# Folic Acid and Methylfolate

## Common Acronyms

| | |
|---|---|
| bw | body weight |
| CNS | Chinese Nutrition Society |
| CRN | Council for Responsible Nutrition |
| DRI | dietary reference intake |
| EC SCF | European Commission Scientific Committee on Food |
| EFSA | European Food Safety Authority |
| EVM | Expert Group on Vitamins and Minerals |
| HOI | highest observed intake |
| ICMR-NIN | Indian Council of Medical Research - National Institute of Nutrition |
| IOM | Institute of Medicine |
| IU | international unit |
| KNS | Korean Nutrition Society |
| LOAEL | lowest observed adverse effect level |
| NDA | EFSA Panel on Nutrition, Novel Foods and Food Allergens |
| NIH | National Institute of Health |
| NOAEL | no observed adverse effect level |
| RCT | randomized clinical trial |
| SUL | safe upper level |
| UF | uncertainty factor |
| UL | tolerable upper intake level |

## Introduction

Vitamin B9 is a water-soluble vitamin that is commonly referred to as “folate” (IOM 1998). The generic term, folate, refers to the naturally occurring compounds in foods that contain aromatic pteridine rings linked to a para-aminobenzoic acid that is bound to two to eight glutamic acid groups (Herbert 1999; EFSA 2023a). Natural folates are unstable, and degradation can occur in the presence of light and oxygen and at high temperatures (EFSA 2023a). Another form of

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vitamin B9 is folic acid, a fully oxidized pteroylmonoglutamic acid, which contains one glutamic acid. Folic acid is a synthetic form of folate (Herbert 1999; EFSA 2023a). Folic acid is the "most chemically stable form" of folate and is not biologically active in this oxidized form (NIH 2022; Martinez-Morata et al. 2024). Rather, folic acid is reduced in the body through dihydrofolate to tetrahydrofolate (THF) (Scaglione and Panzavolta 2014; EFSA, 2023a; Obeid et al. 2020; Martinez-Morata et al. 2024). Subsequently, THF is metabolized to glycine and 5,10-methylene-THF, which is ultimately converted into 5-MTHF, the biologically active form in circulation (Scaglione and Panzavolta 2014). These bioactive forms are involved in a wide variety of biochemical reactions, particularly one-carbon metabolic reactions required for the synthesis of purine and pyrimidine precursors of nucleic acids and homocysteine remethylation (NIH 2022; Scaglione and Panzavolta 2014). A deficiency of folic acid impairs DNA synthesis and cell division; the common clinical manifestation of severe folic acid deficiency is megaloblastic anemia (larger than normal but fewer red blood cells), which exhibits hematological similarities to vitamin B12 deficiency-induced anemia (NIH 2022; Drake 2023). In addition, sufficient intakes of folate during pregnancy are recommended to reduce the risk of neural tube defects (NTDs) and other birth defects (e.g., congenital heart defects and cleft lip palate) (NIH 2022). Elevated plasma homocysteine is one of the primary consequences of folate deficiency. Older individuals with low folate status are at higher risk of cognitive impairment, dementia, and/or Alzheimer's disease, and it has been postulated that the effect of folate deficiency on brain function is mediated by homocysteine (Quadri et al. 2004; Wang et al. 2001; Clarke et al. 1998).

Marketed dietary supplements contain folic acid and 5-methyltetrahydrofolic acid (5-MTHF, also referred to as methylfolate) with recommended dose levels ranging from 0.4 to 0.8 mg per day (NIH 2022). Several forms of synthetic folates are used in supplements, many of which are approved by the EFSA for use as a food additive or food supplement, including folic acid, calcium l-methylfolate, and (6S)-5-methyltetrahydrofolic acid, glucosamine salt (EFSA 2004, 2013, 2023a,b). In addition, the monosodium salt of l-5-methyltetrahydrofolic acid has been approved by the EFSA (2023b) for use as a novel food with "intended uses as a partial or complete substitute for folic acid and other sources of added folate."

Calcium l-methylfolate, (6S)-5-methyltetrahydrofolic acid, and the glucosamine and monosodium salts of l-5-methyltetrahydrofolic acid are all structurally similar and contain the

biologically active form (i.e., THF) of folate/folic acid, with the exception of an added methyl group to the pteridine ring (EFSA 2023a; Martinez-Morata et al. 2024). For each of these forms, when in solution, the salts dissociate from the MTHF and the MTHF directly enters circulation (EFSA 2004; EFSA 2013). The EFSA concluded that the bioavailabilities of these different MTHF salts are comparable (EFSA 2023b).

