

SCIENTIFIC OPINION

Orotic acid salts as sources of orotic acid and various minerals added for nutritional purposes to food supplements¹

Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food (ANS)

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion on the safety of magnesium orotate, zinc orotate, calcium orotate, chromium orotate, copper orotate, iron orotate, manganese orotate, potassium orotate, sodium orotate and choline orotate added for nutritional purposes as a source of magnesium, zinc, calcium, chromium, copper, iron, manganese, potassium, sodium and choline in food supplements and on the bioavailability of these cations from these sources.

¹ For citation purposes: Scientific Opinion of the Panel on Food Additives and Nutrient Sources added to Food (ANS) on orotic acid salts as sources of orotic acid and various minerals added for nutritional purposes to food supplements, following a request from the European Commission. *The EFSA Journal* (2009) 1187, 1-25.

The present opinion deals only with the safety of orotate sources of the ten cations mentioned above and the bioavailability of the nutrient cations from these sources, intended for use in food supplements. The safety of the nutrient cations themselves, in terms of amounts that may be consumed, is outside the remit of this Panel.

Orotic acid is an intermediate in the pyrimidine biosynthesis, which is required for DNA and RNA synthesis. It was originally introduced as a vitamin, called vitamin B₁₃, but essentiality has not been demonstrated.

Orotic acid occurs mainly in milk from ruminants, with highest amounts being found in animals which are deficient in arginine and uridine-5'-monophosphate activity. In cows' milk amounts of 20-100 mg/L are found and somewhat higher amounts in goat's and sheep's milk. Orotic acid has also been detected in infant formula in amounts of 15-118 mg/L, which is reflecting the range of cow's milk.

No use levels are provided for sodium or choline orotate. For the other orotate sources, there is a large difference concerning the daily doses proposed by the petitioners: 1.8 - 6206 mg/day. Largest exposures to orotate from an orotate source would result from the proposed uses for calcium and magnesium orotate, providing the amount of 800 mg calcium/day (equivalent to 6.2 g orotate or about 100 mg/kg bw/day), and magnesium of 250 mg/day (equivalent to 3.2 g orotate/day or 53 mg/kg bw/day). For consumers who would consume several nutrients with orotates as source, the total anticipated combined exposure would amount over 11 g/day.

Orotic acid is synthesised *in situ* from carbamoyl phosphate and aspartic acid through dihydroorotic acid. Orotic acid is then transformed to orotidin-5'-phosphate and further to uridine-5'-monophosphate. When there is insufficient capacity for detoxifying the load of ammonia presented for urea synthesis, carbamoyl phosphate leaves the mitochondria and enters the pyrimidine pathway, where orotic acid biosynthesis is stimulated; orotic acid excretion in urine then increases. Orotic acid synthesis is abnormally high in hereditary deficiencies of urea-cycle enzymes or uridine monophosphate synthase.

Little documentation on the bioavailability of the mineral salts of orotic acid has been submitted, but one study reveals that the bioavailability of zinc from zinc orotate is comparable to that of zinc from another organic source, as well as an inorganic salt. The Panel concludes that this will probably also be the case for the bioavailability of the cations from the other orotate salts, for which no documentation has been supplied.

The petitioners have submitted only few data concerning the toxicity of orotic acid and none on the salts. Orotic acid has a low acute toxicity. In repeated doses orotic acid induces fatty livers in the rat, but not in other species tested. No data were submitted on reproductive and developmental toxicity and only irrelevant data on genotoxicity.

Several studies have shown that repeated dosing of orotic acid to rats and some other species promotes the formation of tumours initiated by various known carcinogenic substances. The usual concentration to promote tumours has been 1 % in the diet, but also 0.5 and 0.2 % in the diet has been shown to have promoting effect, while 0.1 % in the diet did not have effect within the time span tested (up to 20 weeks). A No Observed Adverse Effect Level for this effect can thus be determined to be 50 mg/kg bw/day, while the Lowest Observed Effect Level is 100 mg/kg bw/day.

In a long-term feeding study in rats, 1 % orotic acid in the diet without any initiation, increased the frequency of tumours likely due to a promoting effect of orotic acid on spontaneously arising and/or diet induced altered cells.

The Panel considers that it is not appropriate to conclude on the safety for a combination between chromium and a tumour promoter as long as it is not clear whether chromium is genotoxic or not.

The Panel concludes that in the light of the tumour-promoting effect of orotic acid in animal experimentation, the small margin of safety to this effect from foreseeable exposure, and the absence of any relevant studies on genotoxicity and of any developmental studies, the use of orotate as a source of the eight other minerals and choline at the proposed levels of use is of safety concern.

Key words:

Orotic acid, orotate, magnesium orotate, zinc orotate, calcium orotate, chromium orotate, copper orotate, iron orotate, manganese orotate, potassium orotate, sodium orotate, choline orotate; CAS Registry Numbers: 65-86-1; 73-97-2; 22454-86-0; 94333-35-4; 61573-60-2; 85187-45-7; 34717-03-8; 94333-38-7; 24598-73-0; 154-85-8; 60388-02-5; 68399-76-8; 24381-49-5

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The European Community legislation lists nutritional substances that may be used for nutritional purposes in certain categories of foods as sources of certain nutrients.

The Commission has received a request for the evaluation of magnesium orotate, zinc orotate, calcium orotate, chromium orotate, copper orotate, ferrous orotate, manganese orotate, potassium orotate, sodium orotate and choline orotate added for nutritional purposes to food supplements.

The relevant Community legislative measures are:

- Directive 2002/46/EC of the European Parliament and of the Council on the approximation of the laws of the Member States relating to food supplements².

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide a scientific opinion, based on its consideration of the safety and bioavailability of magnesium orotate, zinc orotate, calcium orotate, chromium orotate, copper orotate, ferrous orotate, manganese orotate, potassium orotate, sodium orotate and choline orotate added for nutritional purposes to food supplements.

