

PRELIMINARY RISK ASSESSMENT - BIOTIN

General Information

Chemistry

D-Biotin (biotin, coenzyme R, vitamin H) is a water-soluble vitamin. It has a bicyclic ring structure. One ring contains a ureido group and the other contains a heterocyclic sulphur atom and a valeric acid side-group.

Natural occurrence

Biotin is derived from *de novo* synthesis by bacteria, primitive eukaryotic organisms including yeasts, moulds and algae, and some plant species.

Occurrence in food, food supplements and medicines

Biotin is widely distributed in natural foodstuffs but at very low levels compared to other water-soluble vitamins. Foods relatively rich in biotin include egg yolk, liver, kidney, muscle and organ meats, and some vegetables. Liver contains approximately 1 mg/kg biotin, whereas fruits and most other meats contain approximately 0.01 mg/kg biotin. Biotin, usually either in the form of crystalline D-biotin or brewer's yeast, is included in many dietary supplements, infant milk formulas and baby foods, as well as various dietetic products. The maximum dose in supplements sold in the UK is 0.15 mg. Several medicines containing biotin, which are available only from pharmacies, are licensed for the prevention and treatment of nutrient deficiency, supplementation of special diets and malabsorption. The maximum daily dose of biotin in licensed medicines is 0.50 mg.

Recommended amounts

Due to insufficient data, COMA (DH, 1991) was unable to set Dietary Reference Values for biotin, but considered that intakes between 0.010 and 0.20 mg/day are both safe and adequate.

Analysis of tissue levels and biotin status

Measurement of biotin in plasma is not a reliable indication of status. Changes in urinary excretion of biotin, or its metabolites bisnorbiotin, 3-hydroxyisovaleric acid and 3-methylcrotonylglycine, are good indicators of biotin status.

Brief overview of non-nutritional beneficial effects

Biotin has been claimed to be beneficial in the treatment of brittle nails, hyperinsulinaemia and impaired glucose tolerance and sternocostoclavicular hyperostosis. Biotin supplements are also indicated in the management of inborn biotin-associated enzyme abnormalities such as deficiency of biotinidase, holocarboxylase synthetase and the individual carboxylase enzymes.

Function

Biotin acts as an essential cofactor for the acetyl-CoA, propionyl-CoA, β -methylcrotonyl-CoA and pyruvate carboxylase enzymes, which are important in the synthesis of fatty acids, the catabolism of branched-chain amino acids and the gluconeogenic pathway. Biotin may also have a role in the regulation of gene expression arising from its interaction with nuclear histone proteins.

Deficiency

Biotin deficiency has been observed in individuals maintained on total parenteral nutrition, people who consume large amounts of uncooked egg white, sufferers of inherent or acquired biotin malabsorption, haemodialysis patients, and individuals receiving some forms of long-term anticonvulsant therapy. Pregnancy may be associated with marginal biotin deficiency in some women. Signs of biotin deficiency include a fine scaly desquamating dermatitis and characteristic skin rash frequently observed around the eyes, nose and mouth, hair loss, conjunctivitis and ataxia. Biotin deficient infants show signs of hypotonia, lethargy, developmental delay and withdrawn behaviour, all of which are characteristic of a biotin deficiency-related neurological disorder. “Egg white injury” may be associated with glossitis, anorexia, nausea, hallucinations, depression and somnolence. Inherited deficiencies in biotinidase and holocarboxylase synthetase result in multiple carboxylase deficiency. These deficiencies and those of specific carboxylase enzymes may produce the same or similar disorders and manifestations of biotin deficiency. Clinical manifestations of biotin deficiency are generally thought to result, directly or indirectly, from deficient activities of the carboxylase enzymes. Biotin deficiency has been shown to cause abnormal foetal development in animals.

Interactions

Some anticonvulsant drugs and alcohol may inhibit intestinal carrier-mediated transport of biotin. Steroid hormones and some anticonvulsant drugs may accelerate the catabolism of biotin in the tissues. Peroxisome proliferators have been shown to accelerate biotin catabolism in rats. However, the human relevance of this finding is questionable. Pantothenic acid and biotin may share common carrier-mediated uptake mechanisms in some tissues but at present there are no known clinical implications of this interaction.

Absorption and bioavailability

Biotin uptake from the small intestine occurs by a carrier-mediated process that operates with a high carrier affinity and also by slow passive diffusion. The carrier is driven by an electron-neutral sodium (Na^+) gradient, has a high structural specificity and is regulated by the availability of biotin, with up-regulation of the number of transporter molecules when biotin is deficient. The colon is also capable of absorbing biotin via a similar transport mechanism. Approximately 80% of biotin in plasma is in the free form and the remainder is either reversibly or covalently bound to plasma proteins. The existence of a specific biotin carrier protein in plasma is a subject of debate.

Factors determining the bioavailability of biotin present in the diet are uncertain. The bioavailability of biotin that is covalently bound to protein is reduced in individuals suffering from biotinidase deficiency. There are few data concerning the bioavailability of crystalline biotin supplements, but a recent study has suggested that doses as high as 22 mg may be completely absorbed. The nutritional significance of biotin synthesis by bacteria present in the lower gut is a subject of controversy.

Distribution and metabolism

Uptake into tissues occurs by specific transport mechanisms dependent upon Na⁺ gradients. Transplacental transport is thought to involve the active accumulation of biotin within the placenta followed by its passive release into the foetal compartment. Biotin is metabolically trapped within the tissues by its incorporation into carboxylase enzymes. In the normal turnover of cellular proteins, carboxylase enzymes are broken down to biocytin or oligopeptides containing lysyl-linked biotin. Biotin may be released for recycling by the hydrolytic action of biotinidase. Liberated biotin may be reclaimed in the kidney against a concentration gradient. Biotin not incorporated into carboxylase enzymes may be metabolised oxidatively at the sulphur present in the heterocyclic ring and/or at the valeric acid side chain.

Excretion

Biotin metabolites are not active as vitamins and are excreted in the urine. Very little biotin is thought to undergo biliary excretion and the substantial amounts of biotin that appear in the faeces are derived from the colonic bacteria.

Toxicity

Human data

Anecdotal reports suggest that typical daily doses of 10 mg are without adverse effects and toxicity has not been reported in individuals receiving as much as 200 mg per day. Clinical data are limited but studies have reported no biotin-related adverse effects following the administration of 9 mg/day for up to 4 years, 10 mg/day for 15 days, 4 mg/day for 3 weeks or 2.5 mg/day for 6-15 months.

Animal data

The database on the toxicity of biotin in laboratory animals is limited. The acute toxicity in mice and rats after intravenous or oral dosage appears to be low. There is controversy as to whether high doses of biotin given sub-cutaneously cause adverse effects to the reproductive system in laboratory animals. Biotin-related disturbances in oestrus cycle, atrophic changes in ovaries, inhibition of foetal and placental growth and the increased resorption of foetuses were reported following administration of biotin (50 mg/kg) by injection to female Holzman rats, up to 3 weeks prior to mating. These effects were not observed in a similar study conducted in Ibm:RORO_f rats or ICR mice. These studies were not appropriate for risk assessment of oral doses.

Carcinogenicity and genotoxicity.

No carcinogenicity data are available for biotin. It has been shown to be negative in the Ames test. However, further data on the mutagenicity testing of biotin are not available.

Genetic variations

No relevant genetic variations have been identified.

Mechanism of toxicity

No relevant data are available.

Dose-response characterisation

No relevant data are available.

Vulnerable groups

No potential vulnerable groups have been identified.

Studies of particular importance in the risk assessment:

Human data

Maebashi et al., 1993

Biotin (9 mg, in combination with 3 g of an anti-microbial drug, Miya-BM) was administered daily, by the oral route (in three divided doses), for up to 4 years, to 20 patients (sex and age unspecified) suffering from non-insulin dependent diabetes. The number of patients followed up after 24, 30, 36 and 48 months was 15, 15, 10 and 5, respectively. There was no control group included in the study. Fasting blood glucose levels decreased to normal within 2 months and remained normal thereafter with continuing treatment. Serum insulin levels were not significantly changed. The authors reported that there were no observed clinical aggravations or undesirable side effects. This study cannot be used for risk assessment because of the unusual design and the small number of subjects.

Velazquez et al., 1995

In a double blind placebo controlled study, protein deficient children (n=22) were administered 10 mg biotin/day for fifteen days. Plasma biotin concentrations and lymphocyte carboxylase enzymes were measured. The authors reported no adverse effects. This study cannot be used to define the safety of biotin in children with a normal protein intake.

Exposure assessment

Total exposure/intake:

Food mean: 0.033 mg/day (1986/7 NDNS)
 97.5th percentile: 0.066 mg/day

Supplements up to 0.15 mg/day (OTC, 2001)

Estimated maximum daily intake: $0.066 + 0.15 = 0.21$ mg/day

No potential high intake groups were identified.

Risk Assessment

There are relatively few human data available on the oral toxicity of biotin. The data available are in the form of anecdotal case reports or from clinical trials or supplementation studies designed primarily to investigate beneficial effects of biotin. The latter rarely specifically report on the presence or absence of adverse effects.

The animal toxicity database for biotin is very limited, especially by the oral route.

ESTABLISHMENT OF GUIDANCE LEVEL

The data from studies in humans and animals are not adequate for the establishment of a safe upper level.

The numerous clinical case reports in the literature describe the outcome of oral biotin administration to patients (infants, juveniles and adults) with biotin-responsive inborn errors of metabolism and other forms of biotin deficiency. Furthermore, in cases where foetal biotin-responsive disorders have been suspected, biotin has been administered prenatally, via the mother. Typically, doses of 10 mg/day (250 x the average intake of the adult male in the UK) have been found to be therapeutic, without reported adverse side effects.

It appears that doses of 9 mg/day given to human volunteers for up to 4 years have not been associated with any adverse effects. The study is limited in that it was performed in diabetics, there was no control group and only a few individuals remained in the study after 4 years. The authors concluded that there were no adverse effects related to the biotin treatment, although they reported a tendency for the treatment to lower blood sugar. Although adverse effects have not been identified, an uncertainty factor of 10 for inter-individual variation had been applied in this case to allow for inter-individual variation. Thus, for guidance purposes only, a supplemental daily intake of 0.9 mg biotin (equivalent to 0.015 mg/kg bw/day in a 60 kg adult) would not be expected to produce adverse effects, although this value may not be applicable to all life stages. Assuming a maximum intake of 0.066 mg/day from food, an estimated

total intake of 0.97 biotin (equivalent to 0.016 mg/kg bw/day in a 60 kg adult) would not be expected to result in any adverse effects.

References

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PRELIMINARY RISK ASSESSMENT – FOLIC ACID

General information

Chemistry and geochemistry

The term folate is used generically to describe the various derivatives of pteroylglutamic acid (PGA, folic acid), the common pharmaceutical and most stable form of the folate vitamins group, which is composed of three major subunits – pteridine, *p*-aminobenzoic acid, and glutamic acid.

Within this assessment, in accordance with the guidelines of the International Union of Pure and Applied Chemistry and International Union of Biochemistry and Molecular Biology (IUPAC-IUB) advisory panel, the term folic acid is used to indicate the parent compound, pteroylglutamic acid, and folate is used generically, to indicate one or a mixture of pteroylglutamates.

Natural occurrence

Folic acid (PGA) *per se* is not present in significant quantities in foods or in the human body. The derivatives of PGA which are predominantly present in the human body, and in plant- and animal-derived foods, are reduced folates, mostly 5,6,7,8-tetrahydrofolates (THF), and also 7,8-dihydrofolate (DHF). Other modifications occur

Occurrence in food, food supplements and medicines

Folates are present in the majority of natural foods. Liver, yeast extract, green leafy vegetables, legumes and some fruits are especially rich sources. The dietary forms are broken down to monoglutamates during storage, processing and cooking. The synthetic pharmaceutical form used for food fortification and in supplements is folic acid (PGA), as this compound is more stable in comparison to other forms of the vitamin. Folic acid is widely available as a nutritional supplement, either alone or as a component of B-complex or multivitamin preparations, in daily doses of up to 0.40 – 0.50 mg. Preparations providing a daily dose of 5 mg are available on prescription only.

Recommended amounts

Reference Nutrient Intakes (RNI) for folate are 0.05, 0.07, 0.10 and 0.15 mg/day for infants and children ≤ 12 months, 1 - 3 years, 4 - 6 years and 7 - 10 years, respectively. The RNI for adults and children ≥ 11 years old is 0.200 mg/day. Recommendations to allow for increased requirements during pregnancy and lactation are increments of 0.10 and 0.060 mg/day, respectively, i.e. 0.30 mg/day for pregnant women and 0.26 mg/day for breastfeeding women. The UK Department of Health also recommends that women who are trying to conceive should take 0.40 mg/day folic acid, in addition to normal dietary folate intake, until the 12th week of pregnancy. An additional dose of 5 mg/day is recommended for women at high risk of a neural tube defect (NTD)-affected pregnancy.

Analysis of tissue levels and folate status

Folate status is usually measured by determination of serum and/or red cell folate levels, in which the predominant species is 5-methyl-THF. Serum folate is a short-term indicator of folate status; levels are normally within the range of 5-16 ng/mL (11-36 nmol/L folic acid activity). Red cell levels are more stable and reflect long term intake, levels < 140 ng/mL (317 nmol/L) indicate reduced body stores. Thus, negative folate balance is indicated by a serum folate concentration < 3 ng/mL, whilst folate deficiency is indicated by erythrocyte and liver folate levels < 120 ng/mL or 1.6 mg/kg (wet weight), respectively.

Brief overview of non-nutritional beneficial effects

Randomised controlled trials and observational studies indicate that peri-conceptional folic acid supplementation in women is associated with a significant reduction in the incidence of foetal NTDs. It has also been suggested that folic acid supplementation may be associated with a reduction of serum levels of homocysteine, higher levels of which have been implicated as a risk factor for cardiovascular disease.

Function

Folate coenzymes within the cell are involved in one-carbon transfer reactions, including those involved in phases of amino acid metabolism, purine and pyrimidine synthesis, and the formation of the primary methylating agent, S-adenosylmethionine.

Deficiency

Folate deficiency results in reduced *de novo* DNA biosynthesis and, thus, impairment of cell replication, with the most obvious effects relating to rapidly dividing cell-types, such as erythrocytes and other cells generated by the bone marrow, enterocytes and skin cells. The condition causes megaloblastic and macrocytic anaemia. Vitamin B₁₂ deficiency also causes a macrocytic anaemia and should be excluded before folate treatment alone is given.

Nutritional folate deficiency may develop during pregnancy, infection, malignant disease, malabsorption syndromes (e.g. coeliac disease) or alcoholism, during some drug treatments and in the elderly on restricted diets.

Interactions

Folic acid supplementation can interfere with a number of drugs (anti-folate drugs, drugs used to treat epilepsy, anti-inflammatory drugs). Folate and vitamin B₁₂ metabolism are linked *via* the enzyme methionine synthase, which requires vitamin B₁₂ as a cofactor. Some authors have reported a negative effect of folate supplementation on zinc status. Some animal studies have suggested that iron deficiency may cause folate depletion.

Absorption and bioavailability

The majority of dietary folate is absorbed within the proximal region of the small intestine by active, carrier-dependent mechanisms, and also by passive diffusion. Polyglutamate forms are first hydrolysed to monoglutamates by conjugase (hydrolase) enzymes within the enterocyte brush border. Ingested folic acid is enzymatically reduced and methylated within the intestinal lumen and enterocytes, although ingestion of high concentrations (> 0.20-0.30 mg/meal) result in the direct appearance of the compound, unmodified, in the plasma.

The absorption of natural folate from a typical North American diet has been suggested to be in the range of 50 to 75% of the amount ingested. Synthetic folic acid, as a food fortificant or supplement is more highly bioavailable than natural food folate.

Distribution and metabolism

Absorbed folate is carried *via* the portal blood to the liver, where a proportion (approximately 0.1 mg/day) is excreted into the bile and undergoes enterohepatic circulation and reabsorption. The liver is also the main storage site, containing approximately half of the total (5 - 10 mg) body folate. The majority of plasma folate is present as 5-methyl-THF-monoglutamate. Within cells, folate is retained in the cytoplasm by polyglutamation. 5-Methyl-THF is not a good substrate for polyglutamation, and must be first converted, *via* a vitamin B₁₂-dependent reaction, to THF. Alternatively, folic acid can be converted to polyglutamate (i.e. metabolically active) forms *via* a vitamin B₁₂-independent pathway.

Excretion

Folate is excreted in the urine, either as the metabolically active form or as breakdown products, and in the faeces.

