Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission related to Calcium, iron, magnesium, potassium and zinc L-pidolate as sources for calcium, iron, magnesium, potassium and zinc added for nutritional purposes to food supplements and to foods intended for particular nutritional uses


Adopted on 20 March 2007 by written procedure

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods (AFC Panel) has been asked to evaluate the safety and bioavailability of calcium, iron, magnesium, potassium and zinc L-pidolate as sources for calcium, iron, magnesium, potassium and zinc when added for nutritional purposes in foods intended for particular nutritional uses and in food supplements. The safety of these nutrient cations themselves, calcium, iron, magnesium, potassium and zinc, in terms of amounts that may be consumed, is outside the remit of this Panel.

From data provided it can be concluded that calcium, iron, magnesium, potassium and zinc are absorbed from their L-pidolic acid salts. Their bioavailability is comparable to that from other water-soluble and dissociable calcium, iron, magnesium, potassium and zinc salts permitted to be used in food supplements and foods intended for particular nutritional uses.

The safety evaluation of the L-pidolatic acid salts was based on the natural occurrence of L-pidolic acid in foods, its endogenous formation, the limited toxicological data available and on the kinetics and metabolic pathways of L-pidolic acid. On this basis,
the Panel concluded that the use of calcium, iron, magnesium, potassium and zinc L-pidolic acid salts as sources for calcium, iron, magnesium, potassium and zinc added for nutritional purposes to food supplements and of calcium, iron, magnesium and zinc L-pidolic acid salts as sources for calcium, iron, magnesium and zinc added for nutritional purposes to food intended for particular nutritional uses, respectively, is of no safety concern at the maximum use levels indicated, which, under the assumption that all the nutrients are ingested as their pidolic acid salts at the same time, would result in intake of L-pidolic acid of 3 g/day.

KEY WORDS

BACKGROUND
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 requests for the evaluation of calcium, iron, magnesium, potassium and zinc L-pidolate added for nutritional purposes to food supplements and to foods intended for particular nutritional uses. The relevant Community legislative measures are:
• Commission Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutrition.

TERMS OF REFERENCE
In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002, the European Commission asks the European Food Safety Authority to provide scientific opinions, based on its consideration of the safety and bioavailability of calcium L-pidolate, iron L-pidolate, magnesium L-pidolate, potassium L-pidolate and zinc L-pidolate added for nutritional purposes in food supplements and in foods intended for particular nutritional uses.

ASSESSMENT
The present opinion deals only with the safety of certain L-pidolic acid salts as sources of calcium, iron, magnesium, potassium and zinc and with the bioavailability of the nutrient cations from these sources, intended to be used in foods for particular nutritional uses and in food supplements. The safety of these nutrient cations
themselves, calcium, iron, magnesium, potassium and zinc, in terms of amounts that may be consumed, is outside the remit of this Panel.

**Chemistry**

Calcium, iron, magnesium, potassium and zinc L-pidolates are water-soluble salts of L-pidolic acid (2-pyrrolidone-5-carboxylic acid, pyroglutamic acid, 5-oxoproline) with the chemical formula Ca\((C_5H_6NO_3)_2\), Fe\((C_5H_6NO_3)_2\), Mg\((C_5H_6NO_3)_2\), KC\(_3\)H\(_6\)NO\(_3\) and Zn\((C_5H_6NO_3)_2\) respectively. According to the applicant, they are L-pidolates with the CAS Registration Numbers 31377-05-6, 69916-59-2, 62003-27-4, 4810-50-8 and 15454-75-8, respectively. Figure 1 shows the structure of the L-pidolic acid anion (CAS 98-79-3):

![Figure 1: L-pidolic acid anion](image)

The applicant claims that the water solubility of the L-pidolates is high. However the applicant has only provided the dissociation constant for calcium L-pidolate: K = 1.3 \times 10^{-3}. This dissociation constant is higher than those of other sources of calcium such as calcium acetate and calcium propionate.

**Specifications**

According to the applicant, the L-pidolates are respectively more than 97.5% (calcium L-pidolate), 96% (iron L-pidolate), 97.7% (magnesium L-pidolate), 98% (potassium L-pidolate) and 96.6% (zinc L-pidolate) pure on an anhydrous basis. They contain 0.6 - 4% glutamic acid and as secondary reaction products up to 1% N-5(oxo-2-pyrrolidinyl) carbonyl glutamic acid and up to 0.5% N-5(oxo-2-pyrrolidinyl) carbonyl glutamyl glutamic acid.