**Bioavailability**

Folic acid exhibits higher bioavailability when ingested as a supplement (~100%) or in a fortified food (~85%), when compared to 50% of folate occurring naturally in foods (NIH 2022; Drake 2023; Hoyo et al. 2011). In contrast, natural food-derived folates have variable bioavailability due to strong binding to proteins and carbohydrates in the food matrix. This binding "traps" the folates in the food matrix until released by enzymatic digestion in the gastrointestinal tract, thus affecting bioavailability (EFSA 2023a; Drake 2023). 5-MTHF, when consumed as a dietary supplement or in fortified foods, has higher bioavailability than folic acid when ingested at concentrations greater than 0.4 mg per day as the reduced form does not need to undergo the enzymatic reduction process (EFSA, 2022; EFSA 2004; EFSA 2013). In addition, the mechanism by which folic acid is reduced to THF has been shown to saturate easily, thus further limiting bioavailability relative to 5-MTHF (Scaglione and Panzavolta 2014). The different salt (i.e., calcium, sodium, and glucosamine) versions of 5-MTHF "are expected to be absorbed across the small intestine in a similar manner as 5-MTHF originating from dietary sources" (EFSA 2013).

Food folates must be deconjugated to their monoglutamate form by the intestinal enzyme folate conjugase prior to absorption (NIH 2022). Following absorption, dietary folic acid is activated in the same manner as diet-derived folates. The body has a finite capacity to metabolize folic acid. When higher amounts of folic acid (i.e., >0.2 mg) are consumed, much of the remaining folic acid is not converted to MTHF in first pass metabolism and results in the accumulation of unmetabolized folic acid (UMFA) (Kelly et al. 1997). After absorption, reduction of folate or folic acid to the 5-MTHF form occurs in intestinal cells and in the liver (EFSA 2023a; Obeid et al. 2020; Martinez-Morata et al. 2024). 5-MTHF is found in plasma as free 5-MTHF or 5-

MTHF bound to albumin or soluble folate receptors (EFSA 2023a; Scaglione and Panzavolta 2014; Drake 2023). Folates and folic acid are water soluble, and thus excretion is believed to be relatively straightforward, though this remains to be demonstrated experimentally.

## Safety Considerations

### *Folic Acid*

The administration of high levels of folic acid (5 mg per day or greater) to individuals with pernicious anemia or other causes of vitamin B12 deficiency can mask megaloblastic anemic manifestations associated with vitamin B12 deficiency, while allowing neurological disease (i.e., posterolateral spinal cord degeneration) to progress (Butterworth and Tamura 1989; IOM 1998; NIH 2022; Selhub et al. 2022; EFSA 2023a; Miller et al. 2024). It has also been suggested that high folic acid levels may also cause decreases in circulating vitamin B12 levels; for example, via the depletion of serum holotranscobalamin, as hypothesized by Selhub and coauthors (2022). Serious neurological and/or cognitive symptoms are most often associated with this sustained deficiency and are the result of progressive demyelination leading to peripheral neuropathy, areflexia, loss of proprioception and vibratory sense, and/or dementia-like effects (Hunt et al. 2014; Langan and Goodbred 2017). A recent narrative review summarized the available evidence for masking and/or exacerbation of vitamin B12 deficiency by folic acid and concluded that studies are limited to uncontrolled trials but provide justification for increased efforts to identify vitamin B12 deficiency in those at risk due to age, diet, impaired gastrointestinal absorption, or other causes (Miller et al. 2024; Selhub et al. 2022). The prevalence of vitamin B12 deficiency has been reported to vary by source, with estimates ranging from 6-20% in the United States and United Kingdom, 40% in Latin America, and 70% in Indian adults (Allen et al. 2009; Hunt et al. 2014; Langan and Goodbred 2017). Low or "marginal" vitamin B12 levels have been reported to occur in up to 40% in Western populations (NIH 2025). EFSA (2023a) noted that the prevalence of vitamin B12 deficiency is likely to be underdiagnosed due to limited diagnostic tools.

The masking of these neurological manifestations is not associated with the ingestion of folic acid at intake levels obtained through ordinary diets or from dietary supplements but is well

established for intakes of folic acid at or greater than 5 mg per day. CRN's *Vitamin and Mineral Safety* (3rd edition) discussed two studies that found no significant increase in risk of masking neurological effects with folic acid doses of 1.25 mg per day (Ross et al. 1948; Chodos and Ross 1951), whereas there is some evidence that masking may be a problem with intakes of 1.5 and 2.55 mg based on limited case studies (Victor and Lear 1956). In addition, the previous version of this chapter noted that few early reports showed some response in certain hematological indices for pernicious anemia patients taking folic acid doses as low as 0.1 to 0.8 mg; these effects are sometimes interpreted as indicating possible risk from increased folic acid intakes (Savage and Lindenbaum 1995). The risk, however, was speculative because more than 25 percent of vitamin B12-deficient patients who were not taking folic acid did not have anemia (normal hematocrit and normal mean cell volume) but only neurological signs (Healton et al. 1991).