Toxicity

Human data

Folic acid is generally considered as safe, even at doses of about 10-20 mg/day. Adverse effects may, potentially, occur in specific groups, such as individuals being treated with drugs that interact with folic acid metabolism. "Indirect toxicity" may occur due to folic acid reversal of the haematological signs and symptoms of vitamin B₁₂ deficiency; this masking effect could allow the neuropathy associated with vitamin B₁₂ deficiency to develop untreated. Vitamin B₁₂ deficiency is most prevalent in older people and, in general, in those with impaired absorption.

A small number of case reports have described hypersensitivity reactions to oral folic acid therapy (generally \geq 1 mg/day). One short-term, uncontrolled supplementation trial reported adverse symptoms (mental changes, sleep disturbances and gastrointestinal symptoms) in healthy volunteers given very high doses of folic acid (15 mg/day) for 1 month, but other studies have not observed similar effects.

Supplementation trials

A substantial number of supplementation studies have been carried out or are ongoing to assess the effectiveness of folic acid therapy in disease prevention (generally, either for the prevention of foetal NTD-pregnancies, or cardiovascular disease, in high-risk groups). Many of these trials have shown beneficial effects associated with folic acid supplementation at levels up to 10 mg/day for periods of several weeks or months. Very few adverse effects have been reported, although the majority of studies have not specifically addressed this issue.

Trials have shown that peri-conceptual supplementation with folic acid, or folic acid-containing multivitamin supplements, is associated with a significant reduction in the incidence of foetal NTDs. Consequently, health experts recommend that, for the prevention of foetal NTDs, women who are likely to become pregnant should take a 0.40 mg folic acid supplement daily, in addition to normal dietary folate intake. Women in high risk groups may be advised to take up to 10 times this level of supplementation. Such therapy is generally considered to be without adverse reproductive or developmental effects, although it has been noted that human trials carried out to date were not sufficiently powerful to identify rare or possibly slight adverse effects.

One large study of peri-conceptual multivitamin supplementation (including 0.8 mg/day folic acid) in Hungary (1992) showed reduced incidence of NTDs, but with a significant increase in spontaneous abortions. Thus, it has been suggested that folic acid may prevent NTDs by causing spontaneous abortion of affected fetuses. However, this theory is not supported by recent studies using a genetically-predisposed NTD mouse model, in which the administration of folic acid to embryos *in vitro* normalised neural tube development without stimulating abortion.

Animal data

Data from toxicological studies of folic acid in experimental animals are limited. A number of reports have described nephrotoxicity associated with the parenteral administration of extremely high doses (≥ 75 mg/kg bw) of folic acid in rodents. Additionally, direct injection of high doses of folic acid or folates into the brain or spinal fluid has been shown to produce seizures in rats and mice. One study showed that high-dose dietary folic acid supplementation decreased the dose of pentylenetetrazol required to induce seizures in rats.

Oral folic acid supplementation, alone has generally not shown reproductive or embryotoxic effects in animal models, although studies have shown that supplementation is associated with increased foetal folate levels. One group reported that the fetuses of rats fed diets containing approximately 2 mg/kg bw/day folic acid for 3 weeks during pregnancy showed reduced body weight and vertex-coccyx length compared with a control group of animals given a basal diet, although the validity of the reported statistical analysis is uncertain. Additionally, folic acid treatment has been reported to enhance the embryotoxic effects of certain drugs (pyrimethamine, valproic acid), and of zinc-deficiency.

Carcinogenicity and genotoxicity

There are limited data to suggest that folic acid supplementation, in comparison to deficiency, may be associated with the promotion of tumours in animals that develop spontaneous tumours or are exposed to chemical carcinogens. However, this may be related to the role of folic acid in supporting cell replication. Data from *in vitro* and *in vivo* studies indicate that folic acid is not genotoxic.

Vulnerable groups

Groups vulnerable to adverse effects associated with folic acid supplementation include:

- 1] Individuals at risk of vitamin B₁₂ deficiency (most prevalent in the elderly), in whom folic acid supplementation may mask the haematological signs and symptoms of this deficiency, allowing the associated myeloneuropathy to develop.
- 2] Patients treated with drugs which interfere with folate metabolism, and in whom folic acid supplementation may be associated with reduced effectiveness of the therapy or increased incidence of side effects.

Genetic variations

Population groups with a genetically determined increase in susceptibility to folic acid toxicity have not been identified. In contrast, some individuals have a genetic predisposition to deficiency. Subjects homozygous for a variant of the enzyme 5,10-methylene tetrahydrofolate reductase (MTHFR) show reduced activity of this enzyme, resulting in altered cellular distribution of one-carbon units, and associated with low plasma folate status and hyperhomocysteinaemia. Defects of other enzymes involved in homocysteine metabolism (e.g. cystathionine β-synthase, methionine synthase) may also be associated with pathology related to low folate intake/status. In addition, congenital errors of various enzymes involved in folate metabolism have been described, associated with functional folate-deficiency.

Mechanisms of toxicity

Folic acid is generally considered safe and no likely mechanisms for toxicity have been hypothesised. The metabolism of folate and vitamin B₁₂ are linked by the enzyme methionine synthase, which is vitamin B₁₂ dependent and results in polyglutamate synthesis. High levels of folate can result in the production of polyglutamates by a vitamin B₁₂ independent mechanism, which may reverse the megaloblastic anaemia caused by vitamin B₁₂ deficiency. This complicates the diagnosis of vitamin B₁₂ deficiency and allows the neurological damage associated with it to continue.

Dose response characterisation

Few systematic data exist regarding the level of folate intake required to mask vitamin B₁₂ deficiency (Koehler *et al.*, 1997). The majority of available information relates to

early case reports of folic acid therapy for the treatment of pernicious anaemia (mostly high doses, for example ≥ 5 mg/day) (Heinle *et al.*, 1947; Vilter *et al.*, 1947; Bethell *et al.*, 1948; Ross *et al.*, 1948; Vilter *et al.*, 1950; Will *et al.*, 1959; Schwartz *et al.*, 1950; Ellison, 1960; Marshall *et al.*, 1960; Baldwin *et al.*, 1961; Hansen & Weinfeld, 1962, Vilter *et al.*, 1963). In general, data taken from these reports suggest that supplementation with ≤ 1 mg/day folic acid does not mask vitamin B₁₂-associated anaemia in the majority of subjects. The effects of doses of between 1 and 5 mg/day are unclear (*cited by* Chanarin, 1994; Bower & Wald, 1995). Supplementation with ≥ 5 mg/day folic acid is reported to reverse the haematological signs of vitamin B₁₂-deficiency in at least 50% of subjects (discussed by Chanarin, 1994; Bower & Wald, 1995; Savage & Lindenbaum, 1995).

Studies of particular importance in the risk assessment

Human data

Weissberg et al., 1950

This was a non-controlled study of the neurological effects of 20 mg/day folic acid supplementation for 6-12 months, in 26 normal volunteers and 22 patients with non-pernicious anaemia. Prior to therapy, 6 of the normal subjects and 7 of the anaemic subjects showed some abnormal neurological signs (but not those of subacute combined spinal cord degeneration), which were not significantly altered during the therapy. Four subjects (1 normal, 3 anaemic) developed central nervous system changes during the folic acid treatment, but these changes were not considered to be related to the therapy.

Harvey et al., 1950

In this open, uncontrolled study, oral folic acid supplementation (20 mg/day for 3 - 12 months) produced no indications of spinal cord or peripheral nerve damage in 40 healthy subjects without pernicious anaemia (13 subjects had mild hypochromic anaemia).

MRC Vitamin Study Research Group, 1991

This was a multicentre, randomised, double-blind, placebo-controlled trial carried out in the UK, in which a total of 1817 women with a previous NTD-affected pregnancy were assigned to 1 of 4 supplementation groups:

- A] 4 mg/day folic acid
- B] 4 mg/day folic acid + multivitamins (daily – 4000 IU vitamin A, 400 U vitamin D, 1.5 mg each vitamins B₁ and B₂, 1.0 mg vitamin B₆, 40 mg vitamin C, 15 mg nicotinamide)
- C] placebo
- D] multivitamins (as B], without folic acid).

The duration of therapy was from the date of randomisation until the 12th week of pregnancy. Statistical analysis showed a significantly reduced relative risk for NTDs associated with folic acid supplementation compared with no folic acid

supplementation, i.e. groups A and B compared with groups C and D (RR = 0.28; 95% CI, 0.12-0.71). The authors also reported that possible adverse effects of folic acid to the foetus and the mother were examined. The authors concluded that there was no demonstrable harm from the folic acid supplementation, although the ability of the study to detect rare or slight adverse effects was limited. The incidences of general side effects (e.g. infertility, irregular menses, vomiting in pregnancy, upper respiratory illness) reported by women taking part in the trial were similar in all 4 groups.

Animal data

Chung et al., 1993

In a study of the synergistic effects of folic acid and the anti-malarial drug, pyrimethamine (an inhibitor of dihydrofolate reductase), groups of 10 pregnant female rats were supplemented with combinations of the anti-malarial drug, pyrimethamine, and/or folic or folinic acid, from days 7 - 17 of gestation, by gavage. Folic acid treatment (50 mg/kg bw/day) alone showed no significant maternal or embryotoxicity, as compared with vehicle-only treatment.

Exposure assessment

Total exposure/intake:

Food	Mean: 0.261 mg/day 97.5 th percentile: 0.489 mg/day (1990 NDNS)
Supplements	up to 0.40 mg in OTC supplements for males up to 0.70 mg in OTC supplements for females (OTC, 2001)
Estimated maximum daily intake:	0.889 mg/day for males 1.189 mg/day for females

No potential high intake groups have been identified.

Implications of food fortification of wheat flour

During the 1990s, the public health policy with regard to preventing NTDs aimed to ensure that women of childbearing age were aware of the importance of acquiring sufficient folic acid in the diet at the time of conception to minimise the risk of NTDs in their offspring. In the UK, as in the US, there have been calls for more active public policy, including food fortification to address this. The Committee on Medical Aspects of Food and Nutrition Policy (COMA) reviewed the links between folates, including folic acid, and disease (COMA, 2000), and:

- confirmed the link between low folate status and the risk of NTDs;
- concluded that there is insufficient evidence on which to establish a conclusive link with cardiovascular disease;

- acknowledged that increasing the intake of folic acid might pose a risk to people with undiagnosed vitamin B₁₂ deficiency, particularly older people, and advised clinical vigilance to avoid any delays in diagnosis;
- recommended that the current policy of encouraging women who could become pregnant to take 0.40 mg folic acid as a supplement should continue

COMA further concluded that universal fortification of flour at 0.24 mg per 100 g in food products as consumed would result in:

- a significant effect in preventing NTD-affected births and pregnancies without resulting in unacceptably high intakes in any group of the population. It is estimated that this would reduce the incidence of NTD-affected pregnancies by 41% and would have, for example, prevented 38 of the 93 NTD-affected births in England and Wales in 1998, 30 of the 74 in Scotland in 1997, and 6 of the 14 in Northern Ireland in 1998.
- the average intake of folic acid of women aged 16-45 years would increase by 0.201 mg/day, leading to a total folate intake of 0.405 mg/day
- Approximately 7% of women in this age group would have total folate intakes in excess of 0.600 mg/day.
- Approximately 0.6% of people aged over 50 years would be exposed to levels of folic acid intake greater than 1 mg/day

For the purposes of this consultation, it is assumed that COMA's conclusions apply only to wheat flour.

This leads to a theoretical exposure assessment of:

0.368 mg/day (upper level intake for females)

plus 0.201 mg/day from fortified flour

plus 0.700 mg/day from supplements

The potential maximum daily intake would be **1.269 mg/day** for female adults

Risk assessment

Folic acid is generally considered as safe. Adverse effects may, potentially, occur in specific groups, such as individuals being treated with drugs that interact with folic acid metabolism. Women at risk of a NTD-affected pregnancy appear to be able to take folate supplements at up to 4 mg/day, without adverse reproductive or developmental effects.

Folic acid may lead to reversal of the symptoms of vitamin B₁₂ deficiency, potentially allowing the neuropathy associated with vitamin B₁₂ deficiency to develop untreated. Vitamin B₁₂ deficiency is most prevalent in elderly subjects.

Few data are available from toxicological studies of folates in animals.

ESTABLISHMENT OF GUIDANCE LEVEL

There are insufficient data from animal or human studies to establish a safe upper level for folic acid.

Health experts recommend that, for the prevention of foetal NTDs, women who are likely to become pregnant should take a 0.40 mg folic acid supplement daily, in addition to normal dietary folate intake. Women in high risk groups may be advised to take up to 10 times this level of supplementation. Such therapy is generally considered to be without adverse reproductive or developmental effects.

The main concern regarding ingestion of excess folic acid is the consequential masking of vitamin B₁₂ deficiency. Data taken from case reports suggest that supplementation with ≤ 1 mg/day folic acid does not mask vitamin B₁₂-associated anaemia in the majority of subjects, whereas supplementation with ≥ 5 mg/day folic acid does. The effects of doses of between 1 and 5 mg/day are unclear. No other significant adverse effects have been associated with ingestion of folic acid.

For guidance purposes only, in the general population a supplemental dose of 1 mg/day (equivalent to 0.017 mg/kg bw/day in a 60 kg adult) would not be expected to cause adverse effects. Assuming a maximum intake from food of approximately 0.55 mg/day, a total dose of 1.55 mg/day (equivalent to 0.026 mg/day in a 60 kg adult) would not be expected to have any adverse effects.

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PRELIMINARY RISK ASSESSMENT –NIACIN (NICOTINIC ACID AND NICOTINAMIDE)

General information

Chemistry

Niacin (vitamin B₃) is the generic term for nicotinic acid (pyridine 3-carboxylic acid) and nicotinamide (nicotinic acid amide), and the coenzyme forms of the vitamin. Nicotinamide is the active form, which functions as a constituent of two coenzymes, namely, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). These coenzymes in their reduced states (NADH/NADPH) are the principal forms of niacin that exist in animal tissues.

Natural occurrence

Free nicotinic acid and nicotinamide are present in nature in only small amounts. Nicotinic acid is mainly bound to macromolecules in plants, while nicotinamide is usually a component of NADP in the animal world. Nicotinic acid can be formed in humans from the metabolism of dietary tryptophan, and so niacin is not really a vitamin providing adequate tryptophan is available.

Occurrence in food, food supplements and medicines

Important sources of preformed niacin include beef, pork, wheat flour, maize (corn) flour, eggs and cows milk. Human milk contains a higher concentration of niacin than cows' milk. In unprepared foods, niacin is present mainly in the form of the cellular pyridine nucleotides NAD and NADP. Enzymatic hydrolysis of the coenzymes can occur during the course of food preparation.

In the UK there is mandatory fortification of flour (except wholemeal and certain other specified types) with nicotinic acid at a level of not less than 1.6 mg/100 g flour for restoration purposes.

In the UK, niacin supplements are generally in the form of nicotinamide. Levels range from 0.25 to 150 mg per daily dose in multi-nutrient supplements. Nicotinamide products with a maximum daily dose of 500 mg are only available from pharmacists, and those preparations used to treat specific metabolic disorders are only available on prescription. Nicotinic acid is present in some General Sales List multivitamin preparations at levels up to 50 mg, and is available from the pharmacist to treat muscle cramp, and on prescription for use in hyperlipidaemias.

Other sources of exposure

No other sources of exposure have been identified.

Recommended amounts

The UK RNI for niacin is 6.6 mg niacin equivalent/1000 kcal, equivalent to 17 and 13 mg/day in adult males and females respectively. An increment of 2.3 mg/day niacin was recommended for lactating women. Under normal conditions, for an adult, the amount of tryptophan present in dietary protein provides adequate niacin without the need for any preformed vitamin.

Analysis of tissue levels and niacin status

There is no established laboratory method for the assessment of niacin status. In experimental animals, measurement of whole blood NADP can provide a sensitive indication of niacin depletion, while the determination of the urinary excretion of *N*-methyl nicotinamide and its metabolite methyl pyridone carboxamide offers the only other method available.

Brief overview of non-nutritional beneficial effects

High dose treatment with nicotinic acid has been shown to reduce plasma cholesterol levels (by an average of 20 to 35%). In the Coronary Drug Project, nicotinic acid was observed to reduce mortality due to a reduction in lethal coronary events. Supplementation studies have been shown to increase NAD⁺ concentrations in lymphocytes. DNA strand breaks in lymphocytes exposed to oxygen radicals were shown to decrease proportionately to NAD⁺ concentrations.

High dose nicotinamide treatment has also been claimed to offer protection against the development of insulin-dependent diabetes mellitus.

