



Comments from the Norwegian Scientific Committee for Food Safety, Panel on Nutrition, dietetic products, Novel Food and Allergy (Panel 7) on the setting of maximum limits for vitamins and minerals in foods

Tolerable Upper Intake Levels/Guidance Levels

EFSA/SCF have established Tolerable Upper Intake Levels for several vitamins, minerals and trace elements.

For those vitamins or minerals where no Tolerable Upper Intake Level has been established, the Expert Group on Vitamins and Minerals (EVM 2003), UK has suggested so called Guidance Levels (GL), and a Danish Expert group has suggested so called temporary Guidance Levels where no GLs have been established (Rasmussen *et al.* 2006) The following is based on studies/evidence referred in the UK report and on the results from the Danish Expert group, but updated with new studies if such have been published the last couple of years. It is important to note that GL values are derived for adults. Work has not started to find GL for children.

Vitamin B₁, thiamin.

Case reports indicate that very high doses of thiamin (> 5000 mg) cause adverse effects like 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 (n = 4) of reported adverse effects following lower doses. No specific toxic effects of thiamine ingestion by humans have been identified. On the other hand, no large controlled human supplementation studies have been conducted.

In a randomised, double-blind placebo-controlled study by Gokhale (Gokhale 1996), a daily oral dose of 100 mg thiamine hydrochloride was given to 556 young females (12 – 21 y) for 60 – 90 days. No adverse effects were reported. Based on this report, a level of 100 mg/day of supplemental thiamine (equivalent to 1.7 mg kg/kg bw/day supplemental thiamine for a 60 kg adult) would not be expected to result in adverse effects. This level could thus be considered a no-observed-adverse-effect-level (NOAEL).

In the absence of an established lowest-observed-effect-level (LOAEL) and based on the above mentioned study among young girls, Panel 7 suggests a temporary Guidance Level of 50 mg/day. This will allow for inter-individual differences in susceptibility considering that the group of young girls above is not representative of the total population. This is also in accordance with recommendations from the Danish Expert group and is also referred in the US IOM dose-response-assessment of thiamin 2000. They, however argue that supplements

containing up to 50 mg/day of thiamine are widely available without prescriptions, but that effects of intakes at this level or higher do not appear to have been studied systematically.

Vitamin B₂, riboflavin.

There is insufficient data from human and animal studies to establish an Upper Level for riboflavin although the available data indicate that its toxicity is low. In a study from 1998 (Schoenen *et al.* 1998), riboflavin (400 mg) and placebo were given to 55 patients with migraine in a randomized trial of 3 months duration. Using an intention-to-treat analysis, riboflavin was superior to placebo in reducing attack frequency ($p = 0.005$) and headache days ($p = 0.012$). Three minor adverse events occurred, two in the riboflavin group (diarrhoea and polyuria) and one in the placebo group (abdominal cramps). None was serious. If one allows for an uncertainly factor of 10, (because the number of participants was low), supplemental intakes of 40 mg riboflavin/day (equivalent to 0.67 mg/kg bw/day supplemental thiamine for a 60 kg adult) can be considered as a NOAEL. This is in addition to riboflavin provided from the diet.

Panel 7 has not been able to find relevant publications from the last couple of years. Panel 7 supports the UK Expert group in suggesting 40 mg/day as a Guidance Level for vitamin B₂.

Vitamin B₁₂, cobalamin.

Clinical studies have reported no adverse effects following administration of up to 6 mg/day of methylcobalamin for several weeks and up to 1 mg/day cyanocobalamin for several years. In a Swedish study from 1997 (Juhlin & Olsson 1997), 100 patients with vitiligo (loss of pigmentation in skin) were treated with oral folic acid and vitamin B₁₂, the latter in doses of 2 mg cyanocobalamin/day for up to 12 months. There were no reports of adverse effects. A 2 year intervention study on 628 older Japanese patients having had stroke was performed to test the protective effect of folate and vitamin B₁₂ (Sato *et al.* 2005). Vitamin B₁₂ was given in doses of 1500 µg mecobalamin. Twelve patients in the folate and vitamin B₁₂ group experienced anorexia and nausea and one patient experienced itching. All symptoms subsided within a week of discontinuing the supplements. This intake level can be used for guidance purposes. It is equivalent to 0.025 mg/kg bw/day in a 60 kg adult. No uncertainty factor is needed because the number of individuals is in our opinion sufficient. However, it should be noted that the figure is based on patients that may not be representative of the whole population.

