European Food Safety Authority

The EFSA Journal (2008) 760, 1-13

Opinion on Pyridoxal 5'-phosphate as a source for vitamin B_6 added for nutritional purposes in food supplements [1]

Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food

(Question No EFSA-Q-2006-228 and EFSA-Q-2008-026)

Adopted on 8 July 2008

PANEL MEMBERS

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SUMMARY

Following a request from the Commission, the 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 pyridoxal 5'-phosphate as a source for vitamin B_6 when added for nutritional purposes in food supplements.

Vitamin B_6 has been evaluated by the Scientific Committee on Food in 2000 which defined tolerable upper intake levels of vitamin B_6. The tolerable upper intake level amounted to 25 mg per day for adults, with tolerable upper intake levels for children defined based on body weight differences compared to adults.

Vitamin B_6 is the collective term for a family of chemical substances that are structurally related and includes pyridoxine, pyridoxine 5'-phosphate, pyridoxal, pyridoxal 5'-phosphate, pyridoxamine, and pyridoxamine 5'-phosphate which can be converted into one another.

The present opinion deals only with the safety and bioavailability of pyridoxal 5'-phosphate as a particular source of vitamin B_6, intended for the general population, to be added for nutritional purposes to food supplements. The safety of vitamin B_6, in terms of amounts that may be consumed, is outside the remit of this Panel.

[1] For citation purposes: Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Foods (AFC) on a request from the Commission on Pyridoxal-5'-phosphate as a source for vitamin B_6 added for nutritional purposes in food supplements. *The EFSA Journal* (2008) 760, 1-13.

The toxicity of vitamin B_6 has been evaluated in several opinions. The principal toxicity of concern associated with excess intakes of vitamin B_6 (as pyridoxine hydrochloride) is neuronal damage and sensory and motor effects. None of these effects have been specifically linked to the pyridoxal 5'-phosphate form of the vitamin.

Pyridoxal 5'-phosphate did not cause clinical signs or lesions similar to those produced by pyridoxine even when injected in maximum tolerated doses.

The Panel considers that bioavailability and safety of pyridoxal 5'-phosphate will not be significantly different from those of other forms of vitamin B_6. Therefore and because of the fact that pyridoxal 5'-phosphate is one of the vitamin B_6 vitamers the safe upper use levels defined for vitamin B_6 can be used for judging the safety of pyridoxal 5'-phosphate.

The Panel concluded that the use of pyridoxal 5'-phosphate as a source for vitamin B_6 in food supplements intended for the general population would be of no safety concern if use levels were in compliance with defined upper safe use levels.

However, the Panel is concerned that the use levels of pyridoxal 5'-phosphate proposed by the petitioners are 50 and 90 mg/day and are substantially higher than the tolerable upper intake levels defined by the SCF in 2000 of 25 mg/day for adults and 5-20 mg for children depending on their body weight.

The Panel noted that the specifications of pyridoxal 5'-phosphate as commercialised should comply with the specifications for vitamin B_6 and its derivatives used as food additives.

Key words:

Food supplements, pyridoxal 5'-phosphate, CAS N^o 54-47-7, vitamin B_6

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BACKGROUND AS PROVIDED BY THE 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 pyridoxal 5'-phosphate added for nutritional purposes to food supplements. The relevant Community legislative measure is:

- 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 supplements[2].

TERMS OF REFERENCE AS PROVIDED BY THE 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 pyridoxal 5'-phosphate added for nutritional purposes in food supplements.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion:

F. Aguilar, D. Boskou, D. Gott, S. Grilli, R. Guertler, K. Hulshof, J.C. Larsen, J.C. Leblanc, C. Leclercq, A. Mortensen, D. Parent-Massin, I. Pratt, I. Rietjens, P. Tobback, G. Speijers, F. Toldra.

[2] OJ L 183, 12.7.2002, p.51.

ASSESSMENT

1. Introduction

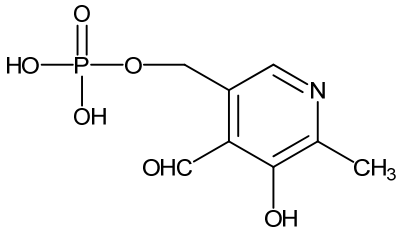
The present opinion deals only with the safety and bioavailability of a particular source of vitamin B_6 intended for the general population, to be used in food supplements. The safety of vitamin B_6, in terms of amounts that may be consumed, is outside the remit of this Panel.

2. Technical data

2.1. Chemistry

Vitamin B_6 is the collective term for a family of chemical substances that are structurally related and includes pyridoxine, pyridoxine 5'-phosphate, pyridoxal, pyridoxal 5'-phosphate, pyridoxamine, and pyridoxamine 5'-phosphate which can be converted into one another.