Function

Niacin is the functional factor of two important coenzymes, NAD and NADP, which activate over 200 dehydrogenases essential to electron transport and other cellular respiratory reactions. Despite their structural similarity, NAD and NADP have quite different metabolic roles. NAD functions as an electron carrier for intracellular respiration as well as a co-factor for enzymes involved in the oxidation of fats and carbohydrates, such as glyceraldehyde 3-phosphate, lactate, pyruvate and α -ketoglutarate dehydrogenases. NADP functions as a hydrogen donor in reductive biosynthesis, such as in fatty acid and steroid synthesis and like NAD as a cofactor for enzymes, such as in the oxidation of glucose-6-phosphate to ribose 5-phosphate in the pentose phosphate pathway.

Deficiency

The most common symptoms of niacin deficiency are changes in the skin, mucosa of the mouth, stomach and intestinal tract and the nervous system. The changes in the skin are among the most characteristic in human beings. They are called 'pellagra', which means 'raw skin' and are most pronounced in the parts of skin exposed to sunlight. Other signs and symptoms include dizziness, vomiting, constipation or diarrhoea, and inflammation of the tongue and gastric mucosa. The neurological

symptoms can include fatigue, sleeplessness, depression, memory loss and visual impairment.

Interactions

Interactions of niacin with drugs have been identified. Prolonged treatment of tuberculosis with isoniazid may lead to niacin deficiency due to its competition with pyridoxal phosphate, a co-enzyme necessary for the conversion of tryptophan to niacin.

The nicotinic acid-induced vasodilation, presenting as skin flushing, is inhibited by clonidine, and may exacerbate the vasodilatory effect of ganglion blocking agents. However, ganglion blocking anti-hypertensives are used extremely rarely now.

Absorption and bioavailability

In humans, niacin is rapidly absorbed from the stomach and intestine by a sodium carrier-mediated mechanism at low concentrations.

Distribution and metabolism

Niacin circulates in the plasma in the unbound form as both the acid and the amide. Each enters peripheral tissues by passive diffusion, followed by metabolic trapping by conversion to the pyridine dinucleotides, NAD(H) and NADP(H). Most is found as NAD(H) and the oxidised form NAD.

The plasma half-life of nicotinic acid is relatively short, approximately one hour. Animal studies have shown that nicotinic acid rapidly disappears from the blood and is mainly concentrated in the liver, but also in adipose tissue and in the kidneys.

The main metabolites in humans are *N*-methylnicotinamide, *N*-methyl-2-pyridone-5-carboxamide and *N*-methyl-4-pyridone-5-carboxamide.

Excretion

The pattern of niacin products excreted after ingestion of the vitamin depends largely on the amount and form of niacin ingested and on the niacin status of the individual. However, the two major excretion products in man are *N*-methylnicotinamide and *N*-methyl-2-pyridone-5-carboxamide, with minor amounts of the unchanged vitamin, nicotinamide-*N*-oxide and 6-hydroxynicotinamide also being excreted.

Toxicity

Human data

Reports of nicotinic acid toxicity in humans stem, in the main, from its use in the treatment of hypercholesterolaemia. Most adverse effects are dose related and generally subside with a reduction in dose or the cessation of treatment. Symptoms of acute toxicity include flushing, itching of the skin, nausea, vomiting and gastrointestinal disturbances. Additionally, jaundice, hyperglycemia, abdominal pain,

elevated serum bilirubin, alkaline phosphatase and aminotransferase levels can be seen with ingestion of high levels of nicotinic acid (generally intakes of 3,000 mg/day or more) for long periods of time. In a small number of cases, anorexia, ophthalmological effects, skin hyperpigmentation and precipitation of incipient psychosis have been reported as side effects of nicotinic acid therapy. Sustained release preparations are reported to be more hepatotoxic than the crystalline form.

Evidence is sparse, but there have been case reports of liver dysfunction following long-term high dose (3000–9000 mg) nicotinamide therapy.

Supplementation trials

Hoffer (1969) reported a range of adverse effects, including headaches, heartburn, nausea, gastrointestinal disturbances and fatigue at doses of 3000 mg supplemental nicotinamide/day for 3-36 months. However, few details were provided and comparison with controls was lacking. Other supplementation trials (Vague *et al.*, 1987; Mendola *et al.*, 1989; Chase *et al.*, 1990; Polizzi *et al.*, 1995; Lampeter *et al.*, 1998) have reported no adverse effects at intakes of nicotinamide up to 3000 mg/day. However the studies in which the highest doses were given were primarily of beneficial effect in Type 1 diabetes mellitus patients and it is unclear how information on adverse effects was ascertained. Gastrointestinal effects and flushing have been reported at intakes of 50 mg supplemental nicotinic acid/day and above.

Doses of up to 2000 mg/day of nicotinic acid have reportedly been administered during pregnancy to niacin deficient women in developing countries, without evidence of foetal toxicity (Moghissi, 1981).

Animal data

Few data are available concerning the ingestion of nicotinic acid. Acute effects in dogs, associated with intake of 2000 mg/day for less than 20 days, included weight loss, bloody faeces and convulsions resulting in death with associated gastrointestinal and central nervous system changes. With administration of lower doses (up to 1000 mg/day) for 8 weeks dogs gained weight and appeared in good health. Traces of albumin and sugar were found in urine.

Chronic administration of 1% nicotinamide in the diet to rats has been shown to inhibit growth.

Reproductive toxicity

In rats, ingestion of nicotinamide has been shown to cause growth retardation, which may be due, in part, to the reduced intake of food and water due to the palatability and in part to a deficiency in methionine, which is expended during the methylation of nicotinamide into its metabolites.

Carcinogenicity and genotoxicity

No carcinogenicity data are available for nicotinic acid.

In lifetime studies in the mouse, nicotinamide alone was not carcinogenic. However, in combination with streptozotocin, nicotinamide has been shown to cause islet cell tumours in rats. The resulting oncogenicity is likely to be the result of an imbalance between the degree of DNA damage and the level of inhibition of DNA repair.

No genotoxicity data are available for nicotinic acid or nicotinamide.

Dose response characterisation

Few data are available, but supplemental intakes of nicotinamide of up to 3000 mg/day appear to be well tolerated. Flushing has been reported with bolus doses of nicotinic acid of 10 mg or higher, but flushing is more consistently associated with supplemental intakes of nicotinic acid of 50 mg/day and above.

Mechanisms of toxicity

No relevant data have been identified.

Vulnerable groups

Individuals with hepatic dysfunction or a history of liver disease, diabetes mellitus, active peptic ulcer disease, gout, cardiac arrhythmias, migraine headaches and alcoholism may be particularly susceptible to nicotinic acid.

Genetic variations

No data on genetic variations that increase vulnerability to niacin toxicity have been identified.

Studies of particular importance in the risk assessment

Human data - nicotinic acid

Spies et al., 1938

In an uncontrolled study, 100 adult subjects were given single oral doses of 50 or 100 mg nicotinic acid on an empty stomach. Flushing, burning and itching was reported in 5% of those receiving the 50 mg dose and 50 % of those given 100 mg. Flushing occurred in the majority of subjects receiving 200 mg doses of nicotinic acid and was present “to some extent” in all subjects receiving 500 mg doses. It is unclear how the subjects were divided between dose groups. By studying cutaneous temperatures, it was determined that the increase was most marked over the ears and neck and less pronounced over the trunk, with the extremities being least affected.

Sebrell and Butler, 1938

In an uncontrolled study, groups of six subjects were given 10, 30 or 50 mg nicotinic acid daily for three months. Flushing was reported intermittently in 0, 2 and 4 individuals, respectively. The subjects received an identical diet. The authors noted

that there was considerable inter-individual variation, which was not accounted for by body weight.

The Coronary Drug Project, 1975

The study was conducted to investigate the possible use of nicotinic acid in coronary heart disease to reduce the incidence of a second myocardial infarction. The study demonstrated little immediate beneficial effect, but considerable toxicity. One third to one half of the 1119 patients taking 3000 mg nicotinic acid/day for at least five years had increased serum levels of liver enzymes. An elevation in serum uric acid levels was noted in the nicotinic acid treated group (44% of nicotinic acid treated individuals) and an increased incidence of acute gouty arthritis (6.4% of nicotinic acid treated individuals), compared to controls (4.3% of individuals). Consistent with other reports, side effects included dermatological problems of flushing, itching and rash. Other significant complaints in nicotinic acid-treated individuals included gastrointestinal and urinary tract problems.

Knopp et al., 1985

A study in 71 patients compared the effects of regular nicotinic acid (37 individuals) and sustained release nicotinic acid (34 individuals). Treatment was for six months and subjects received an initial dose of 1500 mg/day increasing to 3000 mg/day from the second month. However due to a higher frequency of side effects among those receiving the sustained release formula, the daily intake was reduced to 2000 mg/day for this group. Subjects treated with sustained release nicotinic acid experienced a significantly higher rate of gastrointestinal effects, compared with those on regular nicotinic acid. Dermatological problems were reported for both treatments.

Fraunfelder et al., 1995

In a retrospective survey, 102 patients taking nicotinic acid medication (3000-8000 mg/day) for hyperlipidaemia were more likely than those taking other lipid lowering agents to report blurred vision (26%), dry eyes (20%), eyelid oedema (10%) and macular oedema (2%). The ocular side effects were all reversible if treatment was discontinued and the authors reported the severity of effects to be dose dependent. For one patient, a reduction in dose from 3000 mg/day to 1500 mg/day was stated to have reversed the effects on her vision.

Human data – nicotinamide

Vague et al., 1987

In a study published as a letter, no adverse effects were reported in a double-blind trial in 16 Type 1 diabetics who received 3000 mg nicotinamide per day or placebo for six months. The average age of the subjects was 22.1 years in the treatment group and 24.8 years in the placebo group. No adverse effects were noted but it is unclear how this was ascertained.

Mendola et al., 1989

In a single-blind trial, twenty newly diagnosed Type I diabetics, received 1000 mg nicotinamide/day or placebo for 45 days. The average age of the subjects was 18.3 years in the treatment group and 15.5 years in the placebo group. No adverse effects were observed when physiological, biochemical and haematological parameters were assessed.

Chase et al., 1990

In a double-blind study in 35 newly diagnosed Type I diabetics, aged between 6 and 18 years, individuals received 100 mg nicotinamide per year of age per day, up to a maximum of 1500 mg/day for 12 months. The authors stated that no significant adverse effects were encountered with the use of nicotinamide, although it is unclear how any information on side effects was ascertained.

Pozzilli et al., 1995

In a double-blind trial following up the work of Mendola, a further 56 newly diagnosed Type I diabetics aged 5-35 years were given 25 mg/kg bw/day or placebo for 12 months. It was stated that biochemical tests including liver and kidney function were normal during follow up and that no adverse effects were noted in either treated or placebo patients.

Lampeter et al., 1998

The Deutsche Nicotinamide Intervention Study (DENIS) evaluated the clinical efficacy of high doses of nicotinamide in children at high risk of developing Type I diabetes. Fifty-five children were randomised into two groups and received either placebo or supplemental nicotinamide (1200 mg/m²/day) for a maximum duration of 3.8 years. Mean treatment time was 2.1 years. If it is assumed that the children in the study were of average height and weight, a total nicotinamide intake, including the contribution of the diet, of 1260 mg/day or 42 mg/kg bw/day can be estimated in subjects receiving the nicotinamide supplement. The rates of diabetes onset were the same throughout the observation period in both groups. All biochemical and haematological parameters (alanine aminotransferase, aspartate aminotransferase, bilirubin, blood sedimentation rate, γ -glutamyl transferase, urea, uric acid, creatinine and lactate dehydrogenase) were within the normal range, and means did not differ between the groups throughout the study.

Exposure assessment

The exposure data below for intake from food are for niacin equivalents. These are defined as the niacin content of the food plus 1/60th the content of tryptophan, as nicotinic acid is formed in the body from the metabolism of tryptophan. It is not possible to distinguish the two forms of niacin in this survey data.

Food	Mean: 34.1 mg/day (from 1986/87 NDNS) 97.5 th percentile: 57.2 mg/day
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Supplements up to 150 mg/day (as nicotinamide, or up to 50 mg/day as nicotinic acid) (OTC, 2001)

Estimated maximum daily intake of niacin equivalents: 57.2 + 150 = 207.2 mg/day.

No potential high intake groups have been identified.

Risk Assessment

Nicotinic acid

Large doses of nicotinic acid are associated with a number of adverse effects in man. These have been identified from the use of nicotinic acid in the treatment of hypercholesterolaemia. The effects reported include flushing, skin itching, nausea, vomiting and gastrointestinal disturbance. The effects are dose related and reversible on cessation of treatment.

At higher intakes of nicotinic acid over long periods of time, liver dysfunction has been reported. Symptoms such as elevated liver enzymes, elevated bilirubin levels and jaundice have been observed. Other adverse effects reported include hyperglycaemia and adverse ophthalmological effects such as blurred vision and cystoid macular oedema. No relevant animal data have been reported and the mechanism for nicotinic acid-induced toxicity is unclear.

Nicotinamide

Fewer data are available on the safety of nicotinamide. Studies in Type I diabetics have suggested that doses of up to 3000 mg/day nicotinamide are not associated with adverse effects, although these investigations involved small numbers of subjects and it is unclear from the studies in which the highest doses were given how adverse effects would have been ascertained. Since the studies were undertaken in diabetics or in individuals at high risk of developing diabetes the applicability of the results to the general population is unclear. No relevant animal data have been identified.

ESTABLISHMENT OF GUIDANCE LEVEL – NICOTINIC ACID

There are insufficient data from human or animal studies to establish a safe upper level for nicotinic acid.

Numerous reports exist, including both case reports and controlled clinical trials, in which doses of approximately 3000 mg/day nicotinic acid have caused apparent hepatotoxic effects. For example, in a randomised double-blind study, approximately one third of 1119 patients who received 3000 mg nicotinic acid/day for up to 5 years were reported to have elevated levels of liver enzymes. Elevations in serum uric acid levels and an increased incidence of gout were also reported.

Flushing has been reported at intakes as low as 10 mg/day, but has been more consistently reported at intakes of 50 mg/day and above (Spies *et al.*, 1938; Sebrell and Butler, 1938). If 50 mg/day is taken as a LOAEL and an uncertainty factor of 3 is applied to extrapolate to a NOAEL, then a guidance level, for supplementation only, of $50/3 = 17$ mg/day (equivalent to 0.28 mg/kg bw/day in a 60 kg adult) for nicotinic acid is derived. This guidance level is given for supplements only, as adverse effects appear to be related to acute, bolus intakes of nicotinic acid, rather than more sustained exposure as would occur with ingestion of nicotinic acid via food, and free nicotinic acid levels in food are low.

It should be noted that this guidance level is based on intakes of conventional formulations of nicotinic acid and, therefore, would not be applicable to sustained release preparations. Nicotinic acid contained in dietary supplements is not in the sustained release form, which is thought to be more hepatotoxic.

ESTABLISHMENT OF GUIDANCE LEVEL- NICOTINAMIDE

There are insufficient data from human or animal studies to establish a safe upper level for nicotinamide.

From the limited existing database nicotinamide toxicity appears to be quite low. Large doses of nicotinamide (up to 3000 mg/day for periods of up to 3 years) appear to be well tolerated, as reported in numerous trials on the possible benefits of nicotinamide in patients with, or at risk of developing, diabetes. These trials, however, usually studied only one dose level, and the numbers of subjects involved in the trials was small. Two of the best conducted studies are those by Pozzilli *et al.* (1995) and Lampeter *et al.* (1998) and we have used these for guidance purposes. In these studies, doses of 25 and 42 mg/kg bw/day did not affect a range of biochemical parameters, including liver and kidney function tests in small groups of Type 1 diabetics (or those at high risk of developing the condition). Although no adverse effects were detected, the nature of the study population and the small numbers involved mean that this may not be applicable to the whole population. Although no adverse effects were identified, it is prudent in this case to apply a UF of 3 to account for inter-individual variability because of the nature of the study population. Thus, $25/3$ results in a guidance value, for supplementation only, of 8.3 mg/kg bw/day for nicotinamide. This is equivalent to 500 mg/day supplemental nicotinamide in a 60 kg adult. Assuming a maximum intake of 57 mg/day from food, a total dose of 557 mg/day (equivalent to 9.3 mg/kg bw/day in a 60 kg adult) would not be expected to result in any adverse effects. There is a lack of data on the safety of nicotinamide in pregnancy, and no relevant animal data. Therefore, this level does not apply to pregnant women.