A recent EFSA (2023a) evaluation analyzed prospective and retrospective case reports to investigate the risk of progression of vitamin B12-dependent neurological symptoms in vitamin B12-deficient patients. The EFSA Panel concluded that dosages up to 1 mg folic acid per day were unlikely to cause the masking of hematological signs of vitamin B12 deficiency based on ~300 retrospective and prospective case reports/series (see also *Official Reviews* section below). Studies were generally conducted with folic acid levels at ≤1 mg per day or ≥5 mg per day; EFSA acknowledged that there were insufficient data to evaluate the doses between 1-5 mg per day folic acid. As reviewed by the EFSA Panel, sixteen publications discussed prospective cases (around 300 cases in total) administering doses of folic acid between 5 and 20 mg per day, for various treatment durations. There were reports of deterioration or onset of neurological manifestations when treated with folic acid at these levels. In some cases, hematological symptoms were absent and there had been reports that neurological manifestations were irreversible despite administration of vitamin B12. One study reported that administration of 5 mg folic acid per day for 3.5 years to 70 patients with pernicious anemia resulted in twelve patients maintaining a "satisfactory health status," while 58 patients relapsed where neurological relapses occurred sooner than hematological relapses (two years vs three years) (Schwartz et al. 1950). Seven publications reported retrospective cases (20 isolated cases) in patients taking folic acid to treat macrocytic anemia (as reviewed by EFSA 2023a). Most patients were admitted to the hospital due to the onset of neurological symptoms, with varying degrees of severity, though

hematological symptoms were not always observed. Patients were estimated to be consuming doses of folic acid between 0.35 and 5 mg per day and neurological symptoms improved with treatment with vitamin B12 (EFSA 2023a). Based on the available data, the Panel determined the level of certainty in a causal relationship between dietary folic acid and the exacerbation of the neurological manifestations of cobalamin deficiency to be low (15%–50%). The Panel also noted that no new evidence was identified from which to better characterize the dose–response between folic acid intake and the resolution of megaloblastic anemia in cobalamin deficient individuals. In addition, the EFSA Panel stated that no new evidence was reviewed indicating that, below doses of ≤1 mg folic acid per day, the correction of hematological signs of cobalamin deficiency would delay diagnosis.

As reviewed previously by CRN (3rd edition) and multiple authoritative bodies, no serious adverse effects have been associated with supplemental intakes of folic acid in human clinical trials (IOM 1998; SCF 2000; EFSA 2023a; EVM 2003). The 3rd edition discussed a study in which folic acid supplement of 4 mg per day (4,000 µg) was used without adverse effect in a seven-nation trial that involved a total of 1,817 women at 33 study centers (Wald et al. 1991). A large volume of human intervention studies published since the 3rd edition were identified with potential relevance to this update (approximately 120).[1] Given that the EFSA Panel (2023a) conducted a comprehensive review of available studies and did not identify any outcomes sufficient for deriving an UL value for folic acid other than progression of neurological manifestations due to masking of vitamin B12 in deficient patients based on case studies (as discussed above), the literature review approach for this nutrient chapter was minimally modified from CRN's established Methodology.

No serious adverse effects or other safety-related outcomes associated with folic acid intervention were reported across all clinical studies identified in the update based on title and abstract only. Twenty-two clinical studies published between 2023 and 2025 (i.e., since EFSA's 2023 UL assessment) were reviewed in detail for this update; 18 of these studies administered folic acid at levels of ≤1 mg per day; the other four included doses of 2 or 5 mg per day. Full text review was also conducted on additional subsets of studies, as described below. A full

[1] Literature search conducted May 2025.

literature review is outside the scope of this chapter; however, an overview of the information reviewed is summarized below.

Consistent with previous reviews, no serious adverse effects were observed up to the highest doses tested in newer clinical trials of 5 mg per day for up to six months and 15 mg per day for 45 days (e.g., Nematollahi-Mahani et al. 2014; Baszczuk et al. 2017; Paniz et al. 2017). The majority of studies were conducted at folic acid doses between 0.4 and 1.0 mg per day (~80 studies) or ≥5 mg per day (~25 studies). Given that CRN, as well IOM (1998) and EFSA (2023a), previously noted a lack of case studies between 1 and 5 mg per day folic acid informing on the critical endpoint (i.e., progression of neurological symptoms in vitamin B12-deficient patients), clinical studies in this dose range underwent full text review as part of this update. Of the six clinical trials identified with dose levels >1 and <5 mg per day, no serious adverse events were reported (Sharpley et al. 2014; Wen et al. 2018; Bortolus et al. 2021; Corsi et al. 2022; Zhang et al. 2022; Fitrikasari et al. 2023). Of note, these studies did not include monitoring specifically for potentially relevant cognitive effects in participants (except for one study in schizophrenic patients; Fitrikasari et al. 2023), nor were they conducted for durations long enough to detect progression of effects related to vitamin B12 deficiency, with the exception of Sharpley et al. (2014). Sharpley et al. administered 0 or 2.5 mg folic acid per day for up to three years to teens and young adults (14-24 years) with familial risk of mood disorder.