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² OJ L 183, 12.7.2002, p.51.

ASSESSMENT

The present opinion deals only with the safety of orotate sources of ten cations (magnesium, zinc, calcium, chromium (III), copper (II), iron, manganese, potassium, sodium and choline) and the bioavailability of the cations from these sources, intended to be used in food supplements. The safety of the nutrients themselves, in terms of amounts that may be consumed, is outside the remit of this Panel.

1. Introduction

Orotic acid is an intermediate in pyrimidine biosynthesis, which is required for DNA and RNA synthesis. It was originally introduced as a vitamin, called vitamin B₁₃, but essentiality has not been demonstrated. This opinion deals with the use of the salts of orotic acid as sources of the various cations.

2. Technical data

2.1. Chemistry

Substances considered in this paper and their CAS Registry Numbers and molecular weights are presented in Table 1. The IUPAC name of orotic acid is 1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinecarboxylic acid.

Table 1. **Molecular formula, CAS Registry Numbers and molecular weights of the orotates**

Orotate	Molecular formula	CAS N ^o (anhydride)	MW (g/mol) (hydrate)
Orotic acid	C ₅ H ₄ N ₂ O ₄	65-86-1	156.1
Orotate	C ₅ H ₃ N ₂ O ₄	73-97-2	155.1
Calcium orotate, dihydrate	C ₁₀ H ₆ CaN ₄ O ₈ *2H ₂ O	22454-86-0	386.29
Chromium(III) orotate, tetrahydrate	C ₁₅ H ₉ CrN ₆ O ₁₂ *4H ₂ O	94333-35-4	589.3
Chromium(III) orotate, hexahydrate	C ₁₅ H ₉ CrN ₆ O ₁₂ *6H ₂ O	94333-35-4	625.4
Copper(II) orotate, dihydrate	C ₁₀ H ₆ CuN ₄ O ₈ *2H ₂ O	61573-60-2	409.76
Iron(II) orotate, trihydrate	C ₁₀ H ₆ FeN ₄ O ₈ *3H ₂ O	85187-45-7	420.1
Magnesium orotate, dihydrate	C ₁₀ H ₆ MgN ₄ O ₈ *2H ₂ O	34717-03-8	370.5
Manganese orotate, dihydrate	C ₁₀ H ₆ MnN ₄ O ₈ *2H ₂ O	94333-38-7	401.2
Potassium orotate	C ₅ H ₃ KN ₂ O ₄	24598-73-0	194.2
Sodium orotate	C ₅ H ₃ NaN ₂ O ₄	154-85-8	177.1
Zinc orotate, dihydrate	C ₁₀ H ₆ N ₄ O ₈ Zn*2H ₂ O	60388-02-5	411.6
		68399-76-8	
Choline orotate	C ₁₀ H ₁₇ N ₃ O ₅	24381-49-5	259.3

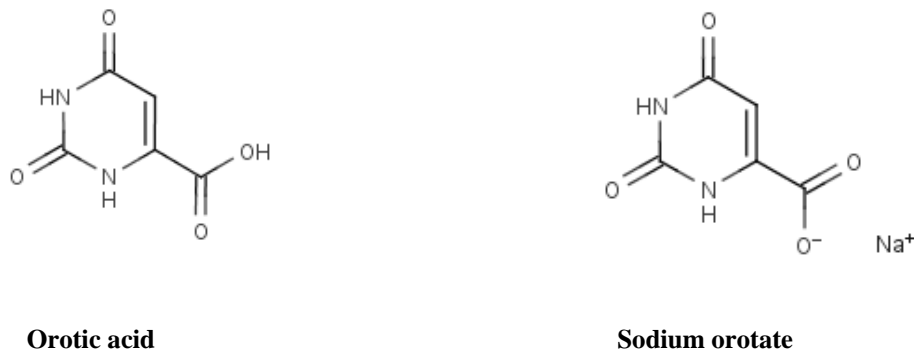


Figure 1. **Structural formulas for orotic acid and one of its salts (From ChemIDplus Lite)**

2.2. Specifications

Calcium, magnesium, sodium, potassium, zinc manganese and choline orotates are described as white to almost white (sodium, off-white; manganese, off-white to yellowish-white) crystalline powders, while chromium orotate is described by one petitioner as greyish to grey-green and by the other petitioners as a yellowish-brown powder. Copper orotate is described as greenish-blue and iron orotate as a yellow-brown powder. All the salts are described as very slightly soluble in water and almost insoluble in ethanol and methanol. However the petitioner for choline orotate mentions that choline orotate “dissolves quite well”.

Specifications are proposed for each of the substances.

2.3. Manufacturing process

Satisfactory descriptions of the manufacturing methods have been submitted. The principle is that a hot solution of orotic acid, which according to one petitioner is manufactured through the oxalacetic ester method, is mixed with a solution of an inorganic salt of the respective cation and the resulting orotate salt is then crystallised and purified.

2.4. Methods of analysis in food

No method for analysis in food or in food supplement preparations was provided. One petitioner gives as an example of how to analyse pure zinc orotate.

Orotic acid can be determined by chromatographic (e.g. HPLC), spectrophotometric and polarographic methods, which have a very wide interval range (19 - 664 mg/L) and a low precision. Also enzymatic methods are used.

2.5. Reaction and fate in foods to which the source is added/Stability

The orotates covered by this opinion are claimed by the petitioners to be stable and some documentation for stability over two years has been submitted.