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PRELIMINARY RISK ASSESSMENT – PANTOTHENIC ACID

General information

Chemistry and geochemistry

Pantothenic acid consists of a pantoic acid moiety amide-linked to a β -alanine subunit. Pantetheine consists of pantothenic acid linked to a β -mercaptoethylamine group. In living systems, the compound is a component of coenzyme A (CoA), which is composed of 4'-phosphopantetheine linked to adenosine 5'-monophosphate, modified by a 3'-hydroxyl phosphate. 4'-Phosphopantetheine is also found covalently linked to various proteins, particularly those involved in fatty acid metabolism.

Natural occurrence

The active vitamin is present in virtually all plant, animal and microbial cells.

Occurrence in food, food supplements and medicines

The majority of pantothenic acid within foods is present as CoA. Chicken, beef, potatoes, oat cereals, tomato products, liver, kidney, yeast, egg yolk, broccoli and whole grains are reported to be major sources of the vitamin, whilst very high levels are present in royal bee jelly (511 mg/kg) and in the ovaries of tuna and cod (2300 mg/kg). Cooking is reported to destroy 15-50% of pantothenic acid in meats and 37 to 78% of pantothenic acid is lost from vegetables during processing. Breakfast cereals may be fortified with 5-6 mg pantothenic acid/100 g.

Pantothenic acid derivatives sold as supplements or medicines are prepared synthetically as calcium pantothenate or panthenol, which are more stable than pure pantothenic acid. In the UK, dietary supplements generally contain 6-10 mg pantothenic acid; licensed medicines may contain a maximum of 50 mg/day pantothenic acid.

Other sources of exposure

The pantothenic acid derivative, panthenol, is added to some cosmetic products.

Recommended amounts

Dietary reference values for pantothenic acid have not been established in the UK. There is no detectable evidence of pantothenic acid deficiency within the general population, even during pregnancy and lactation. Therefore, human requirements for pantothenic acid are considered to be adequately provided by the diet.

Analysis of tissue levels and pantothenic acid status

Blood, urine or tissue pantothenic acid levels may be measured by yeast or *lactobacillus* assay, or by radio-immunoassay. Assays of pantothenic acid in

biological materials other than urine require that the compound be first hydrolysed from CoA.

Brief overview of non-nutritional beneficial effects

It has been claimed that supplementation with pharmacological doses of pantothenic acid may alleviate the symptoms of rheumatoid arthritis and lupus erythematosus.

Function

Pantothenate, usually in the form of CoA-containing species (e.g. acetyl CoA, succinyl CoA), fulfils multiple roles in cellular metabolism and in the synthesis of many essential molecules.

Deficiency

Deficiency of pantothenic acid in humans is extremely rare. Pantothenic acid deficiency has been induced experimentally in human subjects by a diet virtually devoid of the vitamin, or by the administration of a metabolic antagonist (omega-methyl pantothenic acid). Signs and symptoms exhibited by subjects given the antagonist (not all of which were necessarily due to the deficiency) included irritability and restlessness, fatigue, apathy, malaise, sleep disturbances, gastrointestinal complaints such as nausea, vomiting and abdominal cramps, neurological and other clinical effects such as numbness, parasthesia, muscle cramps and staggering gait, hypoglycaemia and an increased sensitivity to insulin. Historically, pantothenic acid deficiency has been implicated in the “burning feet” syndrome experienced by severely malnourished prisoners of war.

Interactions

Reports have indicated a sparing effect of some other vitamins, such as ascorbic acid and other B-vitamins, on pantothenic acid within the body. Biotin and pantothenic acid may share a common carrier-mediated uptake mechanism in the gastrointestinal tract and other tissues, although the physiological relevance of this is unknown. Some earlier reports have suggested that high dietary fat and low dietary protein may exacerbate pantothenic acid deficiency.

Absorption and bioavailability

Pantothenic acid is readily absorbed throughout the gastrointestinal tract. Ingested CoA is hydrolysed within the intestinal lumen, *via* the formation of dephospho-CoA, phosphopantetheine and pantetheine, to pantothenic acid. Uptake of these latter two compounds into intestinal tissues has been demonstrated, and subsequently the enzyme, pantetheinase, can hydrolyse pantetheine to pantothenic acid. Uptake into intestinal cells occurs both by a sodium-dependent active transport mechanism and by passive diffusion. Limited data are available regarding the bioavailability of dietary pantothenic acid. One study found that pantothenic acid in natural foods was approximately 50% bioavailable compared with calcium pantothenate given in a formula diet, as assessed by subsequent urinary excretion of the vitamin.

Distribution and metabolism

Absorbed pantothenic acid is transported to body tissues *via* the blood, primarily as bound forms within erythrocytes. Plasma levels do not correlate well with dietary intake. The majority of tissues import pantothenic acid *via* an active sodium co-transport mechanism. Analysis of rat tissues has shown high concentrations of pantothenic acid in the heart and kidneys. CoA is synthesised from pantothenic acid within cells, with the first, and apparently rate-limiting, step catalysed by pantothenate kinase.

Excretion

Catabolism of CoA leads to the formation of pantothenate, which is excreted in the urine. Excretion levels correlate well with dietary intake.

Toxicity

Human data

Case reports and some much earlier non-controlled studies describe a lack of acute or chronic toxic effects of pantothenic acid compounds (calcium or sodium pantothenate, panthenol) at very high doses (approximately 10,000 mg/day in some cases for a number of years), although such levels have been associated with diarrhoea and gastrointestinal disturbances. In more recent, controlled studies (generally carried out to assess the potential benefits of pantothenic acid supplementation in specific subgroups, for example, arthritic patients) no side effects have been reported for pantothenic acid supplementation at levels up to approximately 2000 mg/day, for periods of several days to several weeks. However, the small numbers of participants and short duration of these studies limit the value of the data regarding any potential rare or long-term toxic effects.

One non-blind, non-randomised, non-placebo controlled trial, designed to investigate the effectiveness of megavitamin therapy in improving the behaviour of 41 children with attention deficit disorders, showed significant increases in serum aspartate transaminase levels (indicative of liver damage) in 17 children after 12 weeks of multivitamin therapy (including doses of calcium pantothenate increasing during the study period to a maximum of 1200 mg/day). This effect may have been associated with the nicotinamide component of the multivitamin supplement, although this could not be confirmed as the vitamins were not given separately.

Animal data

Data regarding the toxicity of pantothenic acid and its commonly-used pharmaceutical forms in experimental animals are limited because of the small numbers of animals used in the studies. In the early 1940's Unna & Greslin reported acute and chronic toxicity tests with D-calcium pantothenate in mice, rats, dogs and monkeys (Unna and Greslin, 1940, 1941). Acute oral LD₅₀ values were very high ($\geq 10,000$ mg/kg bw, mice and rats), with lethal doses producing death by respiratory failure. An oral dose of 1000 mg/kg bw produced no toxic signs in dogs or in one monkey. Oral dosing

(500 or 2000 mg/kg bw/day to rats, 50 mg/kg bw/day to dogs, 200-250 mg/kg bw/day to monkeys) for 6 months produced no toxic signs or weight loss, or evidence of histopathological changes at autopsy. The offspring of rats supplemented with 500 mg/kg bw/day calcium pantothenate were fed diets supplemented with 500 mg/kg bw/day calcium pantothenate from weaning; no evidence of toxicity or reduced weight gain, or histopathological changes were observed. The available data do not indicate reproductive or developmental toxicity of pantothenic acid or its commonly used pharmaceutical forms.

Carcinogenicity and genotoxicity

Calcium pantothenate, sodium pantothenate and panthenol were not mutagenic in bacterial tests. No *in vivo* genotoxicity or carcinogenicity data have been found.

Mechanism of toxicity

No data have been identified.

Dose response characterisation

No data have been identified.

Vulnerable groups

No vulnerable groups have been identified.

Genetic Variations

No genetic variations in the metabolism or effects of pantothenic acid have been identified.

Studies of particular importance in the risk assessment

Human studies

Goldman, 1950; Welsh, 1952, 1954

Supplementation of lupus erythematosus patients with doses of pantothenic acid of 10,000 mg/day or more led to gastrointestinal signs and symptoms.

The General Practitioner Research Group, 1980

This randomised, double-blind, placebo-controlled study was carried out to assess the effects of high-dose pantothenate supplementation in alleviating symptoms of arthritis. A total of 47 (31 completed the trial) patients were treated with calcium pantothenate (total daily dose – 500 mg on days 1-2, 1000 mg on days 3-5, 1500 mg on days 6-9, 2000 mg from day 10 onwards) and 47 (34 completed the trial) with placebo for a period of 8 weeks. The patients were suffering from a variety of arthritides, including 27 patients with rheumatoid arthritis. The authors stated that “... ”

pantothenic acid supplementation in specific subgroups, for example patients suffering joint disease.

Data regarding the toxicity of pantothenic acid and its commonly-used pharmaceutical forms in experimental animals are also limited. However doses of 500 and 2000 mg/kg bw/day in rats and 200-250 mg/kg bw/day in dogs and monkeys, given in the diet for periods of six months, were not associated with adverse effects.

ESTABLISHMENT OF A GUIDANCE LEVEL

There are insufficient data from human or animal studies to establish a safe upper level for pantothenic acid.

There are relatively few human data available on the oral toxicity of pantothenic acid from controlled trials. The limited available data have not identified target organ toxicity and the adverse effects that were noted were transient. The apparent low toxicity of pantothenic acid is supported by the available animal data. The General Practitioner Research Group study suggests that doses of 2000 mg pantothenic acid/day are without effect, although this study investigated relatively few individuals, suffering from a variety of clinical conditions (rheumatoid arthritis, osteoarthritis, gout and spondylitis). Adverse effects were not a primary outcome measure and though the authors specifically noted the absence of side effects in fewer placebo than treated individuals the method by which this data was collected and the specific nature of the side effects noted are not discussed. Based on these data, for guidance purposes only, a supplemental daily intake of 200 mg (equivalent to 3.3 mg/kg bw/day for a 60 kg adult), in addition to that present in the diet, would not be expected to produce adverse effects in the general population. This includes a 10-fold uncertainty factor for inter-individual variation because of the small numbers involved in the study. Assuming a maximum dietary intake of 10 mg/day, this would equate to a total intake of 210 mg/day, or 3.5 mg/kg bw/day for a 60 kg adult.

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PRELIMINARY RISK ASSESSMENT – RIBOFLAVIN

General information

Chemistry

Riboflavin is a water-soluble vitamin of the B group (vitamin B₂). It is stable to mineral acids in the dark at 27°C. Decomposition occurs in both acidic and alkaline solutions.

Natural occurrence

Riboflavin is present as an essential constituent of all living cells, and is therefore widely distributed in small amounts in foods.

Occurrence in food, food supplements and medicines

The major sources of riboflavin are milk, eggs, enriched cereals and grain, ice cream, liver, some lean meats, and green vegetables. Because riboflavin is degraded by light, loss will occur if foods are left out in sunlight, or any UV light. Riboflavin is stable when heated but will leach into cooking water. The pasteurisation process causes milk to lose about 20% of its riboflavin content. Alkalis, such as baking soda, also destroy riboflavin. Riboflavin is a permitted colouring agent in foods and pharmaceuticals. It is permitted at *quantum satis* level in most processed foods, but due to its instability when exposed to light, its use tends to be restricted to relatively few foods, such as salad dressings, confectionery and powdered drinks. It is included in multi-nutrient supplements available over the counter at levels up to 15 mg/daily dose. It is also present in some multi-constituent products which can only be sold in pharmacies because other constituents cannot be sold without the supervision of a pharmacist. These are used for the prevention or treatment of nutrient deficiencies and contain up to 25 mg riboflavin per daily dose.

Other sources of exposure

No other sources of exposure were identified.

Recommended amounts

The minimal requirement for riboflavin to prevent clinical signs of deficiency appears to be less than 0.35 mg/1000 kcal. The Committee on Medical Aspects of Food and Nutrition Policy (COMA) recommended a Reference Nutrient Intake (RNI) of 1.1 mg/day for women and 1.3 mg/day for men. Turnover of riboflavin appears to be related to energy expenditure, and periods of increased physical activity are associated with a modest increase in requirement, but COMA saw no justification for reducing the RNIs for older people below those for younger adults.

Analysis of tissue levels and riboflavin status

Riboflavin and its coenzyme derivatives, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), can be detected with high sensitivity using high pressure liquid chromatography. Riboflavin status can be assessed using the erythrocyte glutathione reductase activation test, in which an activity coefficient (EGRAC) >1.30 is indicative of biochemical riboflavin deficiency.

Brief overview of non-nutritional beneficial effects

Riboflavin has been suggested for the treatment of migraine, carpal tunnel syndrome, cataracts and a variety of skin conditions (acne, dermatitis, eczema, ulceration), and for muscle cramps. However, there is no firm evidence that it is effective in any condition that is not related to riboflavin deficiency.

Function

Clinically, riboflavin promotes normal growth and assists in the synthesis of steroids, red blood cells, and glycogen. FAD also plays roles in oxidation-reduction reactions, interacting with a group of enzymes known as flavoproteins. Riboflavin helps to maintain the integrity of mucous membranes, skin, eyes and the nervous system. It supports the activity of antioxidants and is involved in the production of adrenaline by the adrenal glands. It is thought that riboflavin also aids the body in absorbing iron, since it is common for iron deficiency to accompany a deficiency in riboflavin.

Deficiency

Individuals who have inadequate food intake are at risk of deficiency, particularly children in developing countries. Other groups prone to riboflavin deficiency include older people with poor diet, chronic “dieters”, patients taking tranquillisers, persons who use fibre-based laxatives regularly, patients with hypothyroidism and women who exercise excessively. Riboflavin deficiency may arise in neonates during phototherapy for jaundice. It has also been associated with the development of cataracts and of rheumatoid arthritis. Riboflavin deficiency may occur as a result of inadequate nutrition or intestinal malabsorption. Riboflavin status can markedly influence the activity of hepatic microsomal drug metabolising enzymes.

Deficiency signs and symptoms include dry and cracked skin, sensitivity to bright light, itching, dizziness, insomnia, slow learning, weakness, sore throat, hyperaemia and oedema of the pharyngeal and oral mucous membranes, cheilosis, angular stomatitis, glossitis, seborrhoeic dermatitis, corneal vascularisation and anaemia associated with pure red cell hypoplasia of the bone marrow. The anaemia that develops in riboflavin deficiency is normochromic and normocytic and is associated with reticulocytopenia; leukocytes and platelets are generally normal. Administration of riboflavin to deficient patients causes reticulocytosis, and the concentration of haemoglobin returns to normal.

Interactions

The absorption of iron, zinc and calcium is impaired in riboflavin deficiency. Riboflavin impairs the antibiotic activity of streptomycin, erythromycin, tyrothricin, carbomycin and tetracyclines, but no inactivation occurs with chloramphenicol, penicillin or neomycin. Thyroid hormones, corticotrophin and aldosterone enhance the formation of FMN and FAD from riboflavin, while phenothiazines and possibly tricyclic antidepressants inhibit FAD formation. Ingestion of boric acid increases the excretion of riboflavin. Prior administration of probenecid decreases the renal clearance and gastrointestinal absorption of riboflavin.

A single report (Florsheim, 1994) has found riboflavin to increase the response of mice to ionising radiation. However, Pacernick *et al.* (1975) found that oral riboflavin had no effect on UV-induced skin tumours in hairless mice. Similarly, oral administration of riboflavin did not affect the sensitivity of homozygous or heterozygous Gunn rats to UV light.

Absorption and bioavailability

Riboflavin is readily absorbed from the small intestine, primarily by a specialised transport mechanism involving phosphorylation of the vitamin to FMN. Passive diffusion plays only a minor role at levels ingested in the diet. Riboflavin has been shown to undergo active secretion into, and saturable reabsorption from, the kidney tubules in rat, dog and human.

Distribution and metabolism

Riboflavin is distributed to all tissues. It is present in red blood cells, and appears to bind to a subfraction of immunoglobulins in plasma. Very little riboflavin is stored. Free riboflavin is transformed in the liver to form flavin coenzymes, (FAD and FMN), which are utilised as electron transfer factors in enzymatic reductions.

Excretion

When riboflavin is ingested in amounts approximately equivalent to the minimal daily requirement, only about 10-20% appears in the urine. As the intake is increased above minimal requirements, larger proportions are excreted unchanged.

Riboflavin is also found in faeces, sometimes in quantities exceeding that ingested. This probably represents the riboflavin synthesised by intestinal microorganisms, which is not absorbed.

Toxicity

Human data

No toxic or adverse reactions to riboflavin in humans have been identified. A harmless yellow discoloration of urine occurs at high doses. However, there has been at least one unconfirmed report of dermatitis following oral ingestion of a vitamin B complex that included riboflavin. Because riboflavin is a water-soluble vitamin, excess amounts are excreted.