### *5-Methyltetrahydrofolic acid (5-MTHF or Methylfolate)*

Additional research is needed to determine whether reduced folates, such as 5-MTHF, impart the same vitamin B12-related effects as supplemental folic acid (leading to increased risk of neurological symptoms) and to what extent. The EFSA (2023a) Panel acknowledged the differences in the pharmacokinetics between reduced folates and folic acid, noting that that there is no indication that 5-MTHF salts would impart such effects. Conversely, the Panel also concluded that the safety of 5-MTHF-glucosamine or l-5-MTHF-Ca above the UL for folic acid (1 mg per day) could not be determined, due to a lack of new data. While definitive studies are lacking in humans, the biochemistry of folate and vitamin B12 metabolism suggest that the hematopoietic response to folic acid which leads to masking in pernicious anemia is likely due to circulating UMFA, and therefore unlikely to occur (or to occur to the same extent) with folates

reduced during intestinal absorption, as hypothesized by Selhub et al. (2022). Therefore, one potential advantage of 5-MTHF supplementation over folic acid is that, if ingested in large amounts by vitamin B12-deficient individuals, the 5-MTHF likely would not mask the anemia because in the absence of vitamin B12, it would effectively remain metabolically "trapped" in the same form within cells (Scott et al. 1981).

Of note, Selhub et al. (2022) also noted that vitamin B12 deficiency itself can lead to the same adverse neurological outcomes. One potential mechanism contributing to deficiency is the depletion of serum holotranscobalamin caused by high folic acid intake, leading to reduced circulating vitamin B12; however, additional studies are needed to determine if this process is relevant for reduced folates (Selhub et al. 2022).

Seven clinical trials were identified that tested 5-MTHF, L-5-MTHF, or [6S]-5-MTHF at doses ranging from 0.4-1.13 mg per day (Hekmatdoost et al. 2015; Sicińska et al. 2018; Henderson et al. 2018; Bayes et al. 2019; Cochrane et al. 2023, 2024a,b). No adverse effects were reported in any study; however, not all studies included monitoring for such in the methodology. Two studies administered 5-MTHF at 15 mg per day for up to one consecutive month (Ambrosino et al. 2015; Cagnacci et al. 2015). Ambrosino et al. (2015) demonstrated no adverse effects related to supplementation with 5-MTHF at 15 mg per day in patients with mild or moderate hyperhomocysteinemia (5-MTHF cycled, one month of dosing followed by two months of withdrawal for a total of two years). While this study had 228 participants, no placebo was employed. Healthy postmenopausal women received 0 or 15 mg per day 5-MTHF for three weeks in the study by Cagnacci et al. (2015). No serious adverse effects were reported in this study; however, the methods did not mention monitoring for such.

**Official Reviews**

**IOM (1998).** The IOM established an UL of 1 mg for free folic acid, based on identification of a LOAEL of 5 mg and selection of a UF of 5. The LOAEL was based on case reports (~100 cases) demonstrating neurological manifestations in patients receiving 5 mg or more folic acid without supplemental vitamin B12. For example, three publications reported relapse or progression of neurological manifestations in over half of the subjects (n= 48, 36, and 38,

respectively) receiving 5 mg per day or higher of folic acid for over one year (Schwartz et al. 1950; Will et al. 1959; Spies et al. 1948). The IOM declined to identify a NOAEL, although many of the studies it cited failed to find adverse effects at doses of 1 to 1.25 mg per day folic acid. Ross et al. (1948) reported only one occurrence of relapse or progression of neurological manifestations out of four subjects receiving 1.25 mg per day folic acid for 9-23 months. In another publication, Chodos and Ross (1951) reported three occurrences of relapse or progression of neurological manifestations out of four subjects receiving 1.25 per day mg folic acid for 3.5-26 months, though two of the patients' neurological progressions were deemed as minimal or slight. The IOM noted that there was no record of adverse effects caused by food polyglutamyl folates, perhaps because of the lower bioavailability and/or the limited range of intakes observed. Thus, the IOM UL applies to folic acid "from supplements for fortified foods." The IOM did not evaluate the safety of reduced folates, such as 5-MTHF or its salts.

The IOM also defined dietary folate equivalent (DFE) values to reflect the higher bioavailability of folic acid compared to food folate (1 µg DFE = 1 µg natural food folate; 1 µg DFE = 0.6 µg folic acid from fortified foods or supplements consumed with food; 1 µg DFE = 0.5 µg folic acid from supplements taken on an empty stomach). There was no established conversion factor for 5-MTHF.

**EFSA (2023a).** Previously, the EC SCF (2000) established a UL of 1 mg per day for folic acid, identifying a LOAEL of 5 mg per day and noting that 1 mg per day was "unlikely to cause masking of the haematological signs in PA [pernicious anemia] patients."[2] In 2023, the EFSA delivered a scientific opinion on the revision of the SCF UL for folic acid/folate. During this evaluation, the risk of progression of neurological symptoms in vitamin B12-deficient patients was identified as the critical effect from which to derive a UL for folic acid. The EFSA Panel concluded, based on the prospective and retrospective cases discussed above (see *Safety Considerations* section), that "neurological complications may start or progress in vitamin B12-deficient individuals given supplemental folic acid as the sole treatment." The Panel noted that, although there are reports of neurological manifestations at folic acid doses below 5 mg per day, this evidence is "scarce" and there is not consistent evidence for a dose-response relationship.

