colorectal cancer.

Reference	Topic	Reason for exclusion
Lubecka-Pietruszewska K. et al. (2013) Folic acid enforces DNA methylation-mediated transcriptional silencing of PTEN, APC and RARbeta2 tumour suppressor genes in breast cancer. Biochemical and Biophysical Research Communications 430:623-628.	Folic acid and regulation of tumor suppressor genes.	In vitro study.
Lucock M., Yates Z. (2009) Folic acid fortification: A double-edged sword. Current Opinion in Clinical Nutrition and Metabolic Care 12:555-564.	Folic acid fortification.	A discussion article.
Manizheh S.M. et al. (2009) Comparison study on the effect of prenatal administration of high dose and low folic acid. Saudi Medical Journal 30:88-97	Folic acid supplementation in pregnancy 5 mg/day and 0.5 mg/day.	Cancer is not a topic in this article.
Nan H., Lee J.E., Rimm E.B., Fuchs C.S., Giovannucci E.L., Cho E. (2013) Prospective study of alcohol consumption and the risk of colorectal cancer before and after folic acid fortification in the United States. Annals of Epidemiology 23:558-563	Alcohol consumption, colorectal cancer and folic acid.	Prevention report - low dose of folic acid.
Ortega-Garcia J.A. et al. (2010) Case control study of periconceptional folic acid intake and nervous system tumors in children. Child's Nervous System 26:1727-1733	Periconseptive intake of folic acid and neuroectoderm tumors in children.	Prevention study.
Smith D.E.C. et al. (2013) Folic acid supplementation does not reduce intracellular homocysteine, and may disturb intracellular one-carbon metabolism. Clinical Chemistry and Laboratory Medicine 51:1643-1650	Folic acid supplementation and intracellular Hcy concentration.	Cancer is not a topic in this article.
Taneja S. et al. (2013) Folic acid and vitamin B-12 supplementation and common infections in 6-30-mo-old children in India: A randomized placebo-controlled trial. American Journal of Clinical Nutrition 98:731-737	Folic acid and vitamin B_{12} supplementation and common infections in children performed in India.	Cancer is not a topic in this article.
Vila-Nova C. et al. (2013) Periconceptional use of folic acid and risk of miscarriage - findings of the Oral Cleft Prevention Program in Brazil. Journal of Perinatal Medicine 41:461-466	Periconceptual folic acid supplementation and risk of miscarriages.	Cancer is not a topic in this article.

Reference	Topic	Reason for exclusion
Weggemans R.M. et al. (2009) Toward an optimal use of folic acid: An advisory report of the Health Council of the Netherlands. European Journal of Clinical Nutrition 63:1034-1036	Optimal use of folic acid.	An advisory report of the Health Council of the Netherlands. No original data.
Zappacosta B. et al. (2009) Genotype prevalence and allele frequencies of 5,10-methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms in Italian newborns. Laboratory Medicine 40:732-736	Folic acid supplementation and genotype prevalence.	Cancer is not a topic in this article.
Zhou Y.H. et al. (2011) Effect of folic acid supplementation on cardiovascular outcomes: A systematic review and meta-analysis. PLoS ONE 6	Folic acid and cardiovascular outcomes.	Cancer is not a topic in this article.

Appendix III; Summary Tables

RCTs and prospective studies

Reference	Gao Q.Y., Chen H.M., Chen Y.X., Wang Y.C., Wang Z.H., Tang J.T., Ge Z.Z., Chen X.Y., Sheng J.Q., Fang D.C., Yu C.G., Zheng P., Fang J.Y. (2013) Folic acid prevents the initial occurrence of sporadic colorectal adenoma in Chinese older than 50 years of age: A randomized clinical trial. Cancer Prevention Research 6:744-752.
Study design and type	Prospective, randomised, controlled design comparing daily supplementation with 1 mg folic acid with controls.
Objective	Primary outcome parameter was incidence of any type of CRA (tubulovillous, tubular villous or serrated lesions at 3 year follow-up colonoscopy in both groups. Evaluation of plasma folate at the beginning and end of follow-up period.
Number of participants, country and age	980 individuals from China age more than 50 years.
Baseline characteristics of study subjects/cells	No adenoma confirmed by colonoscopy entered run–in-period. 120 individuals excluded (unable to avoid taken medication or supplements prohibited by the study, decline to continue the study, no adherent, excluded for other reasons. No differences in the baseline characteristics between the folic acid and control group.
Exposure, substance, food, (type and amount)	1 mg/day folic acid supplement, controls treated without folic acid.
Measurement of exposure (biomarker, internal validation)	Adenomas.
Follow-up period, drop-outs	Seven hundred ninety-one (91.98%) underwent colonoscopy after 3 years of follow-up. The remaining 69 participants was lost to follow-up, discontinued the intervention or were unable to avoid regularly taking folic acid for more than 2 weeks and 2 in each group developed CRCs.
Health outcome	See over.
Measurement of outcome	See over.
Statistical analysis	Enough participants were selected to provide power of at least 80% to detect a risk detection with folic acid using two-sided statistical significance level of $p \leq 0.05$. Data collection was conducted using Case Report Form stored in a validated database that conformed GCP requirements. Predefined primary statistical analysis X^2 test with a significant level of 5% comparing the risk of CRA between the two groups. Cox regression methods.

Reference	Gao Q.Y., Chen H.M., Chen Y.X., Wang Y.C., Wang Z.H., Tang J.T., Ge Z.Z., Chen X.Y., Sheng J.Q., Fang D.C., Yu C.G., Zheng P., Fang J.Y. (2013) Folic acid prevents the initial occurrence of sporadic colorectal adenoma in Chinese older than 50 years of age: A randomized clinical trial. Cancer Prevention Research 6:744-752.
Results	After 3 years of follow-up, it was found that folic acid supplementation may decrease the risk of CRA. In the intention –to-treat population, CRA occurred in 64 (14.88%) participants in the folic acid group and in 132 (30.70%) in the control group. 3 or more adenomas occurred in 14 participants in the folic group and 20 in the control group. Adenoma occurred on the left side of the colon in 42 participants in the folic acid group and 78 in the control group. There was no such trend in the right –sided adenoma. Adjustment for age, sex, clinical centre and duration of follow –up at baseline did not substantially affect the results. Baseline folic acid levels did not differ between the two groups, but did show a difference between the participants who developed CRA and those who did not. The results indicate that there may be an effective therapeutic level of folic acid and that people who do not achieve this level may still be at risk of CRA.
Conclusion	Participants with low plasma folate may have a high risk of CRA. Primary prevention with 1 mg /day folic acid supplementation could reduce the incidence of CRA, especially left-sided and advanced disease with no previous adenomas.
Confounders adjusted for	Age, sex, clinical centre and duration of follow up.
Relevance for our risk assessment purpose	Yes.