The following metal impurities have been indicated:

- Calcium L-pidolate: up to 50 mg iron and 20 mg other heavy metals/kg;
- Iron L-pidolate: no data;
- Magnesium L-pidolate: up to 200 mg iron, 20 mg other heavy metals and 2 mg arsenic/kg;
- Potassium L-pidolate: up to 20 mg heavy metals and 2 mg arsenic/kg;
- Zinc L-pidolate: up to 200 mg iron and 40 mg lead/kg.

**Manufacturing process**

The manufacturing process has been adequately described.
Methods of analysis in food

Information was not provided by the applicant.

Reaction and fate in foods to which the source is added

Information was not provided by the applicant. The Panel noted that since L-pidolate is a normal constituent of several foods, any reaction products would also be expected to be present as normal food constituents.

Case of need and intended use

Calcium, iron, magnesium, potassium and zinc L-pidolate are intended to be used in food supplements and as sources for calcium, iron, magnesium, potassium and zinc when added for nutritional purposes in foods intended for particular nutritional uses.

Exposure

As regards the daily amounts recommended by the applicant, the amount of calcium L-pidolate ingested as food supplements may vary between 44 and 1200 mg/day, equivalent to 38 to 1046 mg L-pidolic acid/day. The corresponding figures for the other L-pidolates given by the applicant are: 24 -540 mg iron L-pidolate/day, equivalent to 19 - 432 mg L-pidolic acid/day, 18 - 1500 mg magnesium L-pidolate/day, equivalent to 16 -1379 mg L-pidolic acid/day, 198 - 514 mg potassium L-pidolate/day, equivalent to 153 - 397 mg L-pidolic acid/day and 6 - 80 mg zinc L-pidolate/day, equivalent to 5 -59 mg L-pidolic acid/day. In the case that all these nutrients are ingested as their L-pidolic acid salts in supplements at the same time and at the maximum daily amounts recommended by the applicant, the total intake of L-pidolic acid from supplements could amount up to about 3 g/day.

Because of the natural occurrence in numerous plants, there is also an intake of L-pidolic acid from food, e.g. vegetables, honey (Geoffroy, 1991), fruit juices and wine (Pfeiffer and Orben, 2000). Highest concentrations found by Pfeiffer and Orben (2000) were 0.61 g/l in wine and 1.55 g/l in tomato juice. Estimations of exposure from these sources are not available.

Existing authorisations and evaluations

According to the applicant, L-pidolates have been used in the pharmaceutical industry for a number of years. Magnesium L-pidolate is listed in the European Pharmacopoeia (European Pharmacopoeia, 2005). For calcium, magnesium and zinc, the following Tolerable Upper Intake Levels have been established by the SCF: 2500 mg calcium/day for adults from all sources (SCF, 2003a), 250 mg magnesium/day for readily dissociable magnesium salts and compounds like magnesium oxide not including magnesium normally present in foods and beverages for adults and children from 4 years on (SCF, 2001), and 25 mg zinc/day for adults (SCF, 2003b). For iron and potassium, the available data were insufficient to establish a Tolerable Upper Intake Level (EFSA, 2004 and 2005).
Biological and toxicological data

Bioavailability

A number of studies with animals, healthy persons and patients provided in the dossiers submitted to EFSA show that calcium, iron, magnesium, potassium and zinc are absorbed after ingestion of their L-pidolates. The bioavailability of these cations is expected to be similar to that from other water-soluble and dissociable salts of these metals.

Toxicological data

Metabolism and kinetics

L-pidolic acid is a cyclisation product and metabolite of glutamic acid and plays an important role in the endogenous $\gamma$-glutamyl cycle. It is formed from glutamic acid or $\gamma$-glutamyl amino acids by $\gamma$-glutamylcyclotransferase and retransformed to glutamic acid by 5-oxo-prolinase (Geoffroy, 1991). It has been reported to be present in human plasma (Wolfersberger and Tabachnik, 1973), urine, bones and other tissue (Geoffroy, 1991).

Values for the concentration of L-pidolic acid in human blood serum and cerebrospinal fluid were reported to be about 0.02 and 0.06 mmol/l, respectively (Wilk and Orlowski, 1973). Higher values of about 0.2 and 0.3 mmol/l in plasma from man and guinea pig reported by Wolfersberger and Tabachnik (1973) were questioned by other authors (van der Werf and Meister, 1975). The normal urinary excretion in man was reported to be probably in the range of about 0.5-5 mg/day (van der Werf and Meister, 1975).