The UK Expert group suggested 2 mg/day as a Guidance Level for vitamin B₁₂ based on the study on vitiligo patients cited above. The Japanese study has been published later, but involves both folate and cobalamin. Further studies are needed to conclude, but based on the results from the Japanese study it cannot be ruled out that intake of cobalamin above 1500 µg/day may represent a health risk. This should form the basis of a revised Guidance Level, and Panel 7 suggests 1500 µg as a Guidance Level for vitamin B₁₂.

Biotin

Doses of 10 mg/day have been studied in a number of small clinical trials for therapeutic effects, without reported side effects. A supplemental dose of 9 mg/day was given to diabetics for up to 4 years with no associated adverse effects (Maebashi *et al.* 1993). Because all these studies have been small, and even fewer followed up long-term, an uncertainty factor of 10

for inter-individual variation is sensible to apply. Thus, for guidance purposes, 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. Assuming a maximum intake of 0.066mg/day from food, an estimated total intake (from all sources) of 0.97 mg/day biotin would not be expected to result in any adverse effects.

Panel 7 has not been able to find relevant publications from the last couple of years. Panel 7 supports the UK Expert group in suggesting 0.9 mg/day as a Guidance Level for biotin.

Pantothenic acid

There are very few human studies available on the oral toxicity of pantothenic acid from controlled trials. In a randomized, double-blind, placebo-controlled study from 1980, 47 arthritis patients were treated with calcium pantothenate (initially 500 mg/day, later 2000 mg/day) for 8 weeks (General Practitioner Research Group 1980). Thirty-one patients in the intervention group completed the trial. The authors stated that no side effects were recorded in the patients on calcium pantothenate. Because of the low number of participants, an uncertainty factor of 10 should be applied to allow for inter-human variability. 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 a recent Japanese study on rats (Shibata *et al.* 2005), the animals were fed a diet containing 0%, 0.0016% (control group), 1%, or 3% calcium pantothenate for 29 days. In the 3% addition group, enlargement of the testis, diarrhoea, and hair damage were observed, and the weight increase and food reduced compared to the control group. However, abnormality was not seen in the 1% addition group. The researchers conclude that the 3% level in the diet was the LOAEL and the 1% level was the NOAEL. Given as daily intake per kg bodyweight of rat, the NOAEL was around 1000 mg, while the LOAEL was around 3000 mg. With a safety factor of 100, the tolerable upper intake level would be around 10 mg/kg bw/day or 600 mg/day (for a 60 kg adult).

The UK Expert group suggests 200 mg/day as a Guidance Level for pantothenic acid. The Japanese study has been published later and could form the basis of a revised Guidance Level. It is at present not possible to conclude on which level is safe and appropriate, but the studies show that high intakes of pantothenic acid can give adverse effect, and that there is a need for setting of maximum limits. Further studies are needed to conclude on a GL or UL.

Chromium (III)

Overall, there are too few data from human or animal studies to derive a Safe Upper Level of chromium, probably mainly because trivalent chromium from the diet is poorly absorbed and thus the body is naturally protected. In isolated case reports, Cr picolinate supplementation has been said to cause adverse effects, such as anaemia, renal failure, liver dysfunction, and neuronal impairment. The same form of Cr has been associated with oxidative damage to DNA in rats and mutations and DNA fragmentation in cell cultures. Chromium interacts with iron by affecting its binding to transferrin, and has been shown to impair iron metabolism and storage.