Supplementation trials

Few human data are available. However, no toxic symptoms have been reported at doses of up to 400 mg per day for at least 3 months, other than occasional minor side effects that were not clearly attributable to the compound.

Animal data

On the basis of the limited data available, riboflavin appears to be of very low toxicity when administered orally or by injection to animals. It has been reported that administration of an acute oral dose of 10,000 mg/kg to rats resulted in no toxic effects. There are a few studies in which riboflavin has been administered orally to experimental animals (rats, mice, rabbits, dogs) for periods of up to 22 months at doses of up to 25 mg/kg bw per day. No obvious toxicity was observed. However, only a limited number of endpoints was investigated and, overall, the information available on the toxicity of riboflavin in experimental animals is limited. Riboflavin has been reported to be of very low developmental toxicity in experimental animals.

Carcinogenicity and genotoxicity

Riboflavin has not been shown to be carcinogenic, although *deficiency* of riboflavin may predispose to the development of some tumours. In a limited study in rats, oral administration of 1.5 mg/kg per day of riboflavin for 22 months was not carcinogenic.

Riboflavin was not mutagenic in the Ames Salmonella test. One report found no evidence of mutagenesis in the *umu* or SOS chromotest. In another report, riboflavin had no effect on polyploidy in Chinese hamster lung cells. No other adequate reports on the genotoxicity of riboflavin in other test systems could be located.

Mechanism of toxicity

No relevant data have been identified.

Dose response characterisation

No relevant data have been identified.

Vulnerable groups

There is a theoretical possibility that neonates undergoing phototherapy for hyperbilirubinaemia may be at risk at this time from photoactivation of riboflavin.

Genetic variations

No relevant genetic variations have been identified.

Studies of particular importance in the risk assessment

Human data

Zempleni et al., 1996

The pharmacokinetics and utilisation (flavocoenzyme synthesis) of orally and intravenously administered riboflavin were assessed in a study in healthy adults. After the determination of circadian rhythms of riboflavin concentrations in plasma and urine of four males and five females (control period), each subject received three different oral riboflavin doses (20, 40, and 60 mg) and one intravenous bolus injection of riboflavin (11.6 mg). Pharmacokinetic variables were calculated using a two-compartment open model. No adverse effects were reported at any of the dose levels studied.

Schoenen et al., 1998

In a prophylactic study of migraine in 55 patients with placebo control, high doses (400 mg per day) of riboflavin for at least 3 months were well tolerated, only two minor, non-specific adverse events being reported. These could not be attributed unequivocally to treatment.

Exposure assessment

Total exposure/intake:

Food Mean: 1.8 mg/day (from 1986/87 NDNS)
 97.5th percentile: 3.29 mg/day

Supplements up to 15 mg/day (OTC, 2001)

Estimated maximum daily intake: $3.3 + 15 = 18.3$ mg/day

No potential high intake groups have been identified.

Risk assessment

In several human studies riboflavin was well tolerated with no reports of adverse events.

Data on the toxicity of riboflavin in experimental animals are sparse. Acute oral administration of riboflavin to rats produced no adverse effects. Riboflavin has also been administered orally to rats, mice, rabbits and dogs for long periods without obvious toxicity. However, in none of these studies was a full evaluation performed, and in most cases only a very limited number of endpoints was investigated.

ESTABLISHMENT OF GUIDANCE LEVEL

There are insufficient data from human and animal studies to establish a safe upper level for riboflavin, although the available data indicate that it is of low toxicity. The balance of evidence suggests that ingestion of riboflavin over prolonged periods of time is without harmful effects, even at many times the normal level of exposure. In a prophylactic study of migraine, doses of 400 mg riboflavin per day for at least 3 months were well tolerated (Schoenen *et al.*, 1998). Only two minor non-specific adverse effects, which could not be unequivocally attributed to the treatment, were reported in the 55 patients. If an uncertainty factor of 3 is applied, to extrapolate from a LOAEL to a NOAEL, this study suggests that for guidance purposes only, supplemental intakes of about 100 mg riboflavin/day (equivalent to 1.7 mg/kg bw for a 60 kg adult) would be unlikely to result in adverse effects. This is in addition to the riboflavin that is provided by the diet. Assuming a maximum dietary intake of 3.3 mg/day, a total daily intake of 103.3 mg riboflavin (equivalent to 1.72 mg/kg bw/day in a 60 kg adult) would not be expected to result in any adverse effects.

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PRELIMINARY RISK ASSESSMENT - THIAMIN (VITAMIN B₁)

General Information

Chemistry

Thiamin (vitamin B₁) is a relatively heat- and acid-stable, water-soluble compound, containing a pyrimidine and a thiazole nucleus linked by a methylene bridge. Derivatives of thiamin include the mono-, pyro- and triphosphate forms and the synthetic hydrochloride and slightly less water-soluble mononitrate salt. Synthetic non water-soluble derivatives of thiamin are available but these are not used in food supplements.

Occurrence in food, food supplements and medicines

Foods providing rich sources of thiamin include unrefined grain products, meat products, vegetables, dairy products, legumes, fruits and eggs. In the UK there is mandatory fortification of white and brown flour with thiamin, to a level of not less than 0.24 mg/100g flour, to replace losses during production; thus, cereal products are also a rich source of thiamin.

Mononitrate or hydrochloride derivatives of thiamin are present in multi-constituent medicinal products for the prevention (dose 1 – 5 mg daily) or treatment (dose 10 – 35 mg daily) of nutrient deficiencies. Supplements containing thiamin alone are also available (daily doses up to 300 mg).

Recommended amounts

Body stores of thiamin are limited and a regular intake is necessary. Thiamin requirement is related to energy consumption. The Reference Nutrient Intake (RNI) for adults and children ≥ 1 year is 0.4 mg/1000 kcal and 0.3 mg/1000 kcal in infants. Assuming food intakes of 2000 kcal/day and 20% losses through cooking, this can be estimated to be 1.4 and 1 mg/day for adult males and females respectively. In pregnancy and lactation, thiamin requirement increases to approximately 1.6 - 1.8 mg/day.

Analysis of tissue levels and thiamin status

Thiamin status may be assessed by measurement of thiamin levels in blood or by urinary excretion, before and after loading. Erythrocyte transketolase (ETK) activity in haemolysed red blood cells is a functional measure of thiamin status.

Brief overview of non-nutritional beneficial effects

No reports of non-nutritional beneficial effects have been identified. Established therapeutic uses of thiamin supplements are largely related to the treatment or prophylaxis of deficiency. The effects of thiamin on spasmodic dysmenorrhoea, exercise performance, ventricular function, Alzheimer's disease, and leg cramps during pregnancy have been investigated, with inconclusive results.

Function

Thiamin pyrophosphate (TPP) is a co-enzyme in several enzymatic reactions. TPP may also have a non-co-enzymic function during stimulation of neuronal cells and other excitable tissues, such as skeletal muscle.

Deficiency

The biological half-life of thiamin is approximately 10 - 20 days and marginal deficiency can develop quite rapidly. Symptoms of sub-clinical deficiency include headache, tiredness, anorexia and muscle wasting. A regular daily thiamin intake of ≤ 0.2 mg/1000 kcal results in clinical deficiency and the disease known as beriberi, which affects the cardiovascular and nervous systems. Thiamin deficiency can result in a disorder of the central nervous system known as Wernicke's encephalopathy, characterised by confusion, ataxia and coma. This condition is sometimes accompanied by a syndrome known as Korsakoff psychosis. Both conditions are typically found in alcoholics and co-exist in Wernicke-Korsakoff syndrome. In developed countries, most cases of thiamin deficiency are associated with chronic alcoholism where dietary intake of the vitamin may be low and absorption and utilisation impaired.

Thiamin deficiency may also be involved in foetal alcohol syndrome, characterised by growth retardation, psychomotor abnormalities and congenital malformations, in the offspring of alcoholic mothers.

Interactions

Alcohol can impair the uptake and utilisation of thiamin and these effects may contribute to the prevalence of thiamin deficiency in alcoholics. Alcohol also reduces cellular thiamin diphosphokinase activity. Thiamin is an acetylcholine antagonist, and thus may enhance the effect of neuromuscular blocking agents. 5-Fluorouracil appears to be antagonistic to thiamin, possibly through competition for phosphorylation, which is required by both entities for their activation.

Absorption and bioavailability

Thiamin in food appears to be highly available for absorption. Absorption of thiamin hydrochloride and other water-soluble forms of thiamin is dose-dependent. At physiological concentrations, intestinal uptake occurs mainly via a carrier-mediated transport mechanism. However, this process is saturable and at higher concentrations, uptake is predominately by slower passive diffusion.

Distribution and metabolism

In the blood and tissues, thiamin is present as the free form and mono-, di- (pyro) and triphosphorylated forms, which are interconvertible. Free and phosphorylated forms are transported within the erythrocytes, but plasma and cerebrospinal fluid contain only the free and monophosphorylated forms. Within the tissues, most thiamin present is converted to the pyrophosphate form. Liver contains the highest

concentration of thiamin. Catabolic metabolism amounts to approximately 1 mg/day, and most of this occurs in the liver. The mean thiamin content of human breast milk in the UK has been reported to be 0.16 mg/L.

Excretion

Thiamin metabolites and thiamin in excess of requirements are excreted in the urine. The level of unchanged thiamin in the urine increases as intake increases.

Toxicity

Human data

The oral toxicity of thiamin and thiamin derivatives in humans is generally considered very low. Most reports of adverse effects with exposure to thiamin follow parenteral nutrition. High oral doses of thiamin hydrochloride (≥ 7000 mg) may cause headache, nausea, irritability, insomnia, rapid pulse and weakness. These symptoms are relieved following cessation of treatment or reduction of dose. There have been a very small number of reported adverse effects following lower doses from case reports. Three case reports concerned women, one who experienced muscle tremor, rapid pulse and nervous hyperirritability after taking daily doses of thiamin hydrochloride, reported to be 17 mg/day (see note¹). In another case, a patient suffered an anaphylactic reaction and subsequently died following a single oral dose of 100 mg thiamin 2 months after repeatedly taking 100 mg thiamine per day for a period of 15 days. One patient with thiamin-related contact dermatitis experienced an exacerbation of eczema following experimental provocation with an oral dose of 200 mg thiamin. A fourth case-report involved a young man who contracted allergic encephalitis following an oral dose of thiamin (the amount and form are unclear).

No evidence has been identified on reproductive effects of thiamin or thiamin derivatives in humans.

Supplementation trials

In a supplementation study, one isolated individual, who had earlier received parenteral thiamin hydrochloride, experienced nausea and insomnia following a daily dose of 200 mg thiamin hydrochloride per day for less than a week. Symptoms resolved when the dose was halved.

Animal data

The animal toxicity database is limited. Thiamin is of low acute toxicity with single oral doses of 3000-5000 mg/kg bw thiamin/thiamin hydrochloride in rats and mice are lethal. Thiamin nitrate is even less acutely toxic, with no adverse effects being reported in mice following a single oral dose of 5000 mg/kg bw. There is an absence

¹ It is noted that a dose of 17 mg would have been inconsistent with the rate of urinary and likely faecal excretion quoted within the original article. It is suggested, therefore, that this was a text error within the article that should have read '[17 g', equivalent to 17,000 mg.](#)'

of chronic and sub-chronic data for high-dose exposure to the water-soluble thiamin derivatives.

Carcinogenicity and genotoxicity

There has been no study on the carcinogenicity of thiamin. Thiamin hydrochloride has been shown to be non-mutagenic in a range of bacterial mutagenicity and *in vitro* chromosomal aberration tests.

Genetic variations

There are no known genetic variations resulting in increased susceptibility to thiamine toxicity.

Mechanisms of toxicity

No mechanisms of toxicity have been identified.

Dose-response characterisation

No data have been identified.

Vulnerable groups

No vulnerable groups have been identified; however, the clinical trials indicate that there is a possibility that a very small number of people may be particularly sensitive (allergic) to thiamin.

Studies of particular importance in the risk assessment

Human data

Mills, 1941

Thiamin-associated toxicity was reported in a 47 year old woman who had been taking 10,000 mg thiamin hydrochloride daily for 2¹/₂ weeks (presumably by the oral route, although this is unclear). Symptoms were reported to resemble those of over-dosage of thyroid extract: headache, increased irritability, insomnia, rapid pulse, weakness and trembling. Symptoms disappeared within 2 days following cessation of treatment but recurred 4¹/₂ weeks after the patient resumed a dose of 5 mg (see note²) per day. Again, prompt relief soon followed cessation of intake. In the same report, Mills described symptoms similar to those of thyroid hyperactivity, with fine and coarse muscle tremor, rapid pulse and nervous hyperirritability in a young woman receiving an average of 17 mg (see note³) thiamin hydrochloride per day (again, this

² The Mills report states 5 mg/day. However, when citing the Mills data, Iber *et al.* (1982) state 5 g/day.

³ The dose reported here as 17 mg is inconsistent with the quoted rate of urinary and likely faecal excretion and suggests that this is a text error within the Mills report that should read 17 g. Such an error would be consistent with an earlier error within the same report, indicated by Iber *et al* (see notes 14 and 15).

No potential high intake groups have been identified.

Risk assessment

Thiamin present in food is efficiently absorbed. However, water-soluble supplements, such as thiamin hydrochloride and thiamin mononitrate, are poorly absorbed due to saturation of transport mechanisms.

It is generally accepted that ingested thiamin has a very low toxicity in humans. Most data are either in the form of case reports of possible thiamin-associated adverse effects or from thiamin supplementation studies designed primarily to investigate potential beneficial effects. The latter do not always specifically report an absence of adverse effect.

The limited amount of human data indicates that adverse effects are generally CNS-related and occur only at very high doses. A small number of individuals may show an allergic response to lower doses, but reports of these lower dose-related events are rare. It is possible that this sub-population may be the same sub-group that is susceptible to adverse effects, e.g. anaphylaxis etc, following parenteral administration of thiamin.

The animal database is also very limited. A lethal dose of thiamin in rodents is preceded by CNS effects such as shock, muscle tremor, convulsions, respiratory disturbance and collapse, symptoms which are similar to acute thiamin toxicity in humans.

ESTABLISHMENT OF GUIDANCE LEVEL

There are insufficient data to establish a safe upper level for thiamin. The oral toxicity of thiamin and thiamin derivatives in humans is generally considered to be very low. Most available documented data are either in the form of case reports of possible thiamin-associated adverse effects or from thiamin supplementation studies designed primarily to investigate potential beneficial effects. The latter generally involve the use of the synthetic non-water soluble derivatives (not included in this review and not currently found in dietary supplements) and do not always specifically report an absence of adverse effect. Reports of thiamin-associated toxicity in humans are rare and most relate to incidents following parenteral administration of the vitamin. High doses (≥ 5000 mg) of thiamin hydrochloride may cause headache, nausea, irritability, insomnia, rapid pulse and weakness; these symptoms are relieved following cessation of treatment or reduction of dose. There have been a very small number of reported adverse effects following lower doses. These comprise four case reports and one isolated individual taking part in a supplementation study.

No specific toxic effects of thiamin ingestion by humans have been identified. However, there is a paucity of large controlled human supplementation studies. Significant adverse effects have not been noted with the water-soluble forms of

thiamin used in dietary supplements. These forms are poorly absorbed at high doses, which further restricts their toxicity.

One human supplementation study (Meador *et al.*, 1993) reported that graduated doses of thiamin hydrochloride, up to 6000-8000 mg/day for 5-6 months, caused no adverse effects in a very small group of patients. These subjects were reported to have tolerated the doses well, without weakness or other side effects, with the exception of two subjects (out of seventeen) who developed nausea and indigestion at doses of 7000 – 7500 mg. The study may well have under-reported side effects since half of the subjects were suffering from significant mental impairment on objective measures. From the available database, it appears that higher doses (≥ 7000 mg) may be associated with headache, nausea, irritability, insomnia, rapid pulse and weakness.

In a randomised double-blind placebo-controlled study by Gokhale *et al.* (1996), a daily oral dose of 100 mg thiamin hydrochloride (for sixty or ninety days) was given to 556 young females (12 – 21 years). No adverse effects were reported. The thiamin status of the participants is unclear. Based on this study, a level of 100 mg/day (equivalent to 1.7 mg/kg supplemental thiamin for a 60 kg adult) of supplemental thiamin would not be expected to result in adverse effects. Similarly, assuming a thiamine intake of 3 mg/day from food, a total intake of 103 mg/day (1.7 mg/kg bw/day in a 60 kg adult) would not be expected to result in adverse effects. This level is for guidance only and is applicable to the water-soluble forms of thiamin only. It should be noted that the applicability of the study, which was conducted in young women, to the general population is uncertain and the possibility of rare hypersensitivity reactions cannot be excluded.