Reference	Wu K., Platz E.A., Willett W.C., Fuchs C.S., Selhub J., Rosner B.A., Hunter D.J., Giovannucci E. (2009) A randomized trial on folic acid supplementation and risk of recurrent colorectal adenoma. American Journal of Clinical Nutrition 90:1623-1631.
Study design and type	RCT.
Objective	To assess the effect of folic acid supplementation (1mg/day) for 5-6.5 years on recurrence of colorectal adenomas.
Number of participants, country and age	338 in treatment group and 334 in placebo group collected from Health Professionals Follow-up Study and Nurses' Health Study. Age between 50- 78 years. USA.
Baseline characteristics of study subjects/cells	Patients were diagnosed with colorectal adenomas which were removed. Folate status before inclusion was a evaluated.
Exposure, substance, food, (type and amount)	1 mg folic acid supplementation for 5-6.5 years and placebo.
Measurement of exposure (biomarker, internal validation)	Plasma folate concentration.
Follow-up period, drop-outs	5-6.5 years.
Health outcome	Recurrence of colorectal adenomas.
Measurement of outcome	Endoscopies.
Statistical analysis	Intention to treat approach. Risk was evaluated by risk ratios calculated with use of generalised linear model. Adjustments were made for age, sex, extension group, and time between the start and the last follow up endoscopy.
Results	Incidence of at least one recurrent adenomas was 0.82 (0.59,1.13). Among patients with low plasma folate concentration at baseline (≤7.5ng (mL) and receiving folic acid supplementation had a significant reduced risk of recurrence of adenomas 0.61 (0.42,0.90). Neither was there evidence of increased risk of advanced or multiple adenomas with folic acid supplementation.
Conclusion	No increased risk of recurrence of colorectal adenomas with folic acid supplementation of 1 mg/day for 5-6.5 years.
Confounders adjusted for	Analyses were adjusted for age, sex, extension group, and time between the start and the last follow up endoscopy.
Relevance for our risk assessment purpose	Yes, high relevance for this risk assessment.

Reference	Figueiredo J.C., Grau M.V., Haile R.W., Sandler R.S., Summers R.W., Bresalier R.S., Burke C.A., McKeown-Eyssen G.E., Ba J.A. (2009) Folic acid and risk of prostate cancer: Results from a randomized clinical trial. Journal of the National Cancer Institute 101:432-435.
Study design and type	Secondary analysis of a RCT, USA.
Objective	Measure the risk of prostate cancer following supplementation of 1 mg folic acid. Measure to what extent folate status at baseline predicted prostate cancer. The study was carried out between 1994 and 2006.
Number of participants, country and age	651 randomised and 643 included in the analysis.
Baseline characteristics of study subjects/cells	Measured B vitamins, B_{12} concentration at baseline was lower in the folic acid supplementation group.
Exposure, substance, food, (type and amount)	Riboflavin, folate, vitamin B_6 and vitamin B_{12} status at baseline, folic acid supplement use.
Measurement of exposure (biomarker, internal validation)	Intervention (folic acid supplementation or not), vitamin analysis in plasma.
Follow-up period, drop-outs	Median 7 years, for up to 10 years.
Health outcome	Prostate cancer.
Measurement of outcome	Medical reports for prostate cancer that was confirmed by histopathology.
Statistical analysis	Intent to treat. Cox proportional hazards models to measure the association between baseline vitamin B status and prostate cancer incidence and the effect of folic acid supplementation on prostate cancer.
Results	Folic acid supplementation was associated with a an increased risk of prostate cancer (HR 2.63: 95% CI 1.23 to 5.65) Plasma folate concentration was inversely associated with the risk of prostate cancer (borderline significant).
Conclusion	Evidence that daily supplementation with 1 mg of folic acid was associated with an increased risk of prostate cancer.
Confounders adjusted for	Several relevant.
Relevance for our risk assessment purpose	Relevant. Albeit secondary analysis, this is RCT with a long follow up time that demonstrates a substantial and significant effect of folic acid supplementation on cancer risk.

Reference	Milne E., Greenop K.R., Bower C., Miller M., Van Bockxmeer F.M., Scott R.J., De Klerk N.H., Ashton L.J., Gottardo N.G., Armstrong B.K. (2012) Maternal use of folic acid and other supplements and risk of childhood brain tumors. Cancer Epidemiology Biomarkers and Prevention 21:1933-1941.
Study design and type	Case control study.
Objective	The aim of this study was to measure to what extent maternal folic acid supplementation other supplements was associated with childhood brain tumors.
Number of participants, country and age	327 cases from 10 Australian pediatric oncology centres. 867 controls selected randomly. 2005-2010.
Baseline characteristics of study subjects/cells	0-14 years, Australian.
Exposure, substance, food, (type and amount)	Folic acid supplements or other supplements during pregnancy.
Measurement of exposure (biomarker, internal validation)	Mailed questionnaires with questions on folic acid supplements or intake of other vitamins before and / or during pregnancy, specified by trimester. Non compliers were contacted by phone.
Follow-up period, drop-outs	N.a.
Health outcome	Childhood brain tumor.
Measurement of outcome	Children with tumors were recruited from 10 pediatric oncology centers.
Statistical analysis	Unconditional logistic regression. Exposure variables were whether or not folic acid was taken prepregnancy, first trimester or during the last two trimesters of pregnancy. A dose response relation between folic acid and risk of cancer was also estimated.
Results	Prepregnancy supplementation of folic acid was associated with a significant reduction in the odds of childhood brain tumor (OR 0.65 95CI 0.43 to 0.98). The effect of folic acid during pregnancy was lower and did not reach statistical significance. The effect of providing other vitamins or minerals was much less clear and did usually not attain statistical significance.
Conclusion	Inverse association of child brain tumors and prepregnancy use of folic acid. The effect of giving folic acid during pregnancy was less clear but there was some protection when given during early pregnancy.
Confounders adjusted for	Age, state of residence, ethnicity, maternal age, child's birth year, maternal education, and source of data (questionnaire or phone).