2.6. Case of need and proposed uses

The petitioners propose to use the various orotates to provide an alternative source of the respective cations, as well as of orotic acid. The orotates, according to the petitioners, are formulated as tablets, in sachets, as powder or in capsules. The proposed use levels per day are as follows:

- calcium orotate dihydrate: 900 to 7726 mg
- chromium orotate tetrahydrate: 0.7 to 2.3 mg
- copper(II) orotate dihydrate: 5 to 19 mg
- iron(II) orotate trihydrate: 150 mg
- magnesium orotate dihydrate: 900 to 3811 mg
- manganese orotate dihydrate: 10 to 365 mg
- potassium orotate: 300 to 1251 mg
- zinc orotate dihydrate: 40 to 315 mg

No use levels are provided for sodium or choline orotate.

2.7. Information on existing authorisations and evaluations

The Panel notes that for adults the Scientific Committee on Food (SCF) (1993) established Population Reference Intakes (PRI) for copper (1.1 mg/day), magnesium (150 - 500 mg/day), potassium (3100 - 3500 mg/day), calcium (700 mg/day), iron (9 - 20 mg/day) and zinc (7 - 9.5 mg/day) and an acceptable range of intake for manganese of 1.0 - 10 mg/day. For chromium, the societies for Nutrition of Germany (DGE), Austria (ÖGE) and Switzerland (SGE) jointly established an Adequate Intake of 30 - 100 µg/day (D-A-CH, 2000) (Table 2).

Regarding Tolerable Upper Intake Levels (UL), the SCF established for adults an UL for calcium (2500 mg/day; SCF, 2003a), for copper (5 mg/day; SCF, 2003b), for zinc (25 mg/day; SCF, 2003c) and for supplemental magnesium (250 mg/day; SCF, 2001). WHO considered that supplementation of chromium should not exceed 250 µg/day (WHO, 1996). The US Food and Nutrition Board established an UL of 45 mg/day for iron. For manganese and potassium, EVM in 2003 established for guidance purposes a supplemental intake of 0.5-4 mg/day and of 3700 mg/day, respectively (Table 2).

2.8. Exposure

In human milk, concentrations are in general low (under 2 mg/L) but have been observed to be over 4 mg/L in mothers who are smoking (affecting the pyrimidine biosynthesis process leading to increase in the level of orotic acid), having a decreased uridine-5'-monophosphate

(UMP) activity (inhibited by nicotine, heavy metals and nitrosamine derivatives, etc), or having an arginine deficiency (Karatas, 2002).

Various studies investigated the content of orotic acid in milk from various species. The various studies give highly different amounts, but the overall picture is that cow milk contains 20-100 mg/L and sheep and goat milk contains 200-400 mg/L (Anastasi *et al.*, 2000; Hallanger *et al.*, 1953; Münchberg *et al.*, 1971; Indyk *et al.*, 2004; Motyl *et al.*, 1991); in sow milk and milk from the rat orotic acid is not detectable or levels are very low (Hallanger *et al.*, 1953, Larson *et al.*, 1979). The high range in orotic acid concentrations in cow's milk can be explained by race, lactation period and differences in individual cows. It is higher in cows with a partial deficiency of uridine monophosphate synthase and increases with prolonged lactation. Orotic acid was found in yoghurt (156 mg/L) (Motyl *et al.*, 1991), and in reconstituted infant formula a range of 15 - 118 mg/L (Motyl *et al.*, 1991; Ferreira, 2003; Durschlag *et al.*, 1980b), which reflects the range in cow milk.

No exposure data from the diet are available.

Table 2 presents the calculated potential exposure to orotates based on the use levels proposed by the petitioners.

Table 2. Use levels of orotate hydrates proposed by the petitioners and anticipated exposure estimates.

Orotate * hydrate	PRI for nutrient (mg/day)	UL or guidance level (GL) of nutrient (mg/day)	Proposed use levels of orotate salts (mg/day)	Maximum level of nutrient from supplementation (mg/day)	Maximal exposure to orotate (mg/day)	Exposure to orotate mg/kg bw/day (approx)
Ca ²⁺ * 2H ₂ O	700 ³	2500 ⁵	900 - 7726	800	6206	~100
Cr ³⁺ * 4H ₂ O	0.03 - 0.1 ²	0.250 S ⁶	0.7 - 2.3	0.20	1.8	0.03
Cu ²⁺ * 2H ₂ O	1.1 ³	5 ⁷	5 - 19	3	15	0.25
Fe ²⁺ * 3H ₂ O	9 - 20 ³	45 ⁴	150	20	111	2
Mg ²⁺ * 2H ₂ O	150-500 ³	GL: 250 S ⁸	900 - 3811	250	3192	53
Mn ²⁺ * 2H ₂ O	1.0-10 ³	GL: 4 ⁹	10 - 365	24	307	5
K ⁺ *	3100 ³	GL: 3700 ⁹	300 - 1251	252	999	17
Zn ²⁺ * 2H ₂ O	7 - 9.5 ³	25 ¹⁰	40 - 315	50	237	4
Sum orotates					> 11069	> 185

² DACH (2000); ³ SCF (1993); ⁴ IOM (2001); ⁵ SCF (2003a); ⁶ WHO (1996), S = for supplements; ⁷ SCF (2003b); ⁸ SCF (2001); ⁹ EVM (2003) S GL = Guidance Level for supplements; ¹⁰ SCF (2003c)

The estimated potential exposure to orotates for a single-supplement user would range from 1.8 to 6206 mg orotate per day, corresponding to 0.03 to 100 mg/kg bw/day. It was not possible to calculate the exposure to sodium and choline orotate as no use levels are provided.

Assuming a consumption of several supplements containing orotate, the total cumulative potential exposure would be over 11 g/day, corresponding to 185 mg/kg bw/day, as the potential exposure to sodium and choline orotate are not included.

3. Biological and toxicological data

In general, the biological and toxicological data submitted by the petitioners are dealing only to a limited extent with orotic acid and the orotates. The following sections will focus on those data relevant for orotic acid and the orotates. The alleged beneficial/therapeutic effects have not been addressed unless they provide information relevant for the safety assessment.