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PRELIMINARY RISK ASSESSMENT – VITAMIN B₆ (PYRIDOXINE)

General Information

Chemistry and geochemistry

Pyridoxine is a water-soluble vitamin. Pyridoxine is composed of three forms (vitamers), pyridoxine, pyridoxal and pyridoxamine, all of which are normally present in foods. Pyridoxine hydrochloride is photosensitive and will degrade slowly when exposed to light.

Natural occurrence

Pyridoxamine and pyridoxal are found in animal products, and pyridoxine in animals and plants.

Occurrence in food, food supplements and medicines

Pyridoxine is found in chicken (4.2 mg/kg), fish, liver, kidney, pork, eggs (1.1 mg/kg), milk, wheatgerm (11.5 mg/kg) and brewer's yeast (25 mg/kg). Other sources include brown rice (5.5 mg/kg), soybeans (6.3 mg/kg), oats, whole-wheat grains, peanuts and walnuts (7.3 mg/kg). Long-term storage, canning, roasting or stewing of meat and food processing techniques can destroy pyridoxine. Boiling reduces the pyridoxine content of food because of losses into the water. Pyridoxine is present in a number of food supplements generally at doses up to 10 mg/day but some single dose food supplements may contain 50 to 150 mg.

As a licensed medicine, pyridoxine hydrochloride is also present in various multivitamin preparations for the prevention and treatment of vitamin deficiencies (maximum daily doses of 0.5 – 30 mg). Single nutrient products (recommended maximum daily doses of 10 – 400 mg) are available without the supervision of a pharmacist. Products containing pyridoxine (maximum daily dose of 10 mg) combined with other constituents, are available from a pharmacist.

Recommended amounts

Recommended intakes of pyridoxine are based on protein intake. In the UK, the RNI is set at 15 µg/g protein for adults. This is equivalent to approximately 1.4 and 1.2 mg/day in the UK for males and females respectively. In the US, the Recommended Daily Allowance is set at 1.3 mg/day, and it is approximately 1.6 mg/day in Australia. Pregnant and lactating women and older people, who have low vitamin B₆ levels, can usually increase their intake through a high-protein diet.

Analysis of tissue levels and pyridoxine status

Pyridoxal phosphate has been determined enzymatically using tyrosine apodecarboxylase or by fluorimetric methods. The preferred method is high-pressure liquid chromatography. The tryptophan loading test has been used to assess vitamin B₆ status. This test is not a reliable indicator of vitamin B₆ status in persons receiving oestrogens or with increased secretion of glucocorticoids.

Brief overview of non-nutritional beneficial effects

Pyridoxine is an approved treatment for sideroblastic anaemias and pyridoxine-dependent errors of metabolism. Pyridoxine has also been claimed to alleviate the symptoms of premenstrual syndrome, pregnancy sickness, carpal tunnel syndrome, hyperhomocystinaemia (a risk factor for cardiovascular disease) and neuropathies.

Function

The cofactor forms of pyridoxine are pyridoxal-5'-phosphate and pyridoxamine-5'-phosphate. Pyridoxal phosphate is involved as a cofactor particularly in the metabolic transformation of amino acids, including decarboxylation, transamination and racemisation.

Vitamin B₆ is a cofactor in the conversion of tryptophan to 5-hydroxytryptamine and of methionine to cysteine. Pyridoxine can modify the action of steroid hormones *in vivo* by interacting with steroid-receptor complexes. Pyridoxine is essential for the manufacture of prostaglandins and for the formation of red blood cells.

Pyridoxine is involved in cellular replication and antibody production. An adequate supply of pyridoxine is necessary for the function of the nervous system. The vitamin is involved in the biosynthesis of several neurotransmitters, including serotonin, gamma amino-butyric acid (GABA), dopamine and noradrenaline and so has a role in the regulation of mental processes and mood. It is also involved in sodium-potassium balance, histamine metabolism, the conversion of tryptophan to niacin, absorption of vitamin B₁₂ and the production of hydrochloric acid in the gastrointestinal tract.

Deficiency

Pyridoxine deficiency is unusual in humans. Children who had been given milk in which the pyridoxine had been destroyed by overheating, displayed various symptoms, including weakness, irritability, nervousness, susceptibility to noise, weight loss and insomnia. Adult volunteers on a pyridoxine deficient diet became depressed and irritable, with “a loss of sense of responsibility”. They also experienced a greasy rash on the forehead and around the nose and cracking of the lips and tongue.

Pyridoxine dependency is a rare autosomal recessive disorder in which the enzyme glutamate decarboxylase, which is involved in the synthesis of GABA, has a defective binding site for pyridoxal phosphate. Much higher tissue levels of pyridoxal phosphate are necessary for the enzyme to have any significant activity. The condition results in seizures of prenatal or neonatal onset and treatment with large doses of pyridoxine is necessary to prevent severe mental retardation or death.

It has also been suggested that pyridoxine deficiency may be a factor in hyperhomocysteinaemia which is associated with an increased risk of cardiovascular disease.

Pyridoxine deficiency has also been reported to be associated with immune dysfunction, kidney stones, cancer and carpal-tunnel syndrome, although the evidence for these links is variable.

Interactions

Pyridoxine requires riboflavin, zinc and magnesium for its normal function in the body. Pyridoxine reduces the therapeutic effect of levodopa, a naturally occurring amino acid used in treating Parkinson's disease. Pyridoxine also interacts with other drugs such as isoniazid, phenytoin, theophylline and phenobarbitone. It has been claimed that women taking oral contraceptives may have an increased requirement for pyridoxine.

Absorption and bioavailability

The phosphate forms of vitamin B₆ in food are dephosphorylated in the intestinal lumen, and pyridoxine, pyridoxal and pyridoxamine are taken up from the small intestine by an energy-dependent process. All three are converted to pyridoxal phosphate in the tissues.

A proportion of the vitamin B₆ present in plant-based foods is biologically unavailable because it is present as pyridoxine glycosides that are not hydrolysed by intestinal enzymes. These glycosides may therefore be absorbed, but do not act as a coenzyme in the body and are excreted unchanged in the urine.

All three forms of vitamin B₆ (pyridoxine, pyridoxal and pyridoxamine) are readily absorbed in the small intestine. The extent of absorption is decreased following gastric resection or in patients with malabsorption syndromes. Excess pyridoxine is excreted in the urine, and an adequate daily intake is therefore essential.

Distribution and metabolism

Pyridoxine in food is converted to active forms in the liver, a process which requires zinc and riboflavin.

Vitamin B₆ is stored in the liver, with about 50% also being present in muscle, bound to glycogen phosphorylase. Pyridoxine is also stored in the brain. The total body storage for adults is between 6 and 27 mg. Pyridoxine in the form of pyridoxal crosses the placenta, with foetal concentrations being 5 times that of maternal plasma concentrations. In tissue, the three forms of vitamin B₆ are present mainly as 5-phosphorylated derivatives of pyridoxal and pyridoxamine. The half-life of pyridoxine is 15-20 days, and it is not significantly bound to plasma proteins.

Plasma concentrations of pyridoxine vitamers have been shown to reflect closely the concentration of pyridoxine in the liver. Normal levels of vitamin B₆ are 46-72 ng/mL in serum and 31-43 ng/mL in blood.

Pyridoxine, pyridoxal and pyridoxamine are all largely metabolised in the liver through phosphorylation by pyridoxal kinase. Pyridoxine phosphate is oxidised to the active coenzyme form, pyridoxal-5-phosphate, by an enzyme found mainly in liver.

Pyridoxal-5-phosphate interconverts with pyridoxamine-5-phosphate through enzymatic transamination. The phosphorylated forms are hydrolysed by phosphatases.

Pyridoxal is oxidised in the liver to pyridoxic acid.

Excretion

Pyridoxic acid, the main excretory metabolite, is eliminated via the urine.

Toxicity

Human data

Long-term use of pyridoxine (generally in excess of 200 mg/day) has been reported to result in paraesthesiae, somnolence and low folic acid levels. Large doses of pyridoxine (usually quoted as over 2000 mg/day) can cause nerve damage. Symptoms include tingling in the hands and feet, a stumbling gait, perioral numbness, a characteristic "stocking-glove" sensory loss and lack of muscle coordination. Duration as well as dosage is important, with lower doses of pyridoxine (50 to 500 mg/day) consumed for many months or years also being associated with neuropathy. The damage is generally reversible but not in all reported cases. Night restlessness, vivid dreams, sun sensitivity and an acne-like rash may also occur with high doses (of 150 mg/day or more) of pyridoxine.

Supplementation trials

Pyridoxine has been studied in a number of human supplementation trials, investigating the effects of the vitamin on conditions such as carpal tunnel syndrome, pre-menstrual syndrome, morning sickness and hyperhomocysteinaemia. The majority of these trials have not been placebo controlled and have either not considered or not reported adverse effects in detail. The available studies are considered in detail in the review [EVM/00/19](#) with the key studies for the risk assessment being discussed below.

Animal data

Studies in several species have shown the development of symptoms of neuropathy at variable time intervals following doses ranging from 50 mg/kg bw/day to 7000 mg/kg bw/day. Inter-species variability in response is apparent.

No effects on foetal development have been observed in animal studies. Doses of 250 and 500 mg/kg bw/day pyridoxine, administered intraperitoneally, produced histological changes in the testes of rats (reversible at the lower dose), and doses of 50 mg/kg/day administered intraperitoneally to female rats prevented prolactin release.

Carcinogenicity and genotoxicity

No published data on the carcinogenicity or genotoxicity of pyridoxine have been found.

Mechanisms of toxicity

Pyridoxal phosphate is thought to be responsible for the observed toxicity. Schwann cells in culture grow less well when provided with pyridoxal in the culture medium than when the vitamin B₆ source is pyridoxine. The addition of pyridoxal to the culture medium decreased cell survival even in the presence of an adequate concentration of pyridoxine, suggesting a possible neurotoxic action of pyridoxal. It is not known whether pyridoxal is similarly cytotoxic to other cell types in culture.

Dose-response characterisation

The precise dose-response relationship of pyridoxine and peripheral neuropathy is uncertain in humans. Neuropathy has been associated with high doses of pyridoxine (generally over 2000 mg/day), but duration of exposure is also an important part of the relationship, with neuropathy also occurring following long periods of exposure to lower levels (50-500 mg/day) pyridoxine.

Vulnerable groups

No groups particularly vulnerable to pyridoxine-induced toxicity have been identified.

Genetic variations

No genetic variations which increase vulnerability to toxicity of vitamin B₆ have been identified.

Studies of particular importance in the risk assessment

Human data

Parry and Bredesen, 1985

The paper describes 16 patients taking high doses of pyridoxine (200-5000 mg/day) for several months or more. The patients developed unstable gait, perioral numbness and a "stocking-glove" sensory loss. Improvement followed discontinuation of pyridoxine. The authors believed that the toxicity of pyridoxine was manifested through an effect on the dorsal root ganglion. Most patients took 2000 mg/day or more pyridoxine (many of these had started on lower doses) and symptoms became apparent within a year of taking 2000 mg/day or more. Three patients took 200 mg/day for 3 years and 500 mg/day for 1-2 years) and symptoms became apparent after at least one year of consumption.

Del Tredici et al., 1985

This report studied the effect of vitamin B₆ on 24 patients with carpal tunnel syndrome treated for 2-4 months with 150 or 300 mg vitamin B₆ daily. Vitamin B₆ was reported as having a beneficial effect on carpal tunnel syndrome, and no exacerbation of neurological symptoms or signs of peripheral neuropathy were

reported. Distal Motor Latency was measured and patients completed a self-assessment questionnaire numerically ranking the severity of the symptoms.

Dalton and Dalton, 1987

Serum vitamin B₆ levels were measured in all women taking vitamin B₆ supplements at a private clinic. Patients (n=172) who had elevated serum vitamin B₆ levels (above the normal range in humans of 3.16 to 18 ng/mL) were specifically asked to report symptoms such as tingling in the fingers, which could be interpreted as evidence of sensory neuropathy. The patients were all taking <50-<500 mg/day vitamin B₆ for premenstrual syndrome. No control group of patients with similar premenstrual symptoms were asked the same questions. Patients reported symptoms including paraesthesia, hyperaesthesia, bone pains, muscle weakness, numbness and fasciculation that were most marked on the extremities and bilateral. The authors reported findings on neurological examination of weakness, loss of tendon reflexes, positive L'Hermitte's test and sensory loss in a stocking/glove distribution. No neurophysiological tests were undertaken. Symptoms disappeared when vitamin B₆ was withdrawn and reappeared if treatment was resumed. Complete recovery occurred within 6 months of stopping vitamin B₆ treatment. Three women had subnormal serum levels of vitamin B₆ after stopping supplementation due to symptoms of sensory neuropathy. They were advised to take 50 mg vitamin B₆/day, but within a month they again experienced symptoms of neuropathy and had serum vitamin B₆ levels of > 18 ng/mL. The 103 women who presented with symptoms took a mean dose of 117 ± 92 mg vitamin B₆ for a period of 2.9 ± 1.9 years, and in 70% of these subjects, serum levels were > 34 ng/mL (the upper limit of testing). In contrast, the group which did not report symptoms, took a mean dose of 116 ± 66 mg vitamin B₆, for a period of 1.6 ± 2.1 years, and in 55% of these subjects, serum levels were > 34 ng/mL.

Brush, 1988

In a retrospective survey of 336 women treated with pyridoxine alone or in combination with other agents, 5 subjects reported mild tingling and/or numbness at doses of 200 mg/day. The duration of treatment was not reported. Similar side effects were not observed in an earlier group of women treated with up to 150 mg/day.

Bernstein and Lobitz, 1988

In an open study, 16 patients received 150 mg vitamin B₆ daily for 6 months for the treatment of diabetic neuropathy. It was reported that one patient developed increased photosensitivity with increased tanning on minimal exposure to sunlight, but elected to remain in the study. The patients underwent a monthly clinical evaluation by a neurologist, with a detailed electrophysiological study of motor and sensory nerves being conducted. No deterioration of peripheral nerve function was observed. However, examinations were only completed at 4 months (in 10 subjects) and at 5 months (in 5 subjects).

Molimard et al., 1990

In a double-blind study, 69 medical students received a tablet of placebo, 50 or 250 mg vitamin B₆ to be taken twice a day for 10 days (total dose being 100 or 500 mg/day). The subjects were given a simple digit coding task prior to treatment, immediately after treatment and 14 days later. The volunteers were also tested on the course content during the study period and given some numerical problems at the end of treatment. Fifty eight of the volunteers completed the digit coding tests, which showed a highly significant improvement with time (a learning effect) in all dose groups. There were no significant differences in the uncorrected scores, but there was evidence of a dose-related decrease in the learning effect, which was highly statistically significant ($p < 0.002$) in the top dose group compared to controls but was not statistically significant at the 5% level ($p < 0.07$) in the 100 mg/day group. No other differences were apparent. In a second experiment, a group of 30 patients were randomised to receive placebo, 20 mg or 1000 mg/day pyridoxine for 15 days with subjects given a variety of tests before and after treatment. At the high dose, an adverse effect was reported for word recognition, but not for word or visual memorisation. A decrease in the results of visual retention test was also found in the high dose pyridoxine group after treatment.

Berger et al., 1992

In this study, doses of 1000 or 3000 mg/day pyridoxine were administered in a well-designed experimental study to three and two healthy volunteers, respectively, until the development of symptoms of neuropathy. Symptoms occurred within 1.5 and 3.5 months in high-dose subjects. In subjects treated with 1000 mg/day symptoms of neuropathy, including numbness and pins and needles sensation in the toes, developed in two of the subjects after 4.5 and 7 months of treatment. Treatment of one subject was continued for over 14 months before quantitative sensory threshold results became abnormal, an early indicator of neuropathy. When calculated on a body weight basis, a dose-response relationship was observed with regard to the development of toxicity. Clinical symptoms continued to intensify for some time after pyridoxine was withdrawn.

Bernstein and Dinesen, 1989

This paper reported the effect of vitamin B₆ in the treatment of carpal tunnel syndrome. Sixteen patients received 200 mg vitamin B₆ per day for 3 months. No signs of peripheral neuropathy were found in the uninvolved ulnar nerves, and there was no evidence of new peripheral neuropathy during the 3 months of treatment.

Animal data

Phillips et al., 1978

Pyridoxine hydrochloride was administered orally in gelatine capsules (0, 50 or 200 mg/kg bw/day) to three groups of female beagles (four control and five per treatment group) over 100-112 days. Four of the five animals in the high dose group showed signs of ataxia and loss of balance after 45 days of treatment, while the fifth showed clinical signs after 75 days. Clinical signs of toxicity were not observed in the 50

mg/kg/day group, but bilateral loss of myelin in the dorsal nerve roots was observed histologically.