[2] The EFSA did not specify a NOAEL or UFs in its summary.

Citing no basis to change the SCF's previous UL, the EFSA Panel retained the previous UL for the intake of supplemental folate from fortified foods and food supplements of 1 mg per day in adults. Of note, the Panel also concluded that "the level of certainty in a positive and causal relationship between the dietary intake of folic acid and the exacerbation of the neurological manifestations of vitamin B12 deficiency is low (15%–50% probability)."

Other potential adverse health effects that were evaluated for risk included: cognitive impairment in individuals with low cobalamin status, colorectal cancer, prostate cancer, other types of cancer, insulin resistance, impaired immune function, allergies, autism/autism spectrum disorder, and/or anemia. The EFSA Panel concluded that:

- The available body of evidence is insufficient to conclude on a positive and causal relationship between dietary intake of folate and risk of colorectal cancer and prostate cancer.
- The evidence for a positive relationship between "high" folate/folic acid intake or folate status and risk of cancer at other sites is scarce. Further investigation is needed to evaluate the relationship between high folate intake and risk of cancer.
- The available evidence on the relationship between dietary folate intake and risk of insulin resistance cannot be used for establishing a UL for folate.
- The available evidence on the relationship between folate intake and impairment of immune function, risk of allergies, risk of autism/autism spectrum disorder, or risk of anemia cannot be used for establishing a UL for folate.

The EFSA Panel (2004) has evaluated the use of calcium l-methylfolate as a food additive and concluded that the "previously established tolerable upper intake level for folic acid of 1mg/adult person/day would also be applied to the combined intake of folic acid and L-5-MTHF-Ca (expressed as folic acid)." Support of this UL value is based on studies that administered 5-MTHF to hemodialysis or psychiatric patients at doses of 15-17 mg per day for two to six months (Bostom et al. 2000; Perna et al. 1997; Godfrey et al. 1990). The EFSA (2013) also evaluated (6S)-5-methyltetrahydrofolic acid glucosamine as an added nutrient source to food supplements and concluded that the "proposed use and use levels (up to 1 mg per day) of 5-MTHF-glucosamine as an alternative source of folate to be used for the manufacture of food supplements is not of safety concern." The safety of (6S)-5-methyltetrahydrofolic acid

glucosamine was supported by the previous EFSA evaluations for calcium l-methylfolate data and for glucosamine from *Aspergillus niger* (EFSA 2009; EFSA 2013). The EFSA (2023b) also evaluated the safety of the monosodium salt of L-5-MTHF and concluded that the data supporting L-5-MTHF-Ca can be applied to support the safety of the sodium L-5-MTHF salt. The Panel concluded that monosodium salt of L-5-MTHF "is safe under the proposed conditions of use" and further states that the existing UL (i.e., 1 mg per day) for supplemental folate (i.e., the combined intake of folic acid and currently authorized salts) will also apply to the sodium L-5-MTHF salt (EFSA 2023b). Repeated-dose toxicity studies in rodents conducted with 5-MTHF were previously concluded by the EFSA (2004) to support the safety of the substance at doses that were 20,000-fold higher than 1 mg per day. Exposure to calcium, sodium, and glucosamine from these 5-MTHF salts were expected to be insignificant as compared to current intake levels (EFSA 2004; EFSA 2013; EFSA 2023b). The EFSA (2023a) "Panel acknowledge[d] that there are differences in ADME of these forms compared to folic acid and that there is no indication that 5-MTHF salts would correct the haematological manifestations of cobalamin deficiency, thereby increasing the risk of progression of the neurological manifestations of cobalamin deficiency. On the other hand, no new data have been identified regarding 5-MTHF-glucosamine or l-5-MTHF-Ca and the Panel considers that no statement can be made about the safety of 5-MTHF-glucosamine or l-5-MTHF-Ca above the UL for folic acid."

**Expert Group on Vitamins and Minerals (EVM 2003).** Similar to the IOM and the EC SCF, the UK's EVM established a guidance level for supplementation of 1 mg of free folic acid per day for the general population (EVM 2003). The EVM acknowledged that the available case reports indicated that supplementation with 1 mg per day folic acid does not mask vitamin B12-associated anemia in subjects, whereas supplementation with 5 mg per day folic acid does demonstrate masking effects.[3] However, the EVM noted that effects of dosages ranging from 1 mg per day to 5 mg per day remained unidentified. No other significant adverse effects have been associated with ingestion of folic acid according to the EVM (2003). The EVM did not apply UFs due to the consistency of the data from the large number of human studies (EVM 2003). The EVM did not evaluate the safety of reduced folates, such as 5-MTHF or its salts.

[3] The EVM's discussion did not reference specific studies.

**Chinese Nutrition Society (CNS 2023).** The CNS derived an UL value of 1 mg per day folic acid for adults, including those pregnant or lactating. The CNS did not evaluate the safety of reduced folates, such as 5-MTHF or its salts.