A recently published study on rats (Bailey *et al.* 2006) tested large doses of three forms of chromium commonly found in dietary supplements on developing rat foetuses. From gestation days (GD) 6-17, pregnant chromium-deficient (CD) mice were fed diets containing either 200 mg/kg Cr(pic)3, 200 mg/kg CrCl3, 174 mg/kg picolinic acid, or the diet only to determine if Cr(pic)3, CrCl3, or picolinic acid could cause developmental toxicity. Dams were sacrificed on GD 17, and their litters were examined for adverse effects. The incidence of bifurcated cervical arches was significantly increased in fetuses from the Cr(pic)3 group as compared to the diet-only group. Fetuses in the picolinic acid-treated group had an incidence double that of the control group; however, this increase was not statistically significant. Fetuses in the CrCl3 group did not differ from the controls in any variable examined. No maternal toxicity was observed in any of the treatment groups. Conclusions: High maternal oral exposures to chromium picolinate can cause morphological defects in developing offspring of mice.

The doses in this study were very high. This was a clear effect level and can not be used to derive a NOAEL. The dosages correspond to 120 mg/day in a 60 kg adult.

Another study on rats (Anderson *et al.* 1997), looking at the effect of 0 – 100 mg Cr/kg bw/day on outcomes like body weight, organ weights or blood variables, indicated that 15 mg/kg bw/day of chromium (as Cr-chloride) was not associated with adverse effects in the rat. Based on this study, and again allowing for a total uncertainty factor of 100, 0.15 mg/kg bw/day, or 9 mg in a 60 kg person, would be expected to be without adverse health effects.

In a very recently published study on rats (Scibior & Zaporowska 2007), 0.3 mg Cr/kg bw/day resulted in a significant decrease in kidney GSH concentration and GSH/GSSG ratio in both liver and kidney, indicating that Cr has pro-oxidative effects. The impact of this would be a reduced antioxidant defence in the body. If this study is taken as a starting point for establishing a NOAEL, and including the safety factor of 100, it would be equivalent to 0.003 mg/kg bw/day in humans, or a total of 0.18 mg/day (= 180 µg/d). However, Panel 7 believes this study alone is too soft to be used as basis for establishing a GL. Panel 7 has chosen to use it to modify the UK GL suggestion.

The study above, using GSH levels as an outcome measure, confirms that when no adverse effect levels have been found in earlier studies, it may be because we have not known what to look for. In general, all the divalent or trivalent ions that we classify as essential nutrients (Ca, Mg, Fe, Zn, Cu and Cr) have interactive effects on each others absorption, metabolism and/or excretion. They also have in common that the window from RDA to UL is rather narrow. There is no reason to believe this does not apply to chromium.

The UK Expert group suggested 9 mg/day as a Guidance Level for trivalent chromium. The GSH-study has been published later, but the impact on health is uncertain. Further studies are needed to conclude, but based on the results from the GSH study it cannot be ruled out that an intake of chromium much lower than 9 mg/day for adults may represent a health risk. Panel 7 therefore encourages a restrictive Guidance Level for chromium, and with the GSH study in mind thinks, for the time being, that the upper Guidance Level should be maximum 1 mg/day for an adult.

Vitamin K

Panel 7 supports the UK Expert group in suggesting 1000 µg/day as a Guidance Level for vitamin K.

Other vitamins and minerals/trace elements with potential health risk, but no established Upper Tolerable Intake Level from SCF/EFSA

Iron

Panel 7 supports JECFA and the Danish Expert group in suggesting 50 mg/day as a Guidance Level for iron. As is also concluded in the EFSA opinion on Tolerable Upper Intake Level for iron (2004), intake of 50-60 mg non-hem iron has caused gastrointestinal effects.

Manganese

Manganese and iron compete for absorption sites. Thus a high dietary level of one will reduce the absorption of the other. Panel 7 supports the UK Expert group in suggesting 4 mg/day as a Guidance Level for manganese.

Vitamin C

Panel 7 supports the Danish Expert group in suggesting 1000 mg/day as a Guidance Level for vitamin C. As is also concluded in the EFSA opinion on Tolerable Upper Intake Level for vitamin C (2004), intake of 1000 mg vitamin C can cause gastrointestinal effects.

