Risk Assessment — Riboflavin

General information

Chemistry

Riboflavin is a water-soluble vitamin of the B group (vitamin B_2). 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 100 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. COMA recommended a RNI of 1.1 mg/day for women and 1.3 mg/day for men (COMA, 1991). 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 play a role 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

(For full review see http://www.food.gov.uk/science/ouradvisors/vitandmin/evmpapers or the enclosed CD).

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 28 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: 3.3 mg/day
Supplements	up to 100 mg/day (Annex 4)
Estimated maximum intake:	3.3 + 100 = 103 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. No toxicological hazard has been identified for riboflavin from animal studies. 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 28 patients. Although there is no evidence of hazard at higher levels, an uncertainty factor of 10 is applied to allow for inter-human variability because of the small numbers of individuals involved, who may not be representative of the general population, and the incomplete investigation of adverse effects. This suggests that, for guidance purposes only, supplemental intakes of 40 mg riboflavin/day (equivalent to 0.67 mg/kg bw for a 60 kg adult) would be unlikely to result in adverse effects. This is in addition to riboflavin provided by the diet. Assuming a maximum dietary intake of 3.3 mg/day, a total daily intake of 43 mg riboflavin (equivalent to 0.72 mg/kg bw/day in a 60 kg adult) would not be expected to result in any adverse effects. The available database is insufficient for us to comment on the safety of higher intakes, although these may be without adverse effects.

References

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