Reference	Milne E., Greenop K.R., Bower C., Miller M., Van Bockxmeer F.M., Scott R.J., De Klerk N.H., Ashton L.J., Gottardo N.G., Armstrong B.K. (2012) Maternal use of folic acid and other supplements and risk of childhood brain tumors. Cancer Epidemiology Biomarkers and Prevention 21:1933-1941.
Relevance for our risk assessment purpose	Relevant.

Reference	Amigou A., Rudant J., Orsi L., Goujon-Bellec S., Leverger G., Baruchel A., Bertrand Y., Nelken B., Plat G., Michel G., Haouy Chastagner P., Ducassou S., Rialland X., Hemon D., Clavel J. (2012) Folic acid supplementation, MTHFR and MTRR polymorphisms, and the risk of childhood leukemia: The ESCALE study (SFCE). Cancer Causes and Control 23:1265-1277.
Study design and type	Frequency matched case control study.
Objective	To measure the association between maternal folic acid supplementation before and during pregnancy and the risk of childhood acute leukemia. Measure the association between MTHFR and MTRR genetic polymorphisms and acute leukemia.
Number of participants, country and age	764 cases with childhood acute leukemia and 1681 controls.
Baseline characteristics of study subjects/cells	Cases identified from the French National registry of Childhood Hematopoietic Malignancies between January 2003 and December 2003. Cases and controls were less than 15 years. Randomly selected controls.
Exposure, substance, food, (type and amount)	Folic acid supplement used before or during pregnancy.
Measurement of exposure (biomarker, internal validation)	Telephone interview with the mothers 1-24 months after diagnosis (median 4 months) Collected information on other variables as well. Genotyped 97% of the cases and 48% of the controls.
Follow-up period, drop-outs	N.a.
Health outcome	N.a.
Measurement of outcome	Cases (childhood acute leukemia) vs. controls. Also measured the association in various sub-groups based on type of leukemia.
Statistical analysis	Multiple logistic regressions.
Results	Folic acid supplementation before and during pregnancy was associated with a substantial and significant reduction in the odds of childhood acute leukemia. Subtype of leukemia did not modify this association.
Conclusion	Maternal folic acid supplementation before or during pregnancy may reduce the risk of childhood acute leukemia. They also suggest that the genotype homozygous for variants of the genes required for folate metabolism may be a risk factor for acute leukemia.
Confounders adjusted for	Not stated.
Relevance for our risk assessment purpose	Relevant.

Unmetabolised folic acid

Reference	Boilson A., Staines A., Kelleher C.C., Daly L., Shirley I., Shrivastava A., Bailey S.W., Alverson P.B., Ayling J.E., McDermott A.P., MacCooey A., Scott J.M., Sweeney M.R. (2012) Unmetabolized folic acid prevalence is widespread in the older Irish population despite the lack of a mandatory fortification program. American Journal of Clinical Nutrition 96:613-21.
Study design and type	Cross sectional study, Ireland.
Objective	The aim of this study was to measure the basal (fasted) concentrations of unmetabolised folic acid in the plasma of an elderly population group exposed to the liberal voluntary fortification of foodstuffs in Ireland.
Number of participants, country and age	137 subjects were included in this cross sectional study, aged 60-86 years. Ireland with liberal voluntary folic acid fortification.
Baseline characteristics of study subjects/cells	Subjects excluded were those who had high creatinine concentration or high ALAT.
Exposure, substance, food, (type and amount)	Diet and folic acid supplement use.
Measurement of exposure (biomarker, internal validation)	A semi quantitative food frequency questionnaire measuring average intake previous 7 days.
Follow-up period, drop-outs	N.a.
Health outcome	N.a.
Measurement of outcome	Plasma folate and red blood cell folate analyses were conducted by using the *Lactobacillus casei* microbiological assay. UFA was measured by a column-switching HPLC method by using fluorescence detection after postcolumn coulometric oxidation and homocysteine analysis was performed by using a commercially available kit supplied. Genotyping of the *MTHFR* (methylenetetrahydrofolate reductase) 677C>T polymorphism (rs1801133) and the MTHFD1L (mitochondrial 10-formyltetrahydrofolate synthetase) deletion insertion polymorphism [c.781–6823 ATT (7–9); rs3832406] was carried out.

Reference	Boilson A., Staines A., Kelleher C.C., Daly L., Shirley I., Shrivastava A., Bailey S.W., Alverson P.B., Ayling J.E., McDermott A.P., MacCooey A., Scott J.M., Sweeney M.R. (2012) Unmetabolized folic acid prevalence is widespread in the older Irish population despite the lack of a mandatory fortification program. American Journal of Clinical Nutrition 96:613-21.
Statistical analysis	Because a proportion of subjects had no folic acid intake (from vitamins or fortified food), there were many zeros in the data. Thus, exposure was categorised (total) habitual and recent folic acid intake to create two 5-category variables. The first category was no folic acid intake, and the remaining 4 categories were defined by the quartile cutoffs in the nonzero data. Groups were compared by using the independent *t* test or a 1-factor ANOVA as appropriate. Interrelations between the log-transformed variables were examined with scatter plots and Pearson product-moment correlations (excluding pairwise subjects with zero values of folic acid intake). Multiple regressions were performed with logUFA as the dependent variable and continuous variables or dummy indicator variables as predictors.
Results	Unmetabolised folic acid was detected in 94.1% of the cohort with a mean concentration of 0.39 nmol/L (range: 0.07-1.59 nmol/L), accounting for 1.3% of total plasma folate. UMFA correlated significantly with intake of folic acid. In the participant not stating folic acid intake UFA concentrations were significantly lower than in subjects who recently had an intake. In this small study they found no significant correlations of the genotyping with MTHFR 677C>T, DHFR 19-bp interon deletion or MTHFDIL were found.
Conclusion	Unmetabolised folic acid was detected in 94.1% of the cohort with a mean concentration of 0.39 nmol/L (range: 0.07-1.59 nmol/L), accounting for 1.3% of total plasma folate.
Confounders adjusted for	Not stated.
Relevance for our risk assessment purpose	Unmetabolised folic acid is found in a country with voluntary folic acid supplementation (no mandatory folic acid fortification) in the age group 60-86 years.