In a rare metabolic disorder, the pyroglutamic acidemia, L-pidolate is accumulated in blood and tissues and excreted in urine in large amounts. In one of such patients, a concentration of about 50 mg L-pidolate/100 ml serum (3.9 mmol/l) and an urinary excretion of about 35 g L-pidolate in 24 hours were observed (Eldjarn et al., 1973). The primary defect in patients with this disorder is not related to L-pidolates, but is a deficiency of glutathione synthetase. Such a deficiency causes a lack of intracellular glutathione and an increased production of $\gamma$-glutamyl-cysteine, giving rise to an abnormal rate of L-pidolate formation as secondary effect. Cerebral lesions, which are another secondary effect, are not due to the accumulation of L-pidolate but are attributed to a lack of protection by glutathione against oxidative damage (Skullerud et al., 1980; Marstein et al., 1981).

In adult mice, oral administration of 0.5 g L-pidolic acid/kg bw increased plasma levels of L-pidolic acid 56 times from 0.033 to 1.842 mmol/l after 30 minutes and in the brain 4.5 times from 0.17 to 0.76 mmol/l after 60 minutes. The levels of L-glutamic acid were only slightly elevated in plasma and slightly decreased in brain. In 10-day old mice, the increase of the L-pidolic acid level was even more evident with a 69-fold increase in plasma accompanied by a 5.7-fold increase in brain (Caccia et al.,
1983). These results show that L-pidolic acid can pass the blood-brain barrier (Geoffroy, 1991).

Studies with [¹⁴C]- and [⁴⁵Ca]-labelled calcium L-pidolate administered orally to adult rats revealed the highest incorporation of ⁴⁵Ca in the bone and of ¹⁴C from the L-pidolate in liver and bone. In the only young rat tested, 85% of the ¹⁴C-radioactivity in bones was found in the neutral amino acids. Only a small fraction of aspartic and glutamic acid was labelled. An important fraction of radioactivity was found in proline and hydroxyproline (Phan-Dinh-Tuy and Moczar, 1978; Moczar et al., 1979).

**Acute toxicity**

The oral LD₅₀ of L-pidolates are reported to be greater than 10 g calcium-L-pidolate/kg bw (rats and guinea pigs), greater than 2 g iron-L-pidolate/kg bw (rats), greater than 15 and 11.8 g magnesium-L-pidolate/kg bw (rats and mice, respectively), greater than 2 g potassium-L-pidolate/kg bw (rats) and greater than 2 g zinc-L-pidolate/kg bw (rats).

**Subchronic toxicity**

According to an unpublished study in which 10 male and 5 female Wistar rats received 1 g calcium L-pidolate/kg bw/day for 100 days by gastric tube, no significant difference was observed between treated and control group regarding weight gain, behaviour, haematology and microscopic/histological examination of stomach, small intestine, liver, lung, kidneys, testis and ovary was observed (Gazave, 1972).

In another unpublished study, four groups of rats received 0, 50, 250 and 1000 mg magnesium L-pidolate/kg bw/day orally for 3 months (Dossier on magnesium L-pidolate, 2005). As regards changes in body weight, behaviour, haematology and microscopic and histological examination of some organs, no significant difference was observed between the treated groups and the control group (details not given).

**Effects on the central nervous system**

Because glutamic acid and certain structurally or metabolically related compounds are known to exert neurotoxic effects in infant mice, the potential of L-pidolic acid as a metabolite of glutamic acid to induce brain lesions was examined. L-pidolic acid was given orally to groups of 12 infant mice, 10 days old, at single doses of 0, 2 and 4 g/kg bw by intubation of a 1% aqueous solution. Six hours after administration, animals were killed and brains enucleated. No significant differences in the number of necrotic neurons in the arcuate nucleus of the hypothalamus were observed between treated animals and the control group (Caccia et al., 1983).

**Hypersensitivity**

A case of selective hypersensitivity was reported in a 31-year-old woman, who had developed two episodes of generalised urticaria after intravenous injection of magnesium L-pidolate because of suspicion of tetany. Following single-blind placebo-controlled oral challenges with magnesium and calcium L-pidolate, the
patient developed generalised urticaria again, whereas no adverse effect occurred in challenge tests with magnesium chloride and sodium glutamate. A specific allergic mechanism could not be demonstrated since skin tests were negative (Raison-Peyron et al., 2001).

Genotoxicity

Zinc-L-pidolate did not show mutagenic activity in bacterial reverse mutation tests on \textit{Salmonella typhimurium} TA 1535,1537, 98, 100 and 102 and \textit{Escherichia coli} WP2 uvrA with and without metabolic activation by a liver microsomal fraction (S9) of rats induced with Aroclor 1254 (Centre International Toxicol., 1998).