3.1. Bioavailability

Only data on bioavailability of orotic acid and zinc orotate were available. No data on bioavailability of the other orotate sources were available.

3.1.1. Orotic acid and zinc orotate

One petitioner informs that serum concentration curves and half-lives have been estimated following oral administration of 80 mg orotic acid per kg body weight (bw) in adults and 9- to 17-years old children. Peak plasma levels of $4.22 \pm 3.3 \mu\text{g/mL}$ were measured at 4 hours after oral administration. The half-life was about 1 hour (Walther *et al.*, 1976). Hinkel and Le Petit (1973), found slightly different peak concentrations in new-born children having received 100 mg/kg bw of orotic acid in the morning. The peak concentration was reached between 60 and 120 minutes. The enteral absorption for orotic acid amounted to 5 - 6 %. The half-life was on average 5.5 hours (Hinkel *et al.*, 1973).

The protein-binding rate of orotic acid *in vitro* has been measured by means of equilibrium dialysis of human serum proteins. Orotic acid was completely (almost 100 %) unbound in plasma (Walther *et al.*, 1976).

When carboxyl-labelled orotic acid is given to animals or humans, most of the expired radioactivity appears as respiratory CO₂ within a very few hours (Weissman *et al.*, 1962).

One of the petitioners claims for each of the salts considered that orotate is a salt with a high level of bioavailability of the cation. He also claims that the relatively lipophilic orotic acid salt predominantly passes through the gastrointestinal tract in an undissociated form, and is absorbed quickly and in high quantities. The petitioner also indicates that transportation in the blood and ultimately through the cell membranes also appears to be carried out in an undissociated form and that the orotic acid salt is only dissociated upon reaching the mitochondria. No references are given for these statements, but the petitioner claims that for this reason the required amount of the various nutrients will be much lower from the orotates than from other sources.

However, data in rats, submitted by another petitioner, showed that dietary zinc from sulphate, gluconate, orotate, aspartate and histidine is equivalent in meeting the metabolic requirement of zinc (Windisch *et al.*, 2003).

The bioavailability and pharmacokinetics of three zinc salts (zinc pantothenate, zinc sulphate and zinc orotate) was also evaluated in rabbits (Andermann *et al.*, 1982). The purpose of this work was to study the pharmacokinetics and bioavailability of the three zinc salts in relation to their water solubility. Zinc sulphate is regarded as a water-soluble mineral salt, zinc pantothenate is a soluble organic salt, and zinc orotate is an insoluble organic salt. The plasma concentration curve for zinc orotate showed a slower absorption phase after oral

administration, when compared with that of the other two salts, but the overall results with the three salts were not significantly different.

Two of the petitioners conclude from this study that the early theory on an enhanced absorption of orotates, if compared with other salts, could not be verified, and extrapolate this to suggest that the absorption of also other orotates is at least not inferior when compared with other salts of the same nutrient cations.

3.2. Toxicological data

3.2.1. Acute toxicity

One of the petitioners informs that toxicological data on the orotates in general are not available, but quotes a study that zinc orotate has a ten-fold lower toxicity than zinc sulphate (reference not given).

Another petitioner quotes from the Registry of Toxic Effects of Chemical Substances (RTECS) that the oral LD₅₀ for orotic acid is 2 g/kg for the mouse (RTECS, 2005).

3.2.2. Sub-acute and subchronic toxicity

In one study orotic acid was administered p.o. intermittently for 29 days to cats (Van Steenhouse *et al.*, 1999; RTECS, 2005). In this study the cats were dosed according to metabolic rate and not according to mg/kg bw. The cats (n=20) were treated with 0.3 or 0.6 g orotic acid/kg metabolic body weight (BMW) for 29 days. The orotic acid was given either in a liquid suspension or a capsule. The cats receiving the high dose in suspension showed gastrointestinal symptoms, which was not the case for the cats receiving the same dose in capsules. The cats receiving the high dose in capsules developed severe pathological changes of the kidney. This effect was not seen in the cats receiving the dose in suspension and the authors suggest that because of the vomiting the cats receiving the suspension did not receive as high a systemic exposure to the substance as those receiving the capsules (Van Steenhouse *et al.*, 1999 and Dimski *et al.*, 1994).

Several studies have shown that repeated dosing with orotic acid induces fatty liver in the rat. However, the induction seems to be confined to the rat. Thus Durschlag *et al.* (1980b) demonstrate that orotic acid did not induce fatty liver in the guinea pig, mouse, hamster, pig and squirrel monkey. They showed that while mice are absorbing orotic acid faster than the rat, the absorbed orotic acid is not deposited in the liver as it is in rats, but excreted in the urine. Also the rhesus monkey did not show fatty liver after being fed 10 weeks with a basal diet containing 1 % orotic acid (Korycka-Dahl *et al.*, 1979).

3.2.3. Reproductive and developmental toxicity

No data submitted.

3.2.4. Genotoxicity

The Panel notes that no specific genotoxicity tests on the orotate sources have been submitted. One petitioner quotes from RTECS (RTECS, 2005).

Neither of the studies quoted by RTECS was primarily designed to investigate the genotoxicity of orotic acid. The study by Ito *et al.* (1988) reported that orotic acid (one of the many compounds tested) induces preneoplastic glutathione S-transferase placental-form positive foci in rats, but it is stated (without any experimental data) that it is not mutagenic.

3.2.5. Carcinogenicity

None of the petitioners submitted any data on long-term carcinogenicity, neither on orotic acid as such, nor on any of the salts.