Exposure assessment

Total exposure/intake:

Food Mean: 2.0 mg/day
 97.5th percentile: 3.9 mg/day (NDNS, 1986/87)

Supplements up to 30 mg/day (OTC, 2001)

Estimated maximum daily exposure: $3.9 + 30 = 33.9$ mg

No potential high intake groups have been identified.

Risk assessment

The key adverse effect, for vitamin B₆ is neuropathy, which has been demonstrated in both man and laboratory animals. The effect occurs after consumption of high doses and/or long duration. Generally the symptoms are reversible once the exposure is stopped but in some cases involving high doses, the effects are irreversible. Progressive sensory ataxia occurs, presenting initially as unstable gait and numb feet, then numbness in the hands, followed by profound impairment of position sense and vibration sense in the distal limbs. The senses of touch, temperature and pain are less affected. A single report suggests that vitamin B₆ may affect the ability to memorise information.

Data from animal studies also demonstrate neurotoxicity, although some species differences are apparent. Doses as low as 50 mg/kg bw/day have been associated with a loss of myelin. Subtle effects such as changes in startle response have also been observed. The animal data also suggest that duration of exposure is important in the response to vitamin B₆.

ESTABLISHMENT OF SAFE UPPER LEVEL

Main studies used in the risk assessment:

Phillips *et al.* (1978); Parry and Bredesen (1985); Dalton and Dalton (1987); Brush (1988); Bernstein and Lobitz (1988); Molimard *et al.* (1990); Berger *et al.* (1992); Bernstein and Dinesen (1993),

LOAEL: 50 mg/kg bw/day, based on the study by Phillips *et al.* (1978)
 in dogs

Uncertainty Factors: 3 for LOAEL to NOAEL extrapolation
 10 for inter-species variation

10 for inter-individual variation

Safe Upper Level 50/300 = 0.17 mg/kg bw/day supplemental pyridoxine
(for daily consumption (equivalent to 10 mg/day for a 60 kg adult)
over a lifetime):

Excessive quantities of vitamin B₆ result in peripheral neuropathy in both animals and humans. The effect is dependent on both the dose and the duration of exposure.

To determine the levels at which vitamin B₆ can be safely taken requires large well-controlled studies with careful symptomatic observation and attention to critical endpoints. Unfortunately much of the available data are unsatisfactory with retrospective exploration of records, use of unstandardised end points and incomplete follow up, often of small observational groups with short durations of treatment (< 6 months) and without adequate controls.

Daily doses of 1000 mg or 3000 mg were given to five healthy volunteers in a well conducted clinical trial by Berger *et al.* (1992), and sensory symptoms and/or laboratory abnormalities were detected after dosage for 1.5-3.5 months at 3000 mg/day and 4.5 and 7 months in two subjects at 1000 mg/day. The study which reports neuropathy at the lowest dose (50 mg/day) is that of Dalton and Dalton (1987), but this investigation is flawed in a number of ways. For example, there was no control group, and since subjects were questioned about their symptoms, reports may be biased. However, it had the unique factor of being the only study which considered long term vitamin B₆ intake and the plasma levels of the vitamin were measured. In women with elevated plasma levels, longer exposure was associated with clinical symptoms. In the absence of better quality data, it is not possible to dismiss this investigation. Brush (1988) and Parry and Bredesen (1985) reported neuropathy at doses of 200 mg/day, the latter study involving exposure of several years' duration. However, small numbers of patients were involved and few data were provided. Other studies which investigated the effects of low doses of vitamin B₆ (Del Tredici *et al.*, 1985; Benstein and Lobitz, 1988; Bernstein and Dinesen, 1993) were somewhat better conducted but did not consider exposure of the same duration. For example, in Bernstein and Lobitz (1988) patients received 150 mg/day for 6 months, in Del Tredici (1985) patients received 150 or 300 mg/day for 2-4 months and in Bernstein and Dinesen (1989) the subjects received 200 mg/day for 3 months. The latter studies did not have placebo control groups and involved small numbers of patients, as well as being of durations which were insufficient for the development of symptoms, even had the doses been higher. Thus while the results of these studies are negative, they are not necessarily incompatible with those of Dalton and Dalton.

The duration of exposure reported in the studies by Dalton and Dalton, and Parry and Bredesen (mean 2.9 years and > 2 years respectively) was considerably longer than in the studies where neuropathy was not observed. Thus the findings by Dalton and Dalton (1987) and Parry and Bredesen (1985) of neuropathy at low doses of vitamin B₆ are consistent with the overall database, which clearly indicates that duration of exposure as well as dose is important. Therefore, in the absence of better quality data the reports of toxicity following long, low level exposure to vitamin B₆ cannot be dismissed. Overall, the human data are inadequate to establish a Safe Upper Level,

since the effect levels are unclear and the studies at low levels of intake are of limited quality.

A more subtle effect attributed to vitamin B₆ is the adverse effect on memory reported by Molimard *et al.*, 1990. In a double blind study in which vitamin B₆ was taken for only 10 days, doses of 500 mg reduced the ability to perform a digit coding memory task; at doses of 100 mg/day a decrease occurred, which was not statistically significant at the 5% level. There are no other comparable data available, but this study suggests that more subtle neurological effects could occur following relatively short exposure to vitamin B₆.

Neuropathy is also observed in laboratory animals, and the data confirm the importance of dose and duration in determining the effects of vitamin B₆. In the absence of reliable human data at low levels of exposure, the Safe Upper Level is based on animal data, in which histological changes were apparent in the nerves of dogs treated with 50 mg/kg bw/day for 100-112 days (Phillips, 1978). Clinical signs of toxicity were not apparent in this group but were observed in the high dose group which received 200 mg/kg bw/day. Using uncertainty factors of 300 (consisting of 3 for LOAEL to NOAEL extrapolation of a histopathological change, 10 for inter-species and 10 for inter-individual variation) a Safe Upper Level of 0.17 mg/kg bw/day can be derived. This relates to supplemental pyridoxine because the basal pyridoxine content of the diet in the key study is unknown. This SUL is equivalent to 10 mg/day in a 60 kg adult. These uncertainty factors are appropriate because the LOAEL in the dog related to a sub-chronic study and therefore may have underestimated the toxicity during chronic exposure. The need for an inter-species factor is supported by the fact that the LOAEL dose in dogs (50 mg/kg bw/day) is equivalent to an intake of 3000 mg per day in humans, which would produce severe toxicity, suggesting that humans might be more sensitive than dogs. Finally, the factor for human variability is necessary to allow for the greater size and diversity of the human population compared with the small number of dogs (n=5) studied by Phillips *et al.* (1978).

In humans, a supplementary dose of 10 mg/day represents a clear SUL, with no adverse effects being anticipated over a lifetime's exposure. Doses of 200 mg/day vitamin B₆ or more taken for long periods are associated with reports of neuropathy in some human subjects. The effect of taking vitamin B₆ at doses between 10 and 200 mg over lengthy periods of time is unclear, although short term exposure at such levels may represent a negligible risk.

Some isolated reports suggest that neuropathy may occur at doses <200 mg/day but the data are not sufficiently reliable to determine the precise nature of the dose, duration and effect relationship. Symptoms of sensory neuropathy were reported in 103 out of 172 women who had been taking supplemental vitamin B₆, at intakes ranging from less than 50 mg/day to 500 mg/day, for periods ranging from less than 6 months to over five years and who had raised serum vitamin B₆ levels, outside the normal range for humans (Dalton and Dalton, 1987). Twenty percent of those reporting neurological symptoms had a reported vitamin B₆ intake of less than 50 mg/day. However, this was an uncontrolled study in which individual vitamin B₆ intakes were not accurately estimated and duration of exposure varied from less than 6 months to over 5 years. Also, as subjects were specifically asked if they experienced

signs of sensory neuropathy such as tingling of fingers, there is a potential for reporting bias. No adverse effects were observed in small, uncontrolled studies by Bernstein and Lobitz (1988) in which patients received 150 mg/day vitamin B₆ for 6 months, Del Tredici *et al.* (1985), in which patients received 150 or 300 mg/day for 2-4 months and Bernstein and Dinesen (1993), in which the subjects received 200 mg/day for 3 months. The safety of such doses long term cannot be assured.

It is unfortunate that no reliable and controlled studies have been conducted in the past 5 years to establish whether intakes between 10 and 200 mg/day are safe in the long term.

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PRELIMINARY RISK ASSESSMENT -VITAMIN B₁₂

General Information

Chemistry

Vitamin B₁₂ (cobalamin, Cbl) is a water-soluble vitamin and a member of a family of related molecules known as corrinoids which contain a corrin nucleus made up of a tetrapyrrolic ring structure. The centre of the tetrapyrrolic ring nucleus contains a cobalt ion that can be attached to methyl, deoxyadenosyl-, hydroxo- or cyano- groups.

Natural occurrence

Vitamin B₁₂ originates from bacteria, fungi and algae, and is present in virtually all animal tissues. Plants contain no vitamin B₁₂ beyond that derived from microbial contamination.

Occurrence in food, food supplements and medicines

Major dietary sources of vitamin B₁₂, mainly in the forms of methyl, deoxyadenosyl- and hydroxocobalamin, include meat (e.g. > 0.1 mg/kg in lamb) and fish (e.g. 0.03-0.1 mg/kg in salmon, 0.01-0.03 mg/kg in tuna), particularly liver (> 0.1 mg/kg). Hydroxocobalamin and, in particular, cyanocobalamin are synthetic forms used in vitamin supplements, pharmaceuticals and in the fortification of food. Methyl cobalamin has been used therapeutically outside the UK, for example, in Japan.

Recommended amounts

The Reference Nutrient Intake (RNI) for vitamin B₁₂ in adults in the UK is 0.0015 mg/day. There is no increment required during pregnancy but there is a recommended increment of 0.0005 mg/day for breast feeding mothers.

Analysis of tissue levels and vitamin B₁₂ status

Measurement of vitamin B₁₂ in plasma is routinely used to determine deficiency, but may not be a reliable indication in all cases. In pregnancy, for example, tissue levels are normal but serum levels are low. Various other plasma markers have been identified (including methylmalonic acid, homocysteine, holotranscobalamin, anti-intrinsic factor antibodies) and methods devised (Schilling test, cobalamin absorbance test, serum gastrin deoxyuridine suppression test) to distinguish different causes of deficiency.

Brief overview of non-nutritional beneficial effects

Results of studies in humans have suggested that large doses of vitamin B₁₂ (particularly methyl cobalamin) may influence biological rhythms and thus may be beneficial in the treatment of sleep disorders. Vitamin B₁₂ has also been reported to increase light sensitivity by affecting melatonin secretion. Vitamin B₁₂, in

combination with folic acid, has been suggested to be beneficial in certain disorders, such as idiopathic osteoarthritis and vitiligo.

Function

Vitamin B₁₂ serves as cofactor to at least two enzymes, methionine synthase and methylmalonyl CoA mutase. Methionine synthase is pivotal in one-carbon metabolism, being crucial in the synthesis of the universal methyl donor S-adenosyl methionine and in the cellular import and metabolism of folate. Methylmalonyl CoA mutase converts L-methylmalonyl CoA to succinyl CoA and is important in even-chained fatty acid synthesis.

Deficiency

Dietary deficiency is rare in younger people living in the community but occurs more frequently in older people particularly those living in institutional environments. Individuals adhering to vegan diets may also be at risk. Deficiency is mostly attributable to inherited or acquired defects resulting in malabsorption or the impairment of transport of the vitamin within the body. Deficiency impacts on the haematopoietic and nervous systems. Associated diseases include megaloblastic anaemia and neuropathies typically sub-acute combined degeneration of the spinal cord. Vitamin B₁₂ deficiency can lead to moderate hyperhomocysteinaemia, a possible risk factor for occlusive vascular disease.

Oral supplements are indicated prophylactically where there is a likelihood of deficiency in those whose gastrointestinal function is normal e.g. in individuals who are strict vegetarians. Inherited and acquired disorders relating to vitamin B₁₂ malabsorption are usually treated by repeated injection. However, oral administration of very high doses of vitamin B₁₂ has been shown to be effective in the treatment of pernicious anaemia.

Interactions

Steroid drugs, such as prednisone, have been reported to increase the absorption of vitamin B₁₂ in patients with pernicious anaemia. Excessive alcohol consumption and some drugs may decrease absorption of vitamin B₁₂. Oral co-administration with ascorbic acid may result in destruction of vitamin B₁₂. Concurrent administration of chloramphenicol may lead to antagonism of the haematopoietic response to vitamin B₁₂.

Absorption and bioavailability

Vitamin B₁₂ requires intrinsic factor (IF), secreted mainly from the gastric parietal cells, to ensure adequate absorption at normal dietary intake levels. Thus the absorption of physiological doses of vitamin B₁₂ is limited to approximately 0.0015 – 0.002 mg/dose or meal, due to saturation of the uptake system. Regardless of dose, approximately 1.2% of vitamin B₁₂ is absorbed by passive diffusion and consequently this process becomes quantitatively important at pharmacological levels of exposure. Protein binding in certain foods may reduce the bioavailability of the vitamin, particularly in individuals with impaired gastric acid and/or digestive enzyme

secretion. The different forms of crystalline cobalamin appear to be absorbed or retained to different extents, depending on the dose. Differences are most apparent at low doses.

Ingested vitamin B₁₂ is released from the food matrix by the action of digestive enzymes and gastric acid and becomes bound to salivary haptocorrin-binding proteins. As the pH rises further along the gut, and under the influence of pancreatic enzymes, vitamin B₁₂ is released from the salivary haptocorrin and becomes complexed with intrinsic factor (IF). The cobalamin-IF complex binds to a specific cell wall receptor of the ileal enterocyte and is internalised by endocytosis. Once inside the cell, the IF is degraded and the liberated vitamin is converted to the methyl or the deoxyadenosyl form, is bound to transcobalamin II (TC II) binding protein and then exported into the portal blood. In the general circulation, most cobalamin is bound to transcobalamin I (TC I) but the majority of cobalamin available for uptake into the tissues is that bound to TC II.

Distribution and metabolism

Vitamin B₁₂ is distributed into the liver, bone marrow and virtually all other tissues, including the placenta and breast milk of nursing mothers. The liver is the predominant storage site for vitamin B₁₂.

Uptake into cells occurs through receptor mediated endocytosis involving specific TC II cell wall receptors. Once inside the tissues/cells, the complex is degraded by the lysosomes, and the released cobalamin is metabolised either to methyl-cobalamin in the cytosol, where it binds to methionine synthase, or to deoxyadenosyl-cobalamin in the mitochondria, where it binds to methylmalonyl CoA mutase.

Excretion

Excretion occurs mainly via the faeces and urine, but also through the shedding of skin cells. Excretion is very slow, with significant enterohepatic cycling.

Toxicity

Human data

There are a few case reports of adverse effects associated with ingestion of vitamin B₁₂, either as a supplement, or following the consumption of yeast extract products, which also contain cyanocobalamin. Five cases of allergic reactions were reported, three of which were recurrences of symptoms in individuals who had been previously exposed to cobalamin by the parenteral route. One further case reported the occurrence of a skin eruption that resembled acne rosacea. Vitamin B₁₂ exposures were generally not specified.

No adverse effects were reported in an experiment designed to determine the uptake of single oral doses of cyanocobalamin (up to 100 mg). However, only three participants were administered the very high doses.

Oral studies have been conducted to investigate the effects of vitamin B₁₂ on pernicious anaemia. However, although no adverse effects are apparent, these studies are not relevant to the general population since absorption of vitamin B₁₂ is reduced in this condition. The effect of high oral-dose cyanocobalamin on plasma homocysteine levels in healthy females of child-bearing age and the benefits of cyanocobalamin in patients with seasonal affective disorder have been investigated. No adverse effects related to treatment were reported in any study including those in which individuals received up to 4.5 mg/day cyanocobalamin for 14 days, 2.0 mg/day cyanocobalamin for up to one year or 1.0 mg/day cyanocobalamin for several years. Less information is available following the oral administration of the hydroxocobalamin form of vitamin B₁₂. However, no adverse effects were reported in individuals administered 0.3 mg/day for up to 12 months. No adverse effects were reported in a controlled study in which 125 individuals received 6.0 mg/day methylcobalamin for up to 12 weeks.

Adverse reactions (not specified) were reported in one of 16 and in one of 23 oligozoospermia patients given 6 or 12 mg/day methyl cobalamin, respectively, for 16 weeks, presumably via the oral route. However, this study was not controlled.