Reference	Bailey R.L., Mills J.L., Yetley E.A., Gahche J.J., Pfeiffer C.M., Dwyer J.T., Dodd K.W., Sempos C.T., Betz J.M., Picciano M.F. (2010) Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged >60 y in the United States. American Journal of Clinical Nutrition 92:383-389.
Study design and type	Cross sectional study, USA.
Objective	The objective was to examine UMFA concentrations in relation to dietary and supplemental folic acid and status of biomarkers in the US population aged ≥60 y with use of data from NHANES.
Number of participants, country and age	1121 individuals aged ≥60 y. USA and NHANES 2001-2002.
Baseline characteristics of study subjects/cells	All eligible subjects were included, excluding those who had taken a food supplement before the fasting blood drawing, those who had self-reported anemia therapy or those who had high creatinine concentration or high ALAT.
Exposure, substance, food, (type and amount)	One 24-hour dietary recall was made and calculated intake of folate was done from diet and supplements. Information about dietary supplements was collected for the past 30 days.
Measurement of exposure (biomarker, internal validation)	UMFA and 5-methyltetrahydrofolate (5-MTHF) concentrations were determined by using a revised affinity/HPLC method with electrochemical (coulometric) detection Serum folate, red blood cell (RBC) folate and serum vitamin B_{12} were analysed by using the Quantaphase II radioassay from BioRad, Hercules, CA.
Follow-up period, drop-outs	N.a.
Health outcome	N.a.
Measurement of outcome	N.a.
Statistical analysis	Descriptive statistics (means and medians) were estimated for all variables by using PROC REG and PROC DESCRIPT in SAS-callable Sudaan. Any variables with a skewness >4 were log-transformed before group comparison analysis. The range of UMFA was quite large (0–273 nmol/L); the highest 2 data points (273 and 185 nmol/L) were extreme outliers and were winsorised to the next highest value of 85 nmol/L to remove the influence that these points. All statistical comparisons were controlled for sex, age, and race-ethnicity and presented as least-squares means. SEs for all statistics of interest were approximated by Taylor series linearisation, and significance was set at a Bonferroni-adjusted *P* value ≤ 0.006 to account for multiple comparisons.

Reference	Bailey R.L., Mills J.L., Yetley E.A., Gahche J.J., Pfeiffer C.M., Dwyer J.T., Dodd K.W., Sempos C.T., Betz J.M., Picciano M.F. (2010) Unmetabolized serum folic acid and its relation to folic acid intake from diet and supplements in a nationally representative sample of adults aged >60 y in the United States. American Journal of Clinical Nutrition 92:383-389.
Results	UMFA was detected in 38% of the population, with a mean concentration of 4.4 ± 0.6 nmol/L (median: 1.2± 0.2 nmol/L). The group with UMFA (UMFA+) had a significantly higher proportion of folic acid supplement users than did the group without UMFA (60% compared with 41%). UMFA+ men and women also had higher supplemental and total (food + supplements) folic acid intakes than did their counterparts without UMFA. Forty percent of the UMFA+ group was in the highest quartile of total folic acid intake, but total folic acid intake was only moderately related to UMFA concentrations (r^2 = 0.07). Serum folate concentrations were significantly higher in the UMFA+ group and were predictive of UMFA concentrations (r^2 = 0.15). Serum 5-methyltetrahydrofolate and vitamin B-12 concentrations were higher in the UMFA+ group, whereas there was no difference between the two UMFA groups in red blood cell folate, serum homocysteine, or methylmalonic acid concentrations.
Conclusion	Approximately 40% of older adults in the United States have UMFA that persists after a fast, and the presence of UMFA is not easily explained in NHANES by folic acid intakes alone.
Confounders adjusted for	Sex, age, and race-ethnicity.
Relevance for our risk assessment purpose	Unmetabolised folic acid is found in subjects in countries with voluntary folic acid fortification.

Reference	Morris M.S., Jacques P.F., Rosenberg I.H., Selhub J. (2010) Circulating unmetabolized folic acid and 5-methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors. American Journal of Clinical Nutrition 92:1002-1002.
Study design and type	Cross sectional study, USA.
Objective	To look at the associations between higher folate status and anemia and cognitive test performance to circulating unmetabolised folic acid or 5-methyltetrahydrofolate (5MTHF).
Number of participants, country and age	1858 senior participants in NHANES 1999-2002. USA. Aged ≥60 years. The participants had normal renal function and reported no history of stroke, recent anemia therapy, or diseases of the liver, thyroid, or coronary arteries.
Baseline characteristics of study subjects/cells	Subjects excluded were those who had high creatinine concentration or high ALAT.
Exposure, substance, food, (type and amount)	One 24-hour dietary recall was made and calculated intake of folate was done from diet and supplements only from those included in 2001 and 2002. Information about dietary supplements was collected for the past 30 days.
Measurement of exposure (biomarker, internal validation)	The analyses included measurement of serum concentrations of vitamin B_{12} and folate, which were carried out by using the Quantaphase II Radioassay Kit (Bio-Rad Laboratories, Anaheim, CA). Plasma methylmalonic acid (MMA) was measured by gas chromatography–mass spectrometry with cyclohexanol derivatisation. Plasma homocysteine was analysed by using a commercially available fluorescence polarisation immunoassay kit (Abbott Laboratories, Abbott Park, IL) on the Abbott IMx analyser. Serum ferritin was measured by using the QuantaImmune Ferritin IRMA Kit (Bio-Rad Laboratories). Serum C-reactive protein and cystatin C were quantified by particle-enhanced nephelometry, and serum creatinine concentration was based on the Jaffe reaction.
Follow-up period, drop-outs	N.a.
Health outcome	Anemia, macrocytosis and cognitive performance.
Measurement of outcome	The cognitive function of seniors was assessed by using a version of the Digit-Symbol Substitution Test (DSST) of the Wechsler Adult Intelligence Scale III—a screening test designed to detect cognitive impairment in adults and children. For the main data analyses, the score on the test as a continuously scaled term was used. However, for the purpose of presenting results of preliminary data analyses, subjects were classified as having performed poorly or well using a score of 34—the 20th percentile of the distribution—as the cutoff between the 2 categories.