One of the petitioners informs that in one study orotic acid augmented the metastasis of mammary tumours in mice (Chu *et al.*, 1964). In the course of the study on the effect of nucleic acid precursors on viral carcinogenesis, it was observed that an increased number of metastases developed in the lungs of orotic acid-treated mice (1 % orotic acid added to the drinking water) with spontaneous mammary tumours. Furthermore, orotic acid-treated C3H/HeN mice given injections of a cell suspension of mammary tumour cells intravenously, were found to have significantly more lung metastases than control mice which received only intravenous tumour cell injection (Chu *et al.*, 1964).

Other experimental data support the view that orotic acid has tumour-promoting properties, at least for experimental liver carcinogenesis (Laurier *et al.*, 1984; Rogers 1957b; Laconi *et al.*, 1986; Columbano *et al.*, 1982; Lin *et al.*, 1965; Parodi *et al.*, 1986; Rao *et al.*, 1986; Rao *et al.*, 1982; Rao *et al.*, 1983; Rao *et al.*, 1985; Scholz *et al.*, 1988). Anti-tumour effects were seen in rats treated with ethionine (Sidransky *et al.*, 1970), and in mice it was shown that orotic acid reduced the carcinogenetic effects of methylcholanthrene (Rogers, 1957a).

In a rat study designed to elucidate the dose and required time for orotic acid to promote hepatocarcinogenesis (Laconi *et al.*, 1993a) it was shown that 0.5 and 1 % was sufficient to exert a significant promoting effect on the selective growth of diethylnitrosamine-initiated hepatocytes, while higher concentrations of orotic acid (2 and 4 %) were only marginally more effective. It was found that 10-20 weeks of exposure to orotic acid in the diet was sufficient to induce maximum liver tumour promoting effect with 0.5 and 1 % orotic acid in the diet, while 0.1 % in the diet did not show any promoting effect within this time span. This is equivalent to 50 mg/kg bw/day, which may then be considered as the highest No Observed Effect Level (NOEL) for this effect under these test conditions.

In a study designed to determine the long-term effects of orotic acid in rats not exposed to any carcinogen, male Fischer 344 rats (130-150 g) were divided into two groups and given either a semi-synthetic basal diet or the same diet containing 1 % orotic acid. Animals from both groups were killed after 1 or 2 years of treatment. Foci of placental glutathione-S-transferase (GST 7-7) positive hepatocytes were observed in the livers of both basal diet- and orotic acid-fed rats killed after 1 year. However, they were more in number in animals receiving orotic acid (156 ± 21 versus $51 \pm 11/\text{cm}^3$). After 2 years, hepatic nodules were seen in almost all the animals given orotic acid and in around 30 % of the rats given basal diet. The nodules were of two main types: (i) a reddish-brown type, present in 85 % of rats exposed to orotic acid and in 27 % of rats given basal diet, and (ii) a greyish-white type, found in 50 % of animals fed orotic acid and in none of the animals fed basal diet. These two types of lesions were also

histologically different. Reddish-brown nodules were composed of slightly enlarged hepatocytes resembling normal surrounding tissue, while greyish-white nodules were similar in structure and are indistinguishable from hepatic nodules induced by genotoxic chemical carcinogens. The authors interpreted the results to suggest that the foci/nodules seen in orotic acid-fed rats are due to a promoting effect of orotic acid on spontaneously arising and/or diet-induced altered cells (Laconi *et al.*, 1993b).

According to Sarma, concentrations down to 0.2 % orotic acid in the diet have been shown to exert a tumour-promoting effect (Sarma *et al.*, 1986), meaning that 100 mg orotic acid/kg bw can be described as the Lowest Observed Effect Level (LOEL) for this tumour promoting effect.

While most articles on the tumour-promoting and enhancing effect of orotic acid are on liver tumours in rats, also other organs have been shown to be involved.

The effect of orotic acid on the carcinogenicity of N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine (HPOP) in rats was evaluated. A group of 5-week old Sprague-Dawley male rats were placed on a synthetic 20 % protein diet containing 1 % orotic acid. A second group was placed on a regular, orotic acid-free diet of similar composition. Approximately 2 weeks later, animals from both groups were treated with 400 mg/kg HPOP delivered continuously for 14 days via osmotic pumps implanted subcutaneously (s.c.). Rats fed the orotic acid diet were kept under this diet for 13 weeks following initiation of HPOP treatment and subsequently were placed on the regular diet for another 12 weeks, after which they were killed. In the absence of orotic acid, HPOP-treated rats developed adenomas in the kidney and lungs at incidences of 5 and 33 % respectively, while pancreas and liver were unaffected. On the other hand, rats fed the orotic acid diet and treated with HPOP developed renal mesenchymal tumours and pulmonary adenomas at incidences of 70 and 65 %, respectively. In addition, HPOP induced lesions in the pancreas of animals fed the orotic acid diet. The enhancement of the tumorigenic effectiveness of HPOP was at least partly ascribed to the effect of orotic acid treatment on the rate by which carcinogen-induced alkylation of DNA was repaired in various tissues. Accumulation of N-7-methylguanine in kidney, lung and pancreas of rats fed the orotic acid diet was 1.6, 1.9 and 2.4 times higher than in the same organs of animals fed the regular diet. Similarly, concentrations of the pre-mutagenic O⁶-methylguanine (O⁶-MeG) were 3.0, 3.1 and 2 times greater in the kidney, lung and pancreas of rats fed the orotic acid diet than in the corresponding organs of those fed the regular diet. Feeding an orotic acid diet to HPOP-treated rats did not have an effect on either the resistance of the liver to this carcinogen or on the level of O⁶-MeG accumulation in the DNA of this tissue (Kokkinakis *et al.*, 1991).

The effect of dietary orotic acid on liver/pancreas carcinogenesis induced in female Syrian hamsters by HPOP was evaluated. All animals infused with the carcinogen received the same doses. Results of the control group which received no orotic acid or carcinogen were compared with the results of: (a) hamsters treated with HPOP and fed a regular 20 % protein synthetic diet (group 1); (b) hamsters fed the orotic acid diet (1 %) for a brief time period during initiation with the carcinogen (group 2); and (c) hamsters to which orotic acid (1 % in the diet) was administered after carcinogen infusion for life (group 3).