**Indian Council of Medical Research - National Institute of Nutrition (ICMR-NIN 2020).** The ICMR-NIN identified a UL for folic acid of 1 mg per day in adults. The ICMR-NIN noted that, "Folate together with B12 and iron may have adverse effects in the long term. Hence the recommended TUL to be viewed with caution." The Council did not evaluate the safety of reduced folates, such as 5-MTHF or its salts.

**Korean Nutrition Society (KNS 2020).** The KNS published its general approach to evaluating data for setting DRI values. Based on this approach, an UL of 1 mg dietary folate equivalent (DFE) per day was derived for adults (ages 19 and older; 0.9 mg DFE per day for ages 15-18 years). The KNS did not evaluate the safety of reduced folates, such as 5-MTHF or its salts.

## CRN Recommendations

### *Folic Acid*

The goal of the current update to CRN's supplemental UL for folic acid was to determine whether more recent human clinical data are available that might impact the conclusions published in the 3rd edition, which derived a supplemental UL value of 1 mg folic acid per day for adults. While not all human clinical trials are specifically designed to evaluate adverse effects, no new trials were identified following CRN's updated methodology that reported any serious adverse effects associated with folic acid supplementation. As with any assessment in which not all available data are reviewed, inherent uncertainties with the risk assessment and selection of the UL are recognized.

CRN's safety methodology for deriving a supplemental intake UL prioritizes data from human studies, when available. The table below summarizes the key human case reports/series considered in deriving an updated UL for supplemental intakes by CRN according to its principal points of departure for risk assessment (as described in CRN's Methods chapter). Given that the

critical endpoint was identified only in case studies/series, clinical studies are not included in the summary table below. A full literature review is outside the scope of this chapter; therefore, only studies identified in the updated review that are most pertinent[4] to deriving a revised UL for folic acid/folate based on CRN's methodology, are summarized below.

### Key Studies Considered for the CRN UL for Folic Acid in Adults

| Reference | Study Design | Participant Description | No. of Subjects | Dose(s) (mg/day) | Duration (months) | NOAEL (mg/day) | LOAEL (mg/day) |
|---|---|---|---|---|---|---|---|
| Ross et al. 1948 | Case report | Patients with pernicious anemia | 4 | 1.25 | 9-23 | 1.25 | N/A |
| Chodos and Ross 1951 | Case report | Patients with pernicious anemia | 4 | 1.25 | 3.5-26 | 1.25 | N/A |
| Victor and Lear 1956 | Case report | Patients with pernicious anemia | 2 | 1.5 and 2.55 | 10-39 | N/A | 1.5[a] |
| Schwartz et al. 1950 | Case report | Patients with pernicious anemia | 48 | 5 | 48 | N/A | 5 |
| Will et al. 1959 | Case report | Patients with pernicious anemia | 36 | 5-10 | 12-120 | N/A | 5 |
| Spies et al. 1948 | Case report | Patients with pernicious anemia | 38 | ≥10 | 24 | N/A | 5 |

N/A, not applicable

[a] Limited evidence

As summarized in the *Official Reviews* section, UL values derived previously by the IOM (1998), EVM (2003), and SCF (2000) were based on neurological effects in vitamin B12-deficient patients due to supplementation with folic acid. Recently, the EFSA (2023a) conducted a comprehensive review of available studies and case reports and identified the risk of progression of vitamin B12-dependent neurological symptoms in deficient patients as the critical effect, upholding the previous SCF (2000) UL of 1 mg per day. This critical effect was based on case reports and not on controlled clinical studies.

[4] Where numerous relevant case reports/studies were identified, those most pertinent to the UL derivation are included in the table as representative studies. For example, EFSA (2023a) reviewed ~300 cases across 23 studies starting at folic acid doses of 5 mg per day; these are not summarized here. Rather, prioritization was given to studies previously identified in CRN's 3rd edition, as representative studies.

Two case series found no significant increase in risk of masking neurological effects with folic acid doses of 1.25 mg per day; Ross et al. (1948) reported effects in one of four cases and Chodos and Ross (1951) reported slight/minimal effects in three of four cases. Conversely, there was some evidence that masking may occur with intakes of 1.5 and 2.55 mg (one case at each dose; Victor and Lear 1956). In studies at higher folic acid doses, some patients with pernicious anemia experienced relapse or progression of neurological manifestations when administered 5 mg per day doses of folic acid (Schwartz et al. 1950; Will et al. 1959; Spies et al. 1948).