Reference	Morris M.S., Jacques P.F., Rosenberg I.H., Selhub J. (2010) Circulating unmetabolized folic acid and 5-methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors. American Journal of Clinical Nutrition 92:1002-1002.
Statistical analysis	Multiple linear and logistic regression models were created for initial analyses of interaction and thereafter for the full models. All analyses were adjusted for sex, age, race-ethnicity, current smoking status, serum cystatin C, serum C-reactive protein, and an alcohol-intake variable (ie, current alcohol intake for anemia and macrocytosis and self-reported history of alcohol abuse for cognitive test performance). Analyses focused on anemia were additionally adjusted for triceps skinfold thickness and self-reported diabetes status. Analyses focused on macrocytosis were additionally adjusted for body mass index, cancer history, and serum ferritin. Analyses focused on cognitive test performance were additionally adjusted for educational achievement and self-reported history of cancer and diabetes.
Results	Circulating unmetabolised folic acid was detected in ≈33% of the subjects and was related to an increased odds of anemia in alcohol users. In seniors with a serum vitamin B_{12} concentration <148 pmol/L or a plasma methylmalonic acid concentration ≥210 nmol/L, the presence compared with the absence of detectable circulating unmetabolised folic acid was related to lower cognitive test scores and lower mean cell volume. In the same subgroup, higher serum 5MeTHF was related to an increased odds of anemia and a marginally significantly decreased odds of macrocytosis. In seniors with a normal vitamin B_{12} status, a higher serum 5MeTHF concentration was related to higher cognitive test scores.
Conclusion	Unmetabolised folic acid was related to lower cognitive test scores in combination with low B_{12} concentration.
Confounders adjusted for	Sex, age, race-ethnicity, current smoking status, serum cystatin C, serum C-reactive protein, and an alcohol-intake variable See statistics.
Relevance for our risk assessment purpose	Unmetabolised folic acid in combination with low B_{12} concentration was associated with lower cognitive test scores.

Reference	Obeid R., Kasoha M., Kirsch S.H., Munz W., Herrmann W. (2010) Concentrations of unmetabolized folic acid and primary folate forms in pregnant women at delivery and in umbilical cord blood. American Journal of Clinical Nutrition 92:1416-1422.
Study design and type	Cross sectional study, Germany.
Objective	Investigate total folate, tetrahydrofolate (THF), 5-methyltetrahydrofolate (5-MTHF), formyl-THF, 5,10-methenylTHF, and folic acid concentrations in women and in umbilical cord blood at delivery.
Number of participants, country and age	87 pregnant women and 29 cord blood - including 24 mother/child pairs and 25 non-pregnant women who were not taking any supplement containing folic acid were included in the study.
Baseline characteristics of study subjects/cells	Subjects excluded were those who had high creatinine concentration or high ALAT.
Exposure, substance, food, (type and amount)	400 µg of folate daily (n=25) compared to those not supplemented pregnant women (n=61).
Measurement of exposure (biomarker, internal validation)	Just information of supplementation. No information on dietary data was collected.
Follow-up period, drop-outs	N.a.
Health outcome	N.a.
Measurement of outcome	Peripheral venous blood samples were obtained from mothers 1–12 h before birth. Concentrations of tHcy were measured by gas chromatography-mass spectrometry as described elsewhere (21). Concentrations of primary folate forms were measured in serum on an Acquity Ultra Performance LC system (Waters Corporation, Milford, MA) coupled to a MicroMass Quattro Premier XE tandem quadrupole mass spectrometer (Waters Corporation). Validation is described.
Statistical analysis	Comparisons of medians of 2 groups — supplemented and non-supplemented or pregnant and non-pregnant—were performed by using the Mann-Whitney U test. Possible differences in the means of different variables between maternal and cord serum were investigated by using a paired Student's t test. Chi-square tests were performed to test differences in categorical variables. Correlations between different variables were examined by the Spearman's test.

Reference	Obeid R., Kasoha M., Kirsch S.H., Munz W., Herrmann W. (2010) Concentrations of unmetabolized folic acid and primary folate forms in pregnant women at delivery and in umbilical cord blood. American Journal of Clinical Nutrition 92:1416-1422.
Results	Pregnant women who received 400 µg folic acid daily had higher total folate (P = 0.041), 5-MTHF (P = 0.049), and formyl-THF (P < 0.001) concentrations and slightly higher THF (P = 0.093) concentrations than did non-supplemented pregnant women. The measured folic acid concentrations >0.20 nmol/L in 38 (44%) pregnant women and in 55% of the cord serum samples, but these measurements were not explained by maternal supplement use. Concentrations of folic acid were nonsignificantly higher in cord blood from supplemented women than in cord blood from non-supplemented women (P = 0.154). Proportions of folic acid to total folate in cord serum did not differ according to maternal supplement usage (0.54% compared with 0.43% in supplemented and non-supplemented women, respectively). Concentrations of folic acid did not differ between maternal and cord serum. However, folic acid constituted a significantly lower proportion of total folate in cord serum than in maternal serum.
Conclusion	Unmetabolised folic acid in more than one-half of cord blood samples was detected. Folic acid (400 µg/d) supplied during pregnancy is not likely to accumulate in the fetus, in contrast to 5-MTHF and THF, which accumulate in the foetus.
Confounders adjusted for	Not done.
Relevance for our risk assessment purpose	Unmetabolised folic acid does not seem to accumulate in cord blood from supplemented or non-supplemented women.