Animal data

The data-base on the oral toxicity of vitamin B₁₂ in laboratory animals is limited. Doses of 1.5 to 3.0 mg/kg bw by intraperitoneal and subcutaneous administration were found to be acutely toxic in mice (CNS effects; convulsions, cardiac and respiratory failure and ultimately death). However, much higher doses (≥ 5 g/kg bw) cyanocobalamin appeared to be tolerated by mice following oral administration. There is no evidence relating to vitamin B₁₂ and teratogenicity or adverse effects on fertility or post-natal development.

Carcinogenicity and genotoxicity

There is no evidence suggesting that vitamin B₁₂ is carcinogenic or genotoxic *in vitro* or *in vivo*. However, although data are not consistent, there is some limited evidence to suggest that high doses of vitamin B₁₂ may have tumour promoting activity.

Mechanisms of toxicity

No data have been identified.

Dose-response characterisation

No relevant data have been identified.

Vulnerable groups

No vulnerable groups have been identified.

Genetic variations

No genetic variations have been identified.

Studies of particular importance in the risk assessment

Waife et al., 1963

As part of a non-controlled supplementation trial in patients with pernicious anaemia (n=27) no adverse reactions were reported in individuals receiving 0.3 mg hydroxocobalamin/day for up to one year.

Berlin et al., 1968

No adverse effects attributable to vitamin B₁₂ (cyanocobalamin) were recorded in a long-term clinical trial of 64 patients with pernicious anaemia, and other types of vitamin B₁₂ deficiency given a daily oral dose of 0.5 mg rising to 1.0 mg of cyanocobalamin (without intrinsic factor) for 10 to 70 months (42 patients received treatment for over 4 years). However, as noted previously, systemic absorption in these patients is limited.

Juhlin and Olsson, 1997

One hundred patients with vitiligo were treated with oral folic acid (5 mg) and vitamin B₁₂ (1.0 mg cyanocobalamin) twice daily, for up to 12 months. There were no reports of adverse effects. However, 27/100 and 48/100 participants had stopped taking the supplements after 1 - 2 months and 3 - 6 months, respectively but reasons for withdrawal were not stated.

Takahashi et al., 1999

As part of a double-blind study to assess the therapeutic effect of methyl cobalamin on sleep-wake disorder, patients were administered either 6.0 (n=21) or 0.03 mg/day (n=27) methylcobalamin for 8 weeks. The lower dose group was considered as a control group because ethical permission was not granted for the inclusion of a placebo control group. There was no report of any adverse effects. The route of administration was not clear from the information given. Data were not available for all patients at the end of 8 weeks (two of the 6.0 mg/day dose group and three of the 0.03 mg/day group).

Exposure assessment

Total exposure/intake⁴:

Food	Mean: 0.0062 mg/day 97.5 th percentile: 0.0195 mg/day (1986/7 NDNS)
Supplements	up to 0.025 mg/day (OTC 2001)

⁴ The survey data do not distinguish the different forms of vitamin B12. Dietary vitamin B12 is mainly in the methyl, deoxyadenosyl and hydroxocobalamin forms. Hydroxo, and particularly cyanocobalamin are the forms usually present in dietary supplements.

Estimated maximum daily intake: $(0.0195 + 0.025) = 0.0445$ mg/day

No potential high intake groups have been identified.

Risk assessment

Vitamin B₁₂ is a water-soluble vitamin. At physiological doses, as occurs in food, the amount absorbed is largely limited (approximately 0.002 mg/meal) by the capacity of the intrinsic factor-wall receptor uptake system. At pharmacological levels of dosing, diffusion becomes more important as the route of absorption. Vitamin B₁₂ present in excess of the binding capacity of the liver, plasma and other tissues is excreted by glomerular filtration.

It is generally accepted that ingested vitamin B₁₂ (cobalamin) has a very low toxicity in humans. Most available documented data are either in the form of case reports of possible vitamin B₁₂-associated adverse effects or from clinical trials or supplementation studies designed primarily to investigate potential beneficial effects. The latter generally involve the use of the cyanocobalamin or methylcobalamin forms of vitamin B₁₂ and do not always specifically report an absence of adverse effects.

The animal toxicity database for vitamin B₁₂ is very limited. Doses of 1.5 to 3.0 mg/kg bw by intraperitoneal and subcutaneous administration were found to be acutely toxic in mice (CNS effects; convulsions, cardiac and respiratory failure and ultimately death). However, much higher doses (≥ 5 g/kg bw) cyanocobalamin appeared to be tolerated by mice following oral administration.

ESTABLISHMENT OF GUIDANCE LEVEL

There are insufficient data from studies in humans and animals to set a Safe Upper Level for vitamin B₁₂.

Clinical studies have reported no adverse effects following administration of up to 6.0 mg/day of methylcobalamin for several weeks and up to 1.0 mg/day cyanocobalamin for several years. Clinical trials and supplementation studies involving up to 100 individuals to investigate the beneficial effects of oral cyanocobalamin have not reported any treatment-related adverse reactions following doses of 0.3 to 4.5 mg for periods ranging from 14 days to several years. Cyanocobalamin is the type of vitamin B₁₂ most frequently included in supplements in the UK. The study by Juhlin and Olsson (1997), supported by that of Berlin *et al.* (1968), suggests that supplemental intakes of 1.0 mg cyanocobalamin/day should not produce any adverse effects and this intake can be used for guidance purposes. This is equivalent to 0.017 mg/kg bw/day in a 60 kg adult. However, it should be noted that this figure has been established in particular subgroups of the population, i.e. vitiligo sufferers and those treated for pernicious anaemia and may not be completely applicable to the general population. Assuming a maximum dietary intake of 0.02 mg/day, an estimated total intake of 1.02 mg/day (equivalent to 0.017 mg/kg bw/day in a 60 kg adult) would not be expected to result in any adverse effects.

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PRELIMINARY RISK ASSESSMENT – VITAMIN C

General information

Chemistry

Vitamin C is a six-carbon compound structurally related to glucose, consisting of two inter-convertible compounds: L-ascorbic acid, a strong reducing agent, and its oxidised derivative, L-dehydroascorbic acid.

Natural occurrence

Most animals are able to synthesise vitamin C from glucose and galactose. However, primates and guinea pigs are unable to do so.

Occurrence in foods, food supplements and medicines

Food of plant origin, particularly citrus and soft fruits and leafy green vegetables, are major sources of vitamin C. Vitamin C is readily lost during cooking, due to oxidation. Kidney and liver are good animal-derived sources of vitamin C. Ascorbic acid is a permitted anti-oxidant in food with no specified limits on the level of fortification. Vitamin C is present in numerous dietary supplements and in licensed medicines at doses of up to 3000 mg.

Recommended amounts

In 1991, the Committee on Medical Aspects of Food and Nutrition Policy (COMA) recommended a Reference Nutrient Intake (RNI) of 40 mg/day for adults, with an increase in pregnancy to 50 mg/day, and during lactation to 70 mg/day. The US Food and Nutrition Board recently recommended an increase in the US Recommended Dietary Allowance (RDA) of vitamin C from 60 to 120 mg/day.

Analysis of tissue levels and vitamin C status

Plasma and urinary vitamin C are measured but reflect recent dietary intake rather than the level of vitamin C in body stores. Leucocytes contain higher concentrations of vitamin C than plasma, whole blood or serum but measurement of leucocyte vitamin C is technically more difficult. A leucocyte vitamin C level below 0.01 mg per 10^8 cells is generally regarded as deficient.

Brief overview of claimed non-nutritional beneficial effects

It has been claimed that vitamin C protects against the common cold and has wide-ranging anti-oxidant effects. Beneficial effects on conditions such as diabetes, asthma, arthritis, Parkinson's disease, autism and depression have also been suggested.

Function

Vitamin C is a strong reducing agent and antioxidant involved in prevention of the damaging effects of free radicals. Vitamin C is involved in the synthesis of collagen, neurotransmitters and carnitine, is an enzyme co-factor and increases the gastrointestinal absorption of non-haem iron.

Deficiency

Vitamin C deficiency in humans leads to the clinical syndrome of scurvy. Early symptoms in adults include fatigue, weakness, anaemia aching joints and muscles. This is followed in later stages by bleeding from the gums, petechial and sheet haemorrhages, and delayed wound healing.

Interactions

Absorption of metal ions may be impaired by vitamin C. Vitamin C supplementation may increase the effectiveness of the anticoagulant warfarin and the severity of gastric lesions caused by aspirin.

Absorption and bioavailability

Gastrointestinal absorption of vitamin C is efficient and occurs in the small intestine via a saturable active transport mechanism. Absorption efficiency of low doses of vitamin C (4 – 64 mg) may be as high as 98%, but decreases with increasing doses of the vitamin.

Distribution and metabolism

Ascorbic acid is widely distributed in all tissues of the body, with higher levels found in the adrenal glands, pituitary and retina, and lower levels in kidney and muscle tissue.

Vitamin C is hydrolysed to diketogulonic acid and then oxidised to oxalic and threonic acid. Some oxidation to carbon dioxide occurs at high doses.

Excretion

Vitamin C metabolites, primarily oxalate, and unmetabolised ascorbic acid are largely excreted in the urine. Approximately 3% of the oral dose is faecally excreted, either unchanged, or as metabolites. More of the vitamin is excreted unchanged at higher levels of vitamin C intake.

Toxicity

Human data

Gastrointestinal effects are the most common adverse clinical events associated with acute, high doses of vitamin C.

Other reported adverse effects include metabolic acidosis, conditioned need/scurvy (where ingestion of an excessive amount of vitamin C during pregnancy may condition the offspring to require greater than the expected or recommended daily intakes), and changes in prothrombin activity.

Adverse effects related to the urinary route of excretion have been reported. These include renal stones, renal tubular disease and oxaluria. Vitamin C consumption has been suggested to increase oxalate excretion causing the formation of urinary stones, but studies in humans did not reveal a substantial increase in urinary oxalate (moderate increases may be an experimental artefact). Subjects with a pre-disposition to the formation of kidney stones may be more sensitive to increases in urinary oxalate associated with vitamin C.

Vitamin C increases iron uptake from the gut and this may be important in subjects with conditions such as haemochromatosis or in subjects heterozygous for this condition. A dose of 2000 mg/day vitamin C for up to 20 weeks had no effect on healthy volunteers.

Supplementation trials

Vitamin C was administered (in combination with other vitamins and minerals) at doses of up to 1000 mg/day for up to 5 years in two supplementation trials with good compliance and no reported adverse effects. Reduced vitamin B₁₂ levels in 3 (of 90) individuals consuming > 1000 mg/day over a minimum of 3 years were reported in an earlier trial.

Animal data

Vitamin C has low acute toxicity. High doses of vitamin C are associated with decreased growth rates in guinea pigs (50 mg/day), increased cholesterol levels in rats (150 mg/kg bw/day) and interference with trace element metabolism in chicks. No effects on reproductive or developmental parameters have been reported. A conditioned increase in vitamin C requirements has been reported in guinea pigs.

Carcinogenicity and genotoxicity

No data on carcinogenicity have been identified. Some positive *in vitro* mutagenicity tests have been reported, although results are generally mixed. However, the positive results tended to occur when vitamin C was tested in the presence of copper. Short-term vitamin C supplementation has been reported to cause an increase in modified DNA bases due to its pro-oxidant effect. *In vitro*, vitamin C caused an increase in DNA reactive metabolites formed from lipid hydroperoxides. There is no evidence of mutagenicity *in vivo*.

Vulnerable Groups

Individuals unable to regulate iron absorption due to haemochromatosis or thalassaemia may be vulnerable to the enhanced iron absorption caused by vitamin C.

Acidification of urine and uricosuria may promote the development of urinary or renal stones.

Mechanism of toxicity

Urinary stones may result from an increase in urinary oxalate excretion. Adverse effects attributed to vitamin C may be due to increased sensitivity to oxidant stress.

Dose-response characterisation

The dose-response is unclear, as many studies have used only one dose level only. However, adverse effects are generally reported at levels in excess of 1000 mg vitamin C/day.

Genetic variations

No genetic variations have been identified with regard to the metabolism of vitamin C *per se* though certain conditions may increase sensitivity to the adverse effects associated with this compound eg. haemochromatosis, thalassaemia or a pre-disposition to urinary or renal stones.

Studies of particular importance in the risk assessment

Cameron and Campbell, 1974

In a stepped study, healthy human volunteers were given doses of vitamin C, which increased by 1000 mg per week. Abdominal distention, flatulence, diarrhoea and transient colic were described as “fairly frequent” at supplemental dose levels of 3000-4000 mg daily. No other details were provided.

Cook et al., 1984

Seventeen adult volunteers were given 2000 mg vitamin C/day with meals for 16 weeks and 9 subjects continued for 24 months in a study examining the effect of dietary iron absorption and assimilation on body iron status. No subjective side effects were reported. Although vitamin C enhances absorption of non-haem iron, no increase in serum ferritin levels was found, despite wide variation in iron status between the volunteers. The authors concluded that supplemental vitamin C had a negligible effect on iron stores. Supplementation continued for a further 20 months in 4 iron-deficient and 4 iron-replete subjects. No effect of vitamin C on body iron reserves (as measured by serum ferritin levels) was apparent and no intestinal adaptation to the enhancing effect of the vitamin had occurred. The authors considered several possible explanations for their findings. No adverse effects were reported in this study of large supplemental doses of vitamin C but the authors noted that the study did not exclude possible adverse effects in individuals who are heterozygous for the haemochromatosis gene (approximately 10% of the population). The study involved small numbers of participants with variable iron status and was not blinded, though compliance was checked.

Estimated maximum daily intake: $161.5 + 3000 = 3161.5$ mg

Vegetarians are a potential high intake group.

Risk Assessment

Few data suggest that vitamin C is associated with significant toxic effects and there are no obvious specific key toxic endpoints for vitamin C in healthy subjects. High doses of vitamin C are associated with gastrointestinal effects, generally at doses of several grams, but which have also been reported at doses of 1000 mg (1 g). There are few controlled studies specifically investigating this adverse effect. Controlled studies do not support anecdotal reports of other possible adverse effects, such as infertility.

Earlier suspicions of potential adverse effects, such as destruction of vitamin B12, have not been confirmed following subsequent developments in analytical techniques. Data on increased oxalate excretion attributable to vitamin C are conflicting. Some reported increases in urinary oxalate might be attributable to experimental artefact though increased oxalate was apparent at doses of > 500 mg/day in subjects with calcium oxalate stones. In normal subjects, doses of 4000 mg/day vitamin C were not associated with increased urinary oxalate excretion.

Potential vulnerable groups include sufferers from disorders of iron metabolism or storage.

Vitamin C has been reported to produce a variety of pro-oxidant effects. The significance of this for the general population is uncertain.

Vitamin C has very low acute toxicity in animals and no effects on reproductive parameters have been reported. However high doses of vitamin C are associated with decreased growth rates in guinea pigs (50 mg/day) and increased cholesterol levels in rats (150 mg/kg bw/day).

ESTABLISHMENT GUIDANCE LEVEL

There are insufficient data to set a safe upper level for vitamin C. The vitamin may be of low toxicity, although adverse effects have been associated with consumption of large quantities of vitamin C. However, both the nature of these adverse effects and the dose threshold at which they occur is unclear. It is apparent that adverse effects, in particular on the gastrointestinal system, may occur in subjects consuming quantities of vitamin C greater than 1000 mg but the precise dose at which such effects occur is variable. A limited study by Cameron and Campbell (1974) suggests a LOAEL of 3000-4000 mg/day, which is supported by other reports such as those by Stein *et al.* (1976). For guidance purposes a LOAEL of 3000 mg/day can be determined. Applying a UF of 3 for LOAEL to NOAEL extrapolation, a supplemental dose of 1000 mg/day supplement would not be expected to have any significant adverse effects. The dose is equivalent to 17 mg/kg bw/day in a 60 kg adult. A guidance level for total vitamin C intake has not been estimated since adverse effects

appear to follow supplemental, bolus doses rather than intake of vitamin C in food. It should be noted that higher levels of vitamin C may be without adverse effects in many individuals.

A number of potentially vulnerable groups have been identified; these include individuals who are heterozygous for haemochromatosis, and thalassaemia or those with a pre-disposition to urinary or renal stones. Data on the possible adverse effects of vitamin C on these individuals are also conflicting, but, with the exception of urinary stone formation, appear to occur at gram levels of consumption. Doses of 500 mg vitamin C or higher, appear to have increased urinary oxalate in subjects predisposed to urinary stone formation thus increasing the risk of stone formation in the future. Doses of 1000 mg/day have also been reported to have increased oxalate excretion in healthy volunteers, however there are methodological concerns regarding the measurement of urinary oxalate so the significance of these reports is uncertain.

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