All animals of the control group were normal at autopsy, while those in group 1 (HPOP alone) revealed the spectrum of lesions accepted as classical in the multistep hyperplasia-dysplasia-carcinoma *in situ* (CIS) sequence of carcinogenesis. Results of group 2, in light of group 1, revealed an increased incidence of the following lesions in the common pancreatic duct: dilatation, 2.5 times; flat and papillary hyperplasia, 2 times; and dysplasia (atypical hyperplasia), 12 times. No significant increase of CIS and invasive cancer in the body and tail

of the pancreas were observed; in addition, the incidence, nature, and location of pancreatic adenocarcinomas were not affected. Yet, the effect of orotic acid administered after carcinogen infusion (group 3) when compared to group 1 seemed to enhance a further increase in the incidence of practically all lesions throughout the pancreas. An obvious overall step-up incidence along the multistep hyperplasia-dysplasia-CIS-invasive cancer process in the pancreas was observed. The increase in incidence of flat, papillary, and atypical hyperplasia of the epithelium of the common pancreatic duct in group 3 was mild compared to that found in the same duct of group 2, but the increase in incidence of these same three lesions when found in the main ducts was marked: flat hyperplasia, 3-fold; papillary hyperplasia, 2.5-fold; atypical hyperplasia, 3-fold. The increase in incidence of CIS in this group was 5-fold and papillary adenocarcinomas 3-fold, when compared to 5 % found in groups 1 and 2. Hepatic malignancies (cholangiocarcinomas) occurred in 6 % of the cases in group 3 compared to none in group 2; the incidence of malignancy in the gallbladder was the same in groups 2 and 3 but three times greater than that in group 1 (Kokkinakis *et al.*, 1994).

It has been reported that tumour-promoting effects have also been seen in the rat mammary gland (Elliot *et al.*, 1989) and in the rat duodenum (Rao *et al.*, 1986), but this has so far only been reported in abstracts.

While the majority of studies on the tumour-promoting effect of orotic acid have been performed in the rat, similar effects have been seen in at least some strains of other species.

Thus, as also reported by one petitioner, Chu *et al.* (1964) describes a promoting effect also on C3H/HeN mice and in another study the tumour-promoting and enhancing effect were seen in mice on a diet leading to high concentrations of endogenous orotic acid (Vasudevan *et al.*, 1994). However, it was not possible to demonstrate a similar effect in BALB/c mice (Laconi *et al.*, 1990; Chu *et al.* 1964).

The tumour-enhancing effect of orotic acid has also been shown on preneoplastic and neoplastic lesions induced in the pancreas and liver of Syrian hamsters (Kokkinakis *et al.*, 1994).

3.2.6. Human studies

Most articles concerning administration of orotic acid and its salts are focussed on the therapeutic effects and not on possible side-effects. One petitioner quotes one study with magnesium orotate where unexpected toxicity induced by magnesium orotate was reported during treatment of congenital hypomagnesaemia (Guillard *et al.*, 2002). When the patient was 21 years of age, it was decided to substitute magnesium orotate treatment for magnesium glycerophosphate. The patient received magnesium orotate (without vitamin B₆) at a daily dose of 18 g magnesium, i.e. equivalent to that provided by magnesium glycerophosphate administration and equivalent to more than 200 g orotate. Clinical symptoms appeared after one week at the time of dose reduction (day 10). Quite unexpectedly, vomiting occurred twice on the 10th day, as well as diarrhoea and abdominal pain. Later on, constant hyperphosphatemia and a moderate rise in alanine transaminase (ALAT) led the authors to discontinue magnesium orotate and resume treatment by magnesium glycerophosphate plus vitamin B₆.

Liver samples obtained at autopsy from patients with ornithine transcarbamylase (OTC) - deficiency, an urea-cycle disorder that is associated with high levels of orotic acid biosynthesis and excretion, were analysed for nucleotide pools. As a control, liver samples from patients with a deficiency of mitochondrial carbamyl phosphate synthetase (CPS-I),

which is not associated with increased levels of orotic acidurias, were also analysed. The results show that liver tissue from OTC-deficiency patients exhibited an increased ratio of uridine nucleotides to adenosine nucleotides, while in CPS-I-deficiency patients, no such increase was noted. According to the authors, this study indicates that genetic disorders that are associated with increased loads of orotic acid exhibit abnormally high ratios of uridine to adenosine nucleotides in the liver. This type of imbalance is analogous to that seen in the liver of rats and mice exposed to orotic acid supplemented in the diet or an arginine-deficient diet under liver tumour promoting conditions. They therefore concluded that it is likely that an imbalance in nucleotide pools may have a significant role in the pathophysiology associated with these disorders.

3.2.7. Other studies

A study by Laconi *et al.* (1988) was designed to find a mechanism behind the tumour-promoting effect of orotic acid. The authors determined whether orotic acid promotes liver carcinogenesis. The experiments were designed to study the effect of orotic acid on the labelling index of isolated hepatocytes in response to epidermal growth factor. The results indicated that orotic acid added *in vitro* inhibited epidermal-growth-factor-induced labelling index of isolated hepatocytes. In addition, isolated hepatocytes from rats exposed to orotic acid under promoting conditions also exhibited a decreased response to epidermal growth factor. The authors concluded that these data suggest that orotic acid may exert its promoting effect by differentially inhibiting the response of normal hepatocytes to one or more endogenous growth stimuli while permitting the initiated hepatocytes to respond to such stimuli and grow to form hepatic nodules.

4. Discussion

Orotic acid and the ten salts in question (magnesium, zinc, calcium, chromium (III), copper (II), iron(II), manganese, potassium, sodium and choline orotate) are chemically well described and specifications have been suggested.