Reference	Nguyen P., Tam C., O'Connor D.L., Kapur B., Koren G. (2009) Steady state folate concentrations achieved with 5 compared with 1.1 mg folic acid supplementation among women of childbearing age. American Journal of Clinical Nutrition 89:844-852.
Study design and type	Randomised, 2-arm, open-label, intervention study.
Objective	To compare steady state folate concentrations in women of childbearing age who took 5 or 1.1 mg folic acid daily for 30 weeks.
Number of participants, country and age	40 nonpregnant women between 18-45 years. Canada.
Baseline characteristics of study subjects/cells	Women did not take folic acid supplements.
Exposure, substance, food, (type and amount)	5 or 1.1 mg folic acid daily for 30 weeks. Folic acid was part of a multivitamin pill.
Measurement of exposure (biomarker, internal validation)	Plasma and red blood cell (RBC) folate.
Follow-up period, drop-outs	30 weeks. 2 participants dropped out, 1 in each arm.
Health outcome	Plasma and RBC concentrations.
Measurement of outcome	Plasma and RBC folate concentrations measured at baseline and at weeks 2, 4, 6, 12 and 30.
Statistical analysis	The primary analysis was to compare the plasma and RBC folate concentrations between the 2 groups.
Results	Plasma folate concentrations increased to 165.3 + 109.9 nmol/L for the 5 mg folic acid group and to 96.8 + 41.1 nmol/L for the 1.1 mg folic acid group by week 30 (differences were statistically significant). Significant differences were also found in RBC folate concentrations between groups at weeks 4, 6, 12 and 30. Concentrations at week 30 were 2339 $\pm$ 782 and 1625 $\pm$ 339 nmol/L for the 5 and 1.1 mg folic acid groups, respectively. At baseline, unmetabolised folic acid was detected in 70% and 65% of subjects in the 5 and 1.1 mg folic acid groups, respectively. By week 30, unmetabolised folic acid was detected n 58% of both groups.
Conclusion	The use of 5 mg folic acid produced higher blood folate concentrations, with a faster rate of folate accumulation, compared with 1.1 mg folic acid.
Confounders adjusted for	Other vitamins in the supplements were identical in the two groups.
Relevance for our risk assessment purpose	Relevant.

Reference	Sweeney M.R., Staines A., Daly L., Traynor A., Daly S., Bailey S.W., Alverson P.B., Ayling J.E., Scott J.M. (2009) Persistent circulating unmetabolised folic acid in a setting of liberal voluntary folic acid fortification. Implications for further mandatory fortification? BMC Public Health 9:295.
Study design and type	Two cross sectional studies, Ireland.
Objective	To examine the levels of circulatory unmetabolised folic acid in Irish adults and new-born infants before the proposed implementation of mandatory folic acid fortification. A secondary aim was to predict the increase in circulatory unmetabolised folic acid levels after fortification.
Number of participants, country and age	50 adult blood donors – non fasted (42 males – 8 females) (age 27-60 years) 20 mothers delivered by caesarean section and their infants (umbilical cord) fasted for 8 hours (age 26-39 years).
Baseline characteristics of study subjects/cells	The 50 blood donors had consumed their normal diet prior to inclusion The 20 pregnant women had fasted 8 hours prior to surgery.
Exposure, substance, food, (type and amount)	Exposure normal diets which include voluntary fortification of foods like milk, yoghurt, vegetable spreads cereals and bread. None of the pregnant women used folic acid supplementation before delivery (the latest 120 days before).
Measurement of exposure (biomarker, internal validation)	Plasma and red cell folate were analysed by L. casei microbiological assay and unmetabolised folic acid was analysed by HPLC method.
Follow-up period, drop-outs	N.a.
Health outcome	None.
Measurement of outcome	
Statistical analysis	Wilcoxon signed rank test and for prediction regression analyses in SPSS were performed assuming a linear increase as total plasma folate increases.

Reference	Sweeney M.R., Staines A., Daly L., Traynor A., Daly S., Bailey S.W., Alverson P.B., Ayling J.E., Scott J.M. (2009) Persistent circulating unmetabolised folic acid in a setting of liberal voluntary folic acid fortification. Implications for further mandatory fortification? BMC Public Health 9:295.
Results	Folic acid was present in 17 out of 20 babies (CI: 62.1%–96.8%) and 18 out of 20 mothers (fasted) (CI: 68.3%–99.8%) comprising 1.31% of the total plasma folate. There was a significant correlation between the maternal plasma folate concentrations and maternal plasma unmetabolised folic acid concentrations ($p = 0.007$, $r^2 = 0.300$), and maternal habitual folic acid intakes were correlated with maternal plasma folate concentrations ($p = 0.001$). A significant correlation between the maternal folic acid concentrations and cord blood folic acid concentrations ($p = 0.004$, $r^2 = 0.378$) was found. Unmetabolised folic acid was present in 49 out of 50 blood donors. After removing 2 samples, which were outliers, the results show that habitual folic acid intakes are significantly correlated with plasma folate levels ($p = 0.009$ $r^2 = 0.115$). Plasma folate was related to plasma unmetabolised folic acid concentrations ($p = 0.011$, r2 = 0.110). The prediction showed that mandatory fortification of bread would result in an expected mean plasma folate increase of 3.1 µg/L. Unmetabolised folic acid to absolute levels of plasma folate was predicted on a population level of 0.623 µg/L for unmetabolised folic acid concentration, i.e. approximately 12% increase from current levels.
Conclusion	With the voluntary folic acid fortification unmetabolised folic acid was already present in most subjects and with a mandatory fortification a further 12% increase was suggested.
Confounders adjusted for	Is not mentioned.
Relevance for our risk assessment purpose	Unmetabolised folic acid is found in subjects in countries with voluntary folic acid fortification.

Summary Tables Meta-analyses

Reference	Mackerras, J T., C L. (2014) Folic Acid, Selected Cancers and All-cause Mortality: A Meta-analysis. International Food Risk Analysis Journal 4.
Study types included	26 randomised controlled trials.
Aim of review	Systematic review of trials of folic acid supplementation on cancer incidence (total and site specific [colorectal, lung, breast, prostate]). In addition, the relation between folic acid and recurrence of colorectal adenoma, and total mortality. The search did not place any restrictions on health or disease outcome.
Timespan literature search	Search conducted May 2013. MEDLINE search from January 1, 2001 to 16 May 2013. Cochrane CENTRAL was also searched on May 16 2013.
Category of exposure	
Dose range in included studies	0.4-20 mg folic acid/day. Trials were excluded if folic acid was administered in a broad multivitamin/mineral supplement.
Timespan follow-up in included studies	Trials had to administer folic acid and placebos for one year or longer and have a follow-up period of one year or longer.
Comperators (placebo)	Any placebos for folic acid had to be a blank or minimal dose of folic acid; factorial designs testing other substances were permitted.
Evaluated for methodological quality?	
Grading of evidence methodology?	
Statistical analysis	RRs were calculated using counts from each study. An overall weighted (unadjusted) RR for each outcome was calculated de novo from the numbers randomised and event numbers using the DerSimonian-Laird inverse variance random effects method. Its 95%CI was calculated using the Greenland-Robins formula.
Results	No significant associations were found. No association with all-cause mortality. RR for total incident cancer (13 studies): 1.04.
Conclusion	No relation between folic acid supplementation, up to 5 mg per day, and risk of cancer or mortality.
Relevance for our risk assessment purpose	Low relevance as includes multiple vitamin supplementation studies.