Beta-carotene

Panel 7 supports the Danish Expert group in suggesting 5 mg/day as a temporary Guidance Level for beta-carotene. An intake of 5 mg/day represents the average dietary level for beta-carotene, and a temporary GL at this level could therefore result in a zero allowance for fortification of foods with beta-carotene (depending on model for addition of nutrients to foods). Supplemental beta-carotene is contraindicated for use in heavy smokers (SCF opinion 2000). Animal studies in ferrets indicate that beta-carotene *per se* affects cell differentiation and proliferation in the bronchus and substances in cigarette smoke enhance this effect (Wang *et al.* 1999). In a rather large study with 22071 male physicians, 12 years of supplementation with 50 mg beta-carotene on alternate days produced neither benefit nor harm in terms of the incidence of malignant neoplasms, cardiovascular disease or death from all causes (Hennekens *et al.* 1996). Although beta-carotene thus seems innocuous to others than smokers, excessive beta-carotene intake may affect population groups and target organs other than those presently investigated.

General remark

Several previous maximum limits for the different vitamins and minerals were lowered in the latest opinions from SCF and EFSA. New studies, e.g. on vitamin E and chromium, indicate that some of these Tolerable Upper Intake Levels should be revised and lowered even further. When evaluating the need for maximum limits for vitamins with insufficient human or animal data (as the above mentioned) this should be kept in mind.

References

- Anderson RA, Bryden NA, Polansky MM. Lack of toxicity of chromium chloride and chromium picolinate in rats. *J Am Coll Nutr* 1997;16:273-9.
- Bailey MM, Boohaker JG, Sawyer RD, Behling JE, Rasco JF, Jernigan JJ, Hood, RD, Vincent JB. Exposure of pregnant mice to chromium picolinate results in skeletal defects in their offspring. *Birth Defects Res B Dev Reprod Toxicol* 2006;77:244-9.
- Expert Group on Vitamins and Minerals (EVM) Safe Upper Levels for Vitamins and Minerals, UK 2003
- Gokhale LB. Curative treatment of primary (spasmodic) dysmenorrhoea. *Indian J Med Res* 1996;103:227-31.
- Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med* 1996;334:1145-1149.
- Juhlin L & Olsson MJ. Improvement of vitiligo after oral treatment with vitamin B12 and folic acid and the importance of sun exposure. *Acta Derm Venereol* 1997;77:460-2.
- Rasmussen S, Andersen NL, Dragsted LO, Larsen JC. A safe strategy for addition of vitamins and minerals to foods. *Eur J Nutr* 2006;45:123-135.
- Sato Y, Honda Y, Iwamoto J, Kanoko T, Satoh K. Effect of folate and mecobalamin on hip fractures in patients with stroke: a randomized controlled trial. *JAMA* 2005;293:1082-8.
- Schoenen J, Jacquy J, Lenaerts M. Effectiveness of high-dose riboflavin in migraine prophylaxis. A randomized controlled trial. *Neurology* 1998;50:466-70.
- Scibior A & Zaporowska H. Effects of vanadium(V) and/or chromium(III) on L-ascorbic acid and glutathione as well as iron, zinc, and copper levels in rat liver and kidney. *J Toxicol Environ Health A* 2007;70:696-704.
- Shibata K, Takahashi C, Fukuwatari T, Sasaki R. Effects of excess pantothenic acid administration on the other water-soluble vitamin metabolisms in rats. *J Nutr Sci Vitaminol (Tokyo)* 2005;51:385-91.
- US Institute of Medicine. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin and Choline 2000. ISBN 0-309-06554-2.
- Wang XD, Liu C, Bronson RT, Smith DE, Krinsky NI, Russel M. Retinoid signaling and activator protein-1 expression in ferrets given beta-carotene supplements and exposed to tobacco smoke. *J Natl Cancer Inst* 1999;91:60-66.