The available data from human clinical studies demonstrate no serious adverse effects are observed up to the highest doses tested in ~120 trials with folic acid, including 5 mg per day for up to six months and 15 mg per day for 45 days (e.g., Nematollahi-Mahani et al. 2014; Baszczuk et al. 2017; Paniz et al. 2017). Given the gap in case studies between 1 and 5 mg per day folic acid, clinical trials identified in this update with dose levels >1 and <5 mg per day were reviewed to determine if they might provide additional context regarding safety for this dose range (Sharpley et al. 2014; Wen et al. 2018; Bortolus et al. 2021; Corsi et al. 2022; Zhang et al. 2022; Fitrikasari et al. 2023). However, since these studies did not include monitoring specifically for potentially relevant cognitive effects in participants, they provide insufficient evidence regarding risk of the critical neurological endpoints for the purposes of UL development. In addition, only one such clinical study was conducted for a duration long enough to detect any potential progression of effects related to vitamin B12 deficiency (Sharpley et al. 2014); however, this study included teens and young adults (14-24 years), who are at lower risk of undiagnosed vitamin B12 deficiency.

Based on the available data, the risk of progression of vitamin B12-dependent neurologic symptoms is maintained as CRN's critical effect for development of an UL value for folic acid. Due to the prevalence of vitamin B12 deficiency and the serious nature of potential neurological effects, this effect is considered relevant to the general population. Therefore, on the basis of the absence of adverse effects at 1 mg per day and no significant effects up to 1.25 mg per day, 1 mg per day is maintained as the NOAEL for folic acid for adults following the CRN process. As described in CRN's Methods, if the supplemental intake dose-response relationship is identified from the strongest data and assessed conservatively, no additional uncertainty factor is needed (that is, the implicit UF is 1.0). Consistent with CRN's methodology, an UF of 1 is applied to

yield an UL of 1 mg per day for adults for supplemental folic acid.

**Quantitative Summary for Folic Acid in Adults**

| | |
|---|---|
| CRN UL (2025), supplemental intake | 1 mg/day[a] |
| IOM UL (1998), supplemental intake | 1 mg/day |
| EFSA (2023a), supplemental intake | 1 mg/day[a] |
| EVM (2003), guidance level, supplemental intake | 1 mg/day |
| CNS (2023), total intake | 1 mg/day |
| ICMR-NIN (2020), total intake | 1 mg/day |
| KNS (2020), total intake | 1 mg DFE/day (ages 19 and older) |

DFE, dietary folate equivalent
[a] Does not apply to reduced forms of folic acid, such as 5-MTHF
[b] Includes folic acid from 5-MTHF-glucosamine and L-5-MTHF salts (EFSA 2004, 2013, 2023a)

### *5-Methyltetrahydrofolic acid (5-MTHF or Methylfolate)*

The goal of the current update was to develop a CRN supplemental UL for methylfolate (5-MTHF) using recent human clinical data, as an UL was not previously established by CRN. While not all human clinical trials are specifically designed to evaluate adverse effects, no trials were identified following CRN's updated methodology that reported any serious adverse effects associated with 5-MTHF intervention. As with any assessment in which not all available data are reviewed, inherent uncertainties with the risk assessment and selection of the UL are recognized.

CRN's safety methodology for deriving a supplemental intake UL prioritizes data from human studies, when available. The table below summarizes the key human case series/studies and clinical studies considered in deriving a UL for supplemental intakes by CRN according to its principal points of departure for risk assessment (as described in CRN's Methods chapter). A full literature review is outside the scope of this chapter; therefore, only studies identified in the literature search with 5-MTHF intake levels pertinent to developing a UL based on CRN's

methodology are presented.

## Key Studies Considered for the CRN UL for 5-MTHF in Adults

| Reference | Study Design | Participant Description | No. of Subjects | Dose(s) (mg/day)[b] | Duration | NOAEL (mg/day) | LOAEL (mg/day) |
|---|---|---|---|---|---|---|---|
| ***Case studies with folic acid*** | | | | | | | |
| Ross et al. 1948 | Case report | Patients with pernicious anemia | 4 | 1.25 (folic acid) | 9-23 months | 1.25 | N/A |
| Chodos and Ross 1951 | Case report | Patients with pernicious anemia | 4 | 1.25 (folic acid) | 3.5-26 months | 1.25 | N/A |
| Victor and Lear 1956 | Case report | Patients with pernicious anemia | 2 | 1.5 and 2.55 (folic acid) | 10-39 months | N/A | 1.5[a] |
| ***Clinical trials with 5-MTHF*** | | | | | | | |
| Hekmatdoost et al. 2015 | Randomized, double blind, placebo-controlled trial | Women with idiopathic recurrent abortion | 220 | 1 (folic acid); 1 (5-MTHF) | ≥28 weeks[c] | 1 | N/A |
| Henderson et al. 2018 | Randomized, double blind, placebo-controlled trial | Healthy women | 75 | 1 (folic acid); 1.13 (L-5-MTHF) | 12 weeks | 1; 1.13 | N/A |
| Ambrosino et al. 2015 | Clinical trial | Patients with hyperhomocysteinemia | 228 | 15 (L-5-MTHF) | 1 month consecutive[d] | 15 | N/A |
| Cagnacci et al. 2014 | Randomized, double blind, placebo-controlled trial | Postmenopausal women | 30 | 15 (folic acid); 15 (5-MTHF) | 3 weeks | 15 | N/A |