Reference	Qin X., Cui Y., Shen L., Sun N., Zhang Y., Li J., Wang B., Xu X., Huo Y., Wang X. (2013) Folic acid supplementation and cancer risk: A meta-analysis of randomized controlled trials. International Journal of Cancer 133:1033-1041.
Study types included	15 randomised controlled trials.
Aim of review	To systematically evaluate the effect of folic acid on cancer risk, not including post-treatment trial observational follow-up studies.
Timespan literature search	Search of MEDLINE database from January 1966 to October 2012.
Category of exposure	
Dose range in included studies	0.5 – 40 mg/day.
Timespan follow-up in included studies	At least 6 months.
Comperators (placebo)	
Evaluated for methodological quality?	Studies were assessed for quality of randomisation, blinding, reporting of withdrawals, generation of random numbers and concealment of allocation (possible score 0-5). Quality of the 15 trials ranged from 3-5.
Grading of evidence methodology?	
Statistical analysis	RRs were used to measure the effect of folic acid supplementation on risk of cancer using a random-effects model. Possible effect modifications of several variables were examined.
Results	Overall, folic acid supplementation had no significant effect on total cancer incidence, colorectal cancer, other gastrointestinal cancer, prostate cancer, other genitourinary cancer, lung cancer, breast cancer, hematological malignancy and total cancer mortality. A significantly reduced risk was found for melanoma (RR 0. 47; 0.23-0.94). A higher total cancer incidence risk was found in trials with a high percent use of lipid lowering drugs or with lower percent baseline hypertension.
Conclusion	Folic acid supplementation has no significant effect on the incidence of total cancer or those mentioned under results, except for a reduced risk of melanoma.
Relevance for our risk assessment purpose	Low relevance as includes multiple vitamin supplementation studies.

Reference	Vollset S.E., Clarke R., Lewington S., Ebbing M., Halsey J., Lonn E., Armitage J., Manson J.E., Hankey G.J., Spence J.D., Galan P., Bonaa K.H., Jamison R., Gaziano J.M., Guarino P., Baron J.A., Logan R.F.A., Giovannucci E.L., Den Heijer M., Ueland P.M., Bennett D., Collins R., Peto R. (2013) Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: Meta-analyses of data on 50 000 individuals. The Lancet 381:1029-1036.
Study types included	13 randomised trials: 10 on prevention of cardiovascular disease, 3 in patients with colorectal adenoma.
Aim of review	To assess any effects on site-specific cancer rates in randomised trials of folic acid supplementation, at doses higher than those obtained from fortification.
Timespan literature search	All trials completed before 2011.
Category of exposure	
Dose range in included studies	0.5-2.5 mg/day. One trial with 40 mg/day, in renal patients.
Timespan follow-up in included studies	Treatment duration of at least one year. Average treatment duration 5.2 years.
Comperators (placebo)	
Evaluated for methodological quality?	
Grading of evidence methodology?	
Statistical analysis	The investigators obtained individual-level data from each trial, totalling 49 621 participants. Log-rank analyses were used to calculate cancer incidence rate ratio.
Results	Folic acid supplementation had no significant effect on overall cancer incidence during the first 5 years of treatment in the trials of cardiovascular disease prevention (RR=1.06, 95% CI 0.99-1.13). No trends towards greater effect with longer treatment. No significant effect on the incidence of cancer of the large intestine, prostate, lung, breast or any other specific site. In the 3 trials among patients with colorectal adenoma, the meta analysis found a nonsignificant RR= 1.33 (95% CI 0.98-1.80).
Conclusion	Folic acid supplementation had no significant effect on cancer incidence.
Relevance for our risk assessment purpose	Low relevance as includes multiple vitamin supplementation studies.

Reference	Baggott J.E., Oster R.A., Tamura T. (2012) Meta-analysis of cancer risk in folic acid supplementation trials. Cancer Epidemiology 36:78-81.
Study types included	6 randomised placebo-controlled trials of folic acid supplementation.
Aim of review	To evaluate the risk of cancer secondary to folic acid supplementation.
Timespan literature search	Search of PubMed on June 11, 2009. Subsequently, PubMed was searched on a nearly daily basis using the key word 'folate' to update the search – it is not clear which was the last search date.
Category of exposure	
Dose range in included studies	Folic acid: 0.5 – 2.5 mg/day. Supplements included other nutrients.
Timespan follow-up in included studies	Duration of intervention 12 months or more. Average duration 7 years.
Comperators (placebo)	
Evaluated for methodological quality?	Not mentioned.
Grading of evidence methodology?	
Statistical analysis	Weighted analysis of trial-specific effects. RR provided.
Results	One of the six trials found a significantly increased risk of cancer. Overall, cancer incidences were higher in the folic acid-supplemented groups than in the non-folic acid- supplemented groups (RR=1.21; 1.05-1.39).
Conclusion	Overall, cancer incidences were higher in the folic acid-supplemented groups than in the non-folic acid- supplemented groups.
Relevance for our risk assessment purpose	Low relevance as includes multiple vitamin supplementation studies.