Orotic acid occurs mainly in milk from ruminants with highest amounts being found in animals which are deficient in arginine and UMP activity. In cow milk amounts of 20 - 100 mg/L are found and somewhat higher amounts in goat and sheep milk. Orotic acid has also been detected in infant formula in amounts of 15 - 118 mg/L, which is reflecting the range of cow milk.

No use levels are provided for sodium or choline orotate. For the other orotate sources, there is a large difference concerning the daily doses proposed by the petitioners: 1.8 - 6206 mg/day. Largest exposures to orotate from an orotate source would result from the proposed uses for calcium and magnesium orotate, providing the amount of 800 mg calcium/day (equivalent to 6.2 g orotate or about 100 mg/kg bw/day), and magnesium of 250 mg/day (equivalent to 3.2 g orotate/day or 53 mg/kg bw/day). For consumers who would consume several nutrients with orotates as source, the total anticipated combined exposure would amount over 11 g/day.

Orotic acid is an intermediate in the pyrimidine synthesis, which is required for the DNA and RNA synthesis. It is synthesised *in situ* from carbamoyl phosphate and aspartic acid through dihydroorotic acid. Orotic acid is then transformed to orotidin-5'-phosphate and further to

uridine-5'-monophosphate (Scholz, 1991). When there is insufficient capacity for detoxifying the load of ammonia presented for urea synthesis, carbamoyl phosphate leaves the mitochondria and enters the pyrimidine pathway, where orotic acid biosynthesis is stimulated; orotic acid excretion in urine then increases. Orotic acid synthesis is abnormally high in hereditary deficiencies of urea-cycle enzymes or uridine monophosphate synthase (Visek, 1992).

It has been claimed by one of the petitioners that orotic acid binds various metals in such a way that the substance is absorbed in an undissociated form through the gut and cell walls and the nutrient is only released at the site where needed. The petitioner indicated that this implies that the bioavailability would be much larger and doses required smaller than for other sources of the respective cations. This is, however, not reflected in the recommended doses and has not been documented by any of the petitioners. Furthermore, studies on the bioavailability of zinc from zinc orotate, compared with other sources, clearly show that there is no marked difference between the bioavailability from the various sources (Windisch *et al.*, 2003 and Andermann *et al.*, 1982). Two of the petitioners extrapolate that to be the case for the other salts as well.

Very little toxicological documentation has been submitted and most of it has not been on the substances considered in the petitions. Of most importance in the assessment of the safety in use of the orotates as food supplements are the series of studies showing that orotic acid has a promoting and enhancing effect on tumour formation by various known tumour initiators. Effects are primarily seen in rat liver, but also other organs and species have been shown to react in this way to orotic acid exposure in combination with tumour-initiating agents.

The usual dose to investigate this effect has been 1 % orotic acid in the diet. This corresponds to 500 mg/kg bw/day. However, also doses of 0.5 % (250 mg/kg bw/day) (Laconi *et al.*, 1993a) and 0.2 % (100 mg/kg bw/day) (Sarma *et al.*, 1986) were shown to promote formation of the chemically induced tumours. Exposure to 0.1 % orotic acid in the diet did not show any effect within the time of the experiment (10 - 20 weeks) (Laconi *et al.*, 1993a) meaning that an apparent NOAEL for the tumour-promoting effect of 50 mg/kg bw/day can be established.

Although the bulk of experiments has been performed together with a tumour-initiating agent, one study showed that rats fed a diet with 1 % orotic acid without an external initiator, developed tumours.. The authors interpreted the results to suggest that the hepatic foci/nodules seen in orotic acid-fed rats are due to a promoting effect of orotic acid on spontaneously arising and/or diet-induced altered cells (Laconi, 1993b).

Table 3 presents the margins of safety (MOS) that can be derived using the NOAEL of 50 mg/kg bw/day for tumour promotion by orotic acid and the anticipated exposure estimates calculated based on the use levels of orotate sources as proposed by the petitioners.

The Panel noted previously that recent reviews and evaluations of chromium(III) (Eastmond *et al.*, 2008; Levina and Lay, 2008) point at conflicting outcomes of genotoxicity assays and report diverging views and conclusions on the consequences of this genotoxicity issue for the ultimate safety assessment of chromium(III) and that given this situation the safety of chromium(III) might need to be re-evaluated in light of these recent reviews and evaluations. The Panel considers it not appropriate to calculate a margin of safety and conclude on the safety for a combination between chromium and a tumour promoter as long as it is not clear whether chromium is genotoxic or not.

Table 3. Margins of safety derived from the NOAEL of 50 mg/kg bw/day for tumour promotion and the anticipated exposure estimates calculated based on the use levels of orotate sources as proposed by the petitioners.

Orotate * hydrate	Exposure to orotate mg/kg bw/day (approx)	Margin of Safety (MOS)
Ca ²⁺ * 2H ₂ O	~100	~ 0.50
Cu ²⁺ * 2H ₂ O	0.25	200
Fe ²⁺ * 3H ₂ O	2	25
Mg ²⁺ * 2H ₂ O	53	0.94
Mn ²⁺ * 2H ₂ O	5	10
K ⁺ *	17	2.94
Zn ²⁺ * 2H ₂ O	4	12.5
Sum orotates	> 185	< 0.27

Some of the recommended doses could lead to intakes exceeding the NOAEL level of tumour promotion following initiation of 50 mg/kg bw/day, leading to MOS values below 1.

Furthermore, given:

- i) the nature of the adverse effect being tumour promotion,
- ii) the absence of reproduction and developmental toxicity studies of orotate, and
- iii) the absence of genotoxicity studies on orotate,

the Panel concludes that the margins of safety are inadequate and that the use of magnesium orotate, zinc orotate, calcium orotate, copper orotate, iron(II) orotate, manganese orotate, potassium orotate, sodium orotate and choline orotate added for nutritional purposes as a source of magnesium, zinc, calcium, copper, iron, manganese, potassium, sodium and choline in food supplements at the use levels proposed, is of safety concern.