DISCUSSION

L-pidolic acid occurs in numerous plants and is a natural constituent of a number of foods. It is formed in human metabolism from glutamic acid and can be metabolised after oral intake to glutamic acid.

Studies on the kinetics in humans are not available. Nevertheless, it can be expected from data in mice, that orally ingested L-pidolates are absorbed and at certain doses will result in increased plasma levels of L-pidolic acid. The oral dose at which considerable increases in plasma L-pidolic acid level were observed in mice, was as high as 0.5g/kg bw (Caccia et al., 1983), equivalent to 30 g for a 60 kg human adult, whereas, given the daily amounts recommended by the applicant, the maximum total intake of L-pidolates from supplements is estimated by the Panel to be only up to 3 g/day. The Panel noted that the the normal glutamate level in the plasma of mice was only slightly elevated and was even slightly decreased in brain.

The neurotoxic effects of high doses of glutamate in infant mice raised the question whether L-pidolates as structurally related compounds have a neurotoxic potential as well. In a study on the potential neurotoxicity of L-pidolic acid in 10 days old mice, however, no lesions in the brain were observed up to a dose of 4 g/kg bw, equivalent to 240 g for a 60 kg human adult (Caccia et al., 1983).

CONCLUSION

From the data provided it can be concluded that calcium, iron, magnesium, potassium and zinc are absorbed from L-pidolates. Their bioavailability is comparable to that from other water-soluble and dissociable calcium, iron, magnesium, potassium and zinc salts permitted to be used in food supplements and foods intended for particular nutritional uses.

The safety evaluation of the L-pidolates was based on the natural occurrence of L-pidolic acid in foods, its endogenous formation, the limited toxicological data available and on the kinetics and metabolic pathways of L-pidolic acid. On this basis, the Panel concluded that the use of calcium, iron, magnesium, potassium and zinc L-pidolic acid salts as sources for calcium, iron, magnesium, potassium and zinc added
for nutritional purposes to food supplements and of calcium, iron, magnesium and zinc L-pidolic acid salts as sources for calcium, iron, magnesium and zinc added for nutritional purposes to food intended for particular nutritional uses, respectively is of no safety concern at the maximum use levels indicated, which, under the assumption that all the nutrients are ingested as their pidolic acid salts at the same time, would result in intake of L-pidolic acid of 3 g/day.

The Panel noted the inconsistency and the lack of information on specific elements in the specifications of heavy metals in the different dossiers as well as the high levels of lead in the specification of zinc L-pidolate in comparison to other sources.

The Panel also noted that data for the content of cadmium in zinc-L-pidolate was not given.

DOCUMENTATION PROVIDED TO EFSA

Dossier on Calcium L-pidolate regarding the use in food supplements. Submitted by UCIP-SOLABIA Group (May 18, 2005).

Dossier on Calcium L-pidolate regarding the use in food for particular nutritional uses. Submitted by UCIP-SOLABIA Group (September 8, 2005).

Dossier on Iron L-pidolate regarding the use in food supplements. Submitted by UCIP-SOLABIA Group (May 23, 2005).

Dossier on Iron L-pidolate regarding the use in food for particular nutritional uses. Submitted by UCIP-SOLABIA Group (September 9, 2005).

Dossier on Magnesium L-pidolate regarding the use in food supplements. Submitted by UCIP-SOLABIA Group (May 12, 2005).

Dossier on Magnesium L-pidolate regarding the use in food for particular nutritional uses. Submitted by UCIP-SOLABIA Group (September 8, 2005).

Dossier on Potassium L-pidolate regarding the use in food supplements and in food for particular nutritional uses. Submitted by UCIB-SOLABIA Group (March 2006).

Dossier on Zinc L-pidolate regarding the use in food supplements. Submitted by UCIP-SOLABIA Group (May 18, 2005).

Dossier on Zinc L-pidolate regarding the use in food for particular nutritional uses. Submitted by UCIP-SOLABIA Group (September 8, 2005).

REFERENCES


European Pharmacopoeia 5th Edition (2005), Council of Europe (COE) - European Directorate for the Quality of Medicines


Calcium, iron, magnesium, potassium and zinc L-pidolate

http://ec.europa.eu/food/fs/sc/scf/out105 en.pdf

http://ec.europa.eu/food/fs/sc/scf/out194 en.pdf

http://ec.europa.eu/food/fs/sc/scf/out177 en.pdf


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