Reference	Wien T.N., Pike E., Wisloff T., Staff A., Smeland S., Klemp M. (2012) Cancer risk with folic acid supplements: A systematic review and meta-analysis. BMJ Open 2.
Study types included	19 studies, including 12 randomised controlled trials. 10 of the 12 trials reported overall cancer incidence.
Aim of review	To explore if there is an increased cancer risk associated with folic acid supplements.
Timespan literature search	Search conducted March-May 2010, using MeSH and free-text search terms for folate, folic acid, cancer and neoplasm. The following electronic databases were searched: EMBASE, Ovid MEDLINE, Cochrane Library, Centre for Reviews and Dissemination, NHS evidence, Clincial evidence, and others.
Category of exposure	
Dose range in included studies	$\geq$ 0.4 mg/day. Up to 40 mg/day (renal disease patients). Folic acid with or without other B-vitamins.
Timespan follow-up in included studies	
Comperators (placebo)	Any.
Evaluated for methodological quality?	The quality of the included studies was assessed using the Cochrane Collaboration's tool for Risk of Bias assessment for RCTs and checklists for cohort and case-control studies.
Grading of evidence methodology?	
Statistical analysis	When feasible, the authors pooled data by meta-analyses with Cochrane Collaboration software and used random-effects model calculating RRs. PRISMA statement for reporting systematic reviews and meta-analyses. RRs are reported.
Results	In the 10 RCTs reporting overall cancer incidence, RR associated with folic acid supplements = 1.07 (1.00-1.14). Six RCTs reported prostate cancer incidence, with a combined RR=1.24 (1.03-1.49). No significant increased risk for any other cancer type. Six trials reporting overall cancer mortality found no significant differences between folic acid supplementation versus not (RR=1.09, 95% CI 0.90-1.30).
Conclusion	Meta-analysis of 10 RCTs showed a borderline significant increase in incidence of overall cancer in the folic acid group. Prostate cancer was the only site-specific cancer where folic acid significantly increased the risk.
Relevance for our risk assessment purpose	Low relevance as includes multiple vitamin supplementation studies.

Reference	Figueiredo J.C., Mott L.A., Giovannucci E., Wu K., Cole B., Grainge M.J., Logan R.F., Baron J.A. (2011) Folic acid and prevention of colorectal adenomas: A combined analysis of randomized clinical trials. International Journal of Cancer 129:192-203.
Study types included	Including 3 randomised controlled studies.
Aim of review	To determine the effect of folic acid supplementation and the risk of adenoma in patients with an adenoma history.
Timespan literature search	Search was not time limited. Keywords were folic acid supplementation, trial and colorectal adenoma.
Category of exposure	Folic acid supplementation.
Dose range in included studies	0.5 to 1 mg folic acid supplementation per day.
Timespan follow-up in included studies	From 6 months up to 42 months.
Comperators (placebo)	Yes, all were placebo controlled studies.
Evaluated for methodological quality?	The results from the three included studies were adjusted for confounding, but the Meta-analysis by itself was not adjusted. Only one study (the Harvard study) checked for folic acid compliance through blood status, while the two other checked with pill count.
Grading of evidence methodology?	
Statistical analysis	The investigators obtained individual-level data from each trial (n=2632). Standard random-effects meta-analysis methods.
Results	The random-effects meta-analysis the RR was 0.98 (95% CI 0.82,1.17) for all adenomas and 1.06 (0.81,1.39) for advanced lesions., however for those who received folate for over 3 years, the risk for adenomatous lesion was increased. The risk for associated with treatment was the highest for the occurrence of advanced lesions.
Conclusion	Overall, the risk of an adenomatous lesion was not increased or decreased among patients with a history of adenomas and who received folate supplementation for up to 3.5 years.
Relevance for our risk assessment purpose	This is the most relevant meta-analysis for our purpose because it includes only study with folic acid supplementation.

Reference	Fife J., Raniga S., Hider P.N., Frizelle F.A. (2011) Folic acid supplementation and colorectal cancer risk: A meta-analysis. Colorectal Disease 13:132-137.
Study types included	3 randomised or pseudorandomised trials.
Aim of review	To determine the effect of folic acid supplementation on colorectal cancer, the risk of adenoma or advanced adenomatous lesions, including RCR.
Timespan literature search	Search conducted July 2008. MEDLINE, EMBASE, Cochrane and CINAHL.
Category of exposure	
Dose range in included studies	1 mg/day for 3 years, 0.5 mg/day for 3 years, 2.5 mg/day for 5 years (+50 mg B6 and 1 mg B12).
Timespan follow-up in included studies	Supplementation for 3-years (1 mg/day) and 6 or 8 years colonoscopic follow-up, 0,5 mg/day 3 years follow-up, 2.5 mg/day 5 years follow-up.
Comperators (placebo)	
Evaluated for methodological quality?	
Grading of evidence methodology?	
Statistical analysis	Weighted treatment effect (using fixed effects) was calculated across trials using RevMan.
Results	Overall, the risk of an adenomatous lesion was not increased among patients who received folate supplementation for up to 3 years, however for those who received folate for over 3 years, the risk for adenomatous lesion was increased. The risk for associated with treatment was the highest for the occurrence of advanced lesions. After 3 years; OR was 1.09 (0.93-1.28). Above 3 years; OR was 1.35 (1.06-1.70).
Conclusion	Folate status is inversely related to the risk of developing colorectal cancer.
Relevance for our risk assessment purpose	Low relevance as includes multiple vitamin supplementation studies.

Reference	Ibrahim E.M., Zekri J.M. (2010) Folic acid supplementation for the prevention of recurrence of colorectal adenomas: Metaanalysis of interventional trials. Medical Oncology 27:915-918.
Study types included	5 Meta-analysis of interventional randomised, placebo controlled studies. Citations from PubMed, EMBASE, Cochrane databases and abstract from relevant proceedings.
Aim of review	Prospective phase II and III randomised clinical trials that directly compared folic acid supplementation given in a defined dose and a planned duration versus placebo to prevent recurrence of colorectal adenomas.
Timespan literature search	Comprehensive search for citation from PubMed, EMBASE, Cochrane database and abstracts of relevant proceedings. Time span not give
Category of exposure	
Dose range in included studies	0.5 mg/day for one year in one study, 1 mg /day of folic acid in 1 year in one study, for 2 years in a second study and 3 years in a third study, 5 mg/day for 1 year.
Timespan follow-up in included studies	Follow up for 3 years in 2 studies
Comperators (placebo)	
Evaluated for methodological quality?	The heterogeneity in methodologies was however significant.
Grading of evidence methodology?	
Statistical analysis	Data was analysed using RevMan.
Results	Examination of the effect of dose of folic acid showed that the two studies that had used 1 mg/day favoured folic acid over placebo with an odd ratio of 0.62. However the overall effect for all the included studies was not significant. OR was 1.08 (0.87-1.33).
Conclusion	Folate supplementation had no protective effect on the recurrence of colorectal adenomas, nor had it a positive outcome on the number of recurrent polyps pr patients.
Relevance for our risk assessment purpose	Low relevance as includes multiple vitamin supplementation studies.