Pyridoxal 5'-phosphate is the metabolically active from of vitamin B_6. Pyridoxal 5'-phosphate is a synonym for (4-formyl-5-hydroxy-6-methylpyridin-3-yl)methoxyphosphonic acid (IUPAC name). Its CAS registry number is 54-47-7 and the petitioner mentioned 29 different synonyms. The molecular formula proposed by the petitioner corresponds to the monohydrate form, $C_8H_{10}NO_6P \cdot H_2O$, and the corresponding molecular mass is 265.16 Dalton. The molecular structure for the anhydrous form is presented below:



2.2. Specifications

The petitioners provide the following specifications for pyridoxal 5'-phosphate: purity not less than 98.0%. Pale yellowish to light yellow crystalline powder, clear, light yellow to yellow in water solution. This water solution should have a pH between 3.0 and 3.5 upon dissolving 0.1 g pyridoxal 5'-phosphate in 200 ml water. Limits were provided by the petitioners for heavy metals (not more than 10 mg/kg), arsenic (not more than 2 mg/kg), lead (not more than 5 mg/kg), mercury (not more than 1 mg/kg), free phosphoric acid (0.5%) and loss on drying (0.5%).

2.3. Manufacturing Process

The manufacturing process described by both petitioners consists of several steps and is adequately described.

2.4. Methods of analysis in food

There are several HPLC methods for analysis of vitamin B_6 vitamers including pyridoxal 5'-phosphate, pyridoxal, pyridoxamine 5'-phosphate, pyridoxamine, pyridoxine, pyridoxine 5'-phosphate and the degradation product 4-pyridoxic acid, in biological and food matrices (Bisp *et al.*, 2002; Rybak and Pfeiffer, 2004; Gatti and Gioia, 2005).

There are also enzymatic methods for analysis of pyridoxal 5'-phosphate (Fu *et al.*, 2001; Han *et al.*, 2002) as well as microbiological methods in which the growth of specific microorganisms is used to detect and quantify the concentration of the compound and which make it possible to differentiate between the three vitamers of vitamin B_6 by suitable selection of the micro-organisms (Van den Berg, 1996; Bognar and Ollilainen, 1997; Kall, 2003).

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

The petitioners indicate that pyridoxal 5'-phosphate is stable in the food supplements to which it is added. One petitioner provides results from an experiment showing the stability of pyridoxal 5'-phosphate packed in 1 kg tin drums for at least 3 years by evaluating appearance, odour, loss on drying and percentage of pyridoxal 5'-phosphate at initial, 0.5, 1.0., 1.5, 2, 2.5 and 3 years storage.

2.6. Exposure

One petitioner indicates that the proposed level of use of pyridoxal 5'-phosphate is up to 50 mg/day in a food supplement. This would amount to 0.83 mg/kg bw/day for a 60 kg person.

The other petitioner indicates that pyridoxal 5'-phosphate is to be used up to the equivalent of 90 mg/day of vitamin B_6. For a 60 kg adult this would amount to 1.5 mg/kg bw/day.

Previously the Scientific Committee on Food (SCF, 2000) provided estimates on vitamin B_6 intakes in EU countries (Table 1). It was reported that the highest mean and 97.5th percentile intake from dietary sources amount to respectively 3.2 and 5.9 mg vitamin B_6/person/day, whereas due to the use of supplements the intake at the 97.5th percentile may increase up to 30.3 mg vitamin B_6/person/day amounting to about 0.5 mg vitamin B_6/kg bw/day for a 60 kg person.

Table 1. **Vitamin B_6 intake in EU countries from individual based surveys (mg/day) (SCF, 2000)**

	n	Method	Supplements	Mean	97.5 %
Austria [a]	2488	24 h recall	?	1.68	3.43
Ireland [b]	662	7-day record	-	3.2	5.9
	717		-	2.4	4.1
	662		+	3.5	7.6
	717		+	3.6	30.3
Italy [c]	2734	7-day record	+	2.0	3.3
The Netherlands [d]	5958	2-day record	-	1.59	3.01
UK [e]	1087	7-day record	-	2.48	4.47
	1110		-	1.57	2.62
	1087		+	2.68	5.35
	1110		+	2.84	10.46

[a] Elmadfa *et al.*, 1998.
[b] IUNA, 2000.
[c] Turrini, 1994-1996, INRAN.
[d] Hulshof and Kruizinga, 1999.
[e] HMSO, 1990

Vitamin B_6 is found in chicken, fish, liver, kidney, pork, eggs, milk, wheat germ and brewer's yeast. Other sources include brown rice, soybeans, oats, whole-wheat grains, peanuts and walnuts at levels up to 25 mg/kg food. The major forms of vitamin B_6 from animal food products are pyridoxal and pyridoxal 5'-phosphate, whereas pyridoxine, pyridoxine 5'-phosphate, pyridoxamine and pyridoxamine 5'-phosphate are the main forms obtained from plants (Midttun *et al.*, 2007). Long-term storage, canning, roasting or stewing of meat and food processing techniques can destroy pyridoxine. Boiling reduces the pyridoxine content of food because of losses into the water (EVM, 2003).