The Panel notes the possible high intake of orotic acid through infant formula.

CONCLUSIONS

The present opinion deals only with the safety of particular orotate sources of ten cations (magnesium, zinc, calcium, chromium(III), copper(II), iron(II), manganese, potassium, sodium and choline) and the bioavailability of the nutrient cations from these sources, intended for use in food supplements. The safety of the nutrient cations themselves, in terms of amounts that may be consumed, is outside the remit of this Panel.

Little documentation on the bioavailability of the mineral salts of orotic acid has been submitted, but one study reveals that the bioavailability of zinc from zinc orotate is comparable to that of zinc from another organic source as well as an inorganic salt. The Panel concludes that this will probably also be the case for the bioavailability of the cations from the other orotate salts, for which no documentation has been supplied.

The Panel considers that it is not appropriate to conclude on the safety for a combination between chromium and a tumour promoter as long as it is not clear whether chromium is genotoxic or not.

The Panel concludes that in the light of the tumour-promoting effect of orotic acid in animal experimentation, the small margin of safety to this effect from foreseeable exposure, and the absence of any relevant studies on genotoxicity and of any developmental studies, the use of orotate as a source of the eight other minerals and choline at the proposed levels of use is of safety concern.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier documenting the safe and appropriate use of Calcium Orotate Dihydrate for the use in Food Supplements (Dir 2002/46/EU) included in Annex II, vitamin and mineral section. June 2005. Submitted by Pharmorgana GmbH.
2. Dossier documenting the safe and appropriate use of Zinc Orotate for the use in Food Supplements (Dir 2002/46/EU) included in Annex II, vitamin and mineral section. June 2005. Submitted by Pharmorgana GmbH.
3. Dossier documenting the safe and appropriate use of Sodium Orotate for the use in Food Supplements (Dir 2002/46/EU) included in Annex II, vitamin and mineral section. June 2005. Submitted by Pharmorgana GmbH.
4. Dossier documenting the safe and appropriate use of Manganese Orotate Dihydrate for the use in Food Supplements (Dir 2002/46/EU) included in Annex II, vitamin and mineral section. June 2005. Submitted by Pharmorgana GmbH.
5. Dossier documenting the safe and appropriate use of Magnesium Orotate Dihydrate for the use in Food Supplements (Dir 2002/46/EU) included in Annex II, vitamin and mineral section. June 2005. Submitted by Pharmorgana GmbH.
6. Dossier documenting the safe and appropriate use of Potassium Orotate for the use in Food Supplements (Dir 2002/46/EU) included in Annex II, vitamin and mineral section. June 2005. Submitted by Pharmorgana GmbH.
7. Dossier documenting the safe and appropriate use of Ferrous Orotate Trihydrate for the use in Food Supplements (Dir 2002/46/EU) included in Annex II, vitamin and mineral section. June 2005. Submitted by Pharmorgana GmbH.
8. Dossier documenting the safe and appropriate use of Cupric Orotate Dihydrate for the use in Food Supplements (Dir 2002/46/EU) included in Annex II, vitamin and mineral section. June 2005. Submitted by Pharmorgana GmbH.
9. Dossier documenting the safe and appropriate use of Cholin Orotate for the use in Food Supplements (Dir 2002/46/EU) included in Annex II, vitamin and mineral section. June 2005. Submitted by Pharmorgana GmbH.
10. Dossier documenting the safe and appropriate use of Chrome Orotate Hexahydrate for the use in Food Supplements (Dir 2002/46/EU) included in Annex II, vitamin and mineral section. June 2005. Submitted by Pharmorgana GmbH.
11. Orotic acid and its salts: zinc orotate dihydrate, magnesium orotate dihydrate, calcium orotate dihydrate, potassium orotate, ferrous orotate trihydrate, copper orotate dihydrate,

- chromium orotate tetrahydrate and manganese orotate dihydrate. July 2001. Submitted by Lyomark Pharma GmbH.
12. Dossier on Magnesium Orotate Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. May 2005. Submitted by the Health Food Manufacturers Association, UK.
 13. Dossier on Zinc Orotate Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. May 2005. Submitted by Health Food Manufacturers Association, UK.
 14. Dossier on Calcium Orotate Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. May 2005. Submitted by the Health Food Manufacturers Association, UK.
 15. Dossier on Manganese Orotate Proposed for Addition to Annex II of Directive 2002/46/EC of the European Parliament and of the Council Relating to Food Supplements. June 2005. Submitted by the Health Food Manufacturers Association, UK.

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GLOSSARY/ABBREVIATIONS

ALAT	Alanine transaminase
ANS Panel	Scientific Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstract Service
CIS	Carcinoma <i>in situ</i>
DNA	Deoxyribonucleic Acid
EC	European Commission
EFSA	European Food Safety Authority
EVM	UK Expert group on Vitamins and Minerals
HPLC	High-Performance Liquid Chromatography
HPOP	N-nitroso(2-hydroxypropyl)(2-oxopropyl)amine
LD ₅₀	Lethal Dose, 50% i.e. dose that causes death among 50 % of treated animals
LOAEL	Lowest Observed Adverse Effect Level
LOEL	Lowest Observed Effect Level
MOS	Margins Of Safety
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
O ⁶ -MeG	O ⁶ -methylguanine
OTC	Ornithine Transcarbamylase
RNA	Ribonucleic acid
PRI	Population Reference Intake
RTECS	Registry of Toxic Effects of Chemical Substances
SCF	Scientific Committee on Food
UL	Tolerable Upper Intake Level
UMP	Uridine-5'-monophosphate
WHO	World Health Organization