N/A, not applicable

[a] Limited evidence

[b] 1 mg 5-MTHF equimolar to ~0.96 mg folic acid; 1 mg L-5-MTHF equimolar to ~0.88 mg folic acid

[c] At least eight weeks before conception until the 20th week of the pregnancy

[d] One month followed by two months withdrawal, for a total of two years

Available human interventional studies with 5-MTHF and L-5-MTHF have demonstrated a lack of adverse effects at doses ranging from 0.4-15 mg per day (Hekmatdoost et al. 2015; Sicińska et al. 2018; Henderson et al. 2018; Bayes et al. 2019; Cochrane et al. 2023, 2024a,b; Ambrosino et al. 2015; Cagnacci et al. 2015). As described in CRN's Methods, uncertainties in a dataset may be addressed by arranging the data in decreasing order of intake and then selecting downward until confidence in the data is sufficient to justify the selection of a NOAEL with a

UF of 1.0. In the case of 5-MTHF, a UF of 1.0 could be supported by selecting a NOAEL value of ~1 mg per day (Hekmatdoost et al. 2015; Henderson et al. 2018), yielding an UL of 1 mg per day. However, such a UL is considered by CRN to be exceedingly conservative based on the available clinical data for 5-MTHF. Therefore, a NOAEL of 15 mg per day is selected as the basis for deriving the UL for 5-MTHF with UFs applied to account for uncertainty associated with limitations with the two studies at this dose (Ambrosino et al. 2015; Cagnacci et al. 2015). Specifically, the consecutive duration of both studies is too short to support an UF of 1.0 (three weeks and one month, respectively). In addition, Cagnacci et al. (2015) included only 30 participants. While the Ambrosino et al. (2015) study was much larger (n = 228), there was no placebo-control group. As such, an UF of 1.0 could not be confidently selected based on the available studies with a NOAEL of 15 mg 5-MTHF per day. Therefore, following the CRN process, a composite UF of 10.0 is applied to the NOAEL of 15 mg per day to account for uncertainties in the dataset (e.g., study design; UF of 3) and the short duration of the supporting studies (UF of 3), yielding an UL of 1.5 mg per day for adults for supplemental 5-MTHF.[5,6]

The UL derived for 5-MTHF is considered by CRN to be conservative given the absence of any reported adverse effects in clinical trials with 5-MTHF up to the highest dose of 15 mg per day. In the future, the availability of additional, well-designed clinical trials demonstrating a lack of any serious adverse effects at levels >1.5 mg 5-MTHF per day would likely support the derivation of an UL value higher than 1.5 mg per day.

As described above, no treatment-related adverse effects have been reported in human trials conducted with 5-MTHF. CRN's derived UL value of 1.5 mg 5-MTHF per day is independent of the UL developed in this chapter for folic acid (1 mg per day), which is based on the potential for folic acid to mask hematological signs or otherwise contribute to the risk of progression of vitamin B12-dependent neurological symptoms in vitamin B12-deficient patients. While additional data regarding the ability of supplemental 5-MTHF to cause such an effect are

[5] Includes all salt forms of 5-MTHF; the small differences in equimolar equivalence are considered negligible.
[6] No adverse effects have been identified in human studies with 5-MTHF. However, any uncertainty remaining around whether reduced folates, such as 5-MTHF, could potentially impart the same vitamin B12-related effects as supplemental folic acid and to what extent is minimal, an UL value is selected for 5-MTHF, as opposed to a highest observed intake (HOI) level. An HOI is derived when no adverse health effects have been identified. In the future, the availability of additional data confirming such effects do not occur at any level with 5-MTHF would suggest that an HOI may be appropriate.

needed, it is thought to be physiologically implausible based on the biochemistry of folate and vitamin B12 metabolism. In addition, clinical studies administering folic acid and 5-MTHF combined are not available for assessment. As such, the masking effect is not considered relevant to the derivation of an UL for 5-MTHF based on currently available information. In the unlikely scenario that a reduced form, such as 5-MTHF, could lead to a similar mode of action, any effects are expected to be negligible relative to effects associated with UMFA released following folic acid supplementation. Available clinical data demonstrating a lack of adverse effects at supplemental levels much higher than the CRN UL of 1.5 mg 5-MTHF per day (up to 15 mg per day) further help to address any uncertainty.

**Quantitative Summary for 5-MTHF in Adults**

| | |
|---|---|
| CRN UL (2025), supplemental intake | 1.5 mg/day[a] |
| IOM UL (1998), supplemental intake | Not evaluated |
| EFSA (2004; 2023a), supplemental intake | 1 mg/day (total folic acid)[b] |
| EVM (2003), guidance level, supplemental intake | Not evaluated |
| CNS (2023), total intake | Not evaluated |
| ICMR-NIN (2020), total intake | Not evaluated |
| KNS (2020), total intake | Not evaluated |

[a] Independent of UL derived for folic acid

[b] Includes folic acid and folic acid from 5-MTHF-glucosamine and L-5- MTHF salts (EFSA 2004, 2013, 2023a)

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