As a licensed medicine, pyridoxine hydrochloride is also present in various multivitamin preparations for the prevention and treatment of vitamin deficiencies (maximum daily doses of 0.5 – 30 mg). Products containing pyridoxine (maximum daily dose of 10 mg) combined with other constituents are available (EVM, 2003).

2.7. Information on existing authorisations and evaluations

In 1993 the EC Scientific Committee on Food stated that intakes of 50 mg vitamin B_6 per day must be regarded as harmful. A tolerable upper intake level of 25 mg/day for adults was established by the SCF (SCF, 2000). The tolerable upper intake levels for children were based on body weight differences compared to adults (SCF, 2000). Table 2 presents the tolerable upper intake levels defined by the SCF.

Table 2. **Tolerable Upper Intake Levels (UL) for vitamin B_6 in mg/day as defined by the SCF (SCF, 2000) for adults and children of different age.**

Age (years)	Tolerable Upper Intake Level (UL) (mg per day)
1-3	5
4-6	7
7-10	10
11-14	15
15-17	20
adults	25

The Expert Group on Vitamins and Minerals of the Food Standard Agency UK (UK EVM) derived a safe upper level (SUL) of 10 mg/day for a 60 kg adult (EVM, 2003).

The tolerable upper limit has been set by the US FDA at 100 mg/day (FNB 1998, 2000).

The Council for Responsible Nutrition (USA) (CRN) identified 100 mg as the upper level for supplements (ULS) for pyridoxine (Council for Responsible Nutrition, 2004).

The differences originate from different views on what is taken as the appropriate critical study and NOAEL or LOAEL and the safety factors used (Hathcock, 2004).

3. Biological and toxicological data

3.1. Bioavailability of vitamin B_6 from its pyridoxal 5'-phosphate source

There are three forms of vitamin B_6 present in the plasma, namely pyridoxal, pyridoxine and 4-pyridoxic acid which can be phosphorylated to the corresponding 5'-phosphates. Pyridoxal 5'-phosphate is the metabolically active phosphorylated form of vitamin B_6. The phosphorylation is catalyzed by pyridoxal kinase.

The phosphate forms of vitamin B_6 in food are dephosphorylated in the intestinal lumen, and pyridoxine, pyridoxal and pyridoxamine are taken up from the small intestine by an energy dependent process. All three are converted to pyridoxal 5'-phosphate in the tissues (EVM, 2003).

The petitioners provided no data on human plasma levels of vitamin B_6 following oral intake of pyridoxal 5'-phosphate.

As only dephosphorylated vitamers can be transported into the cells (Coburn *et al*., 2003) the bioavailability of intact pyridoxal 5'-phosphate upon oral intake would be low. Bioavailability of vitamin B_6 from pyridoxal 5'-phosphate requires hydrolysis of the phosphate group before absorption through the intestinal layer may occur.

3.2. Toxicological data

The toxicity of vitamin B_6 has been evaluated in several opinions (SCF, 2000; Hathcock, 2004, EVM, 2003). The critical effect associated with excess intakes of vitamin B_6 (as pyridoxine hydrochloride) is neuronal damage and sensory and motor effects. None of these effects have been specifically linked to the pyridoxal 5'-phosphate form of the vitamin.

Pyridoxal 5'-phosphate did not cause clinical signs or lesions similar to those produced by pyridoxine even when injected in maximum tolerated doses.

Several agencies have defined tolerable upper limits for the intake of vitamin B_6 (see under Information on existing authorisations and evaluations).

Given the fact that pyridoxal 5'-phosphate is one of the vitamin B_6 forms, the safe upper use levels defined for vitamin B_6 can be used for judging the safety of pyridoxal 5'-phosphate.

4. Discussion

Only dephosphorylated B_6 vitamers can be transported into the cells (Coburn *et al*., 2003) indicating that the bioavailability of intact pyridoxal 5'-phosphate upon oral intake would be low. Removal of the phosphate group is a function of alkaline phosphatases, which are encoded by at least four different genes producing tissue non specific, intestinal, placental and germ cell alkaline phosphatases (van Hoof and De Broe, 1994). The tissue non-specific alkaline phosphatase is the one predominantly involved in vitamin B_6 metabolism and it is located anchored to the ectoplasmic side of the plasma membrane.

Given that the bioavailability of pyridoxal 5'-phosphate requires hydrolysis of the phosphate group before absorption through the intestinal layer may occur, one can conclude that the bioavailability of vitamin B_6 from pyridoxal 5'-phosphate will be lower than or at best similar to the bioavailability of pyridoxine.

One petitioner indicates that the proposed level of use of pyridoxal 5'-phosphate is up to 50 mg/day in a food supplement. This would amount to 0.83 mg/kg bw/day for a 60 kg person.

The other petitioner indicates that pyridoxal 5'-phosphate is to be used up to the equivalent of 90 mg/day of vitamin B_6. For a 60 kg adult this would amount to 1.5 mg/kg bw/day.

The toxicity of vitamin B_6 has been evaluated in several opinions (SCF, 2000; Hathcock, 2004, EVM, 2003). The critical effect associated with excess intakes of vitamin B_6 (as pyridoxine hydrochloride) is neuronal damage and sensory and motor effects.

Several agencies have defined tolerable upper limits for the intake of vitamin B_6.

The Expert Group on Vitamins and Minerals of the Food Standard Agency UK (UK EVM) derived a safe upper level (SUL) of 10 mg/day for a 60 kg adult (EVM, 2003).

In 1993 the EC Scientific Committee on Food stated that intakes of 50 mg vitamin B6 per day must be regarded as harmful. A tolerable upper intake level of 25 mg/day for adults was established by the SCF (SCF, 2000). The tolerable upper intake levels for children were based on body weight differences compared to adults and amounted to values from 5-20 mg/day (SCF, 2000).

The Panel considers that bioavailability and safety of pyridoxal 5'-phosphate will not be significantly different from those of other forms of vitamin B_6. Therefore and because of the fact that pyridoxal 5'-phosphate is one of the vitamin B_6 vitamers the safe upper use levels defined for vitamin B_6 can be used for judging the safety of pyridoxal 5'-phosphate.

The Panel notes that the tolerable upper intake levels defined by the SCF for vitamin B_6 of 25 mg/day for adults and 5-20 mg/day for children depending on their body weight, is lower than the use levels proposed for pyridoxal 5'-phosphate by the two petitioners which amount to 50 and 90 mg/day, respectively.

CONCLUSION

The present opinion deals only with the safety and bioavailability of pyridoxal 5'-phosphate as a particular source of vitamin B_6 intended for the general population, to be used in food supplements. The safety of vitamin B_6 itself, in terms of amounts that may be consumed, is outside the remit of this Panel.

The Panel considers that bioavailability and safety of pyridoxal 5'-phosphate will not be significantly different from those of other phosphorylated forms of vitamin B_6. Therefore and because of the fact that pyridoxal 5'-phosphate is one of the vitamin B_6 vitamers the safe upper use levels defined for vitamin B_6 can be used for judging the safety of pyridoxal 5'-phosphate.

The Panel concluded that the use of pyridoxal 5'-phosphate as a source for vitamin B_6 in food supplements intended for the general population would be of no safety concern if use levels were in compliance with defined upper safe use levels.

However, the Panel is concerned that the use levels of pyridoxal 5'-phosphate proposed by the petitioners are 50 and 90 mg/day and are substantially higher than the tolerable upper intake levels defined by the SCF in 2000 of 25 mg/day for adults and 5-20 mg for children depending on their body weight.

The Panel noted that the specifications of pyridoxal 5'-phosphate as commercialised should comply with the specifications for vitamin B_6 and its derivatives used as food additives.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier on pyridoxal-5'-phosphate. July 2005. Application for derogation submitted by Solgar Vitamin and Herb.
2. Dossier with additional information on pyridoxal-5'-phosphate proposed for addition to Annex II of Directive 2002/46/EC of the European parliament and of the Council Relating to Food supplements. Submitted by BioCare Ltd.

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GLOSSARY / ABBREVIATIONS

AFC	Scientific Panel on Food additives, Flavourings, Processing aids and Materials in Contact with Food.
bw	body weight
CAS	Chemical Abstract Service
EVM	Expert Group on Vitamins and Minerals, UK
IUPAC	International Union of Pure and Applied Chemistry
LOAEL	Lowest-Observed-Adverse-Effect Level
NOAEL	No-Observed-Adverse-Effect Level
SCF	Scientific Committee for Food
UL	Upper Level