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Dietary Reference Values for vitamin B6

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)

Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) derives Dietary Reference Values (DRVs) for vitamin B6. The Panel considers that plasma pyridoxal 5′-phosphate (PLP) concentration is the biomarker of status suitable for deriving DRVs for vitamin B6. Considering that a plasma PLP concentration of 30 nmol/L, as a population mean, is indicative of an adequate vitamin B6 status, the Panel proposes to use this cut-off value to set Average Requirements (ARs). Population Reference Intakes (PRIs) are derived for adults and children from ARs, assuming a coefficient of variation (CV) of 10%. For women, the AR and PRI are set at 1.3 and 1.6 mg/day. For men, the AR of 1.5 mg/day is derived by an allometric scaling from the AR for women, and a PRI of 1.7 mg/day is set. For all infants aged 7–11 months, an Adequate Intake of 0.3 mg/day is set, averaging the results of two extrapolation approaches based on an allometric scaling: upwards extrapolation from the estimated vitamin B6 intake of exclusively breastfed infants from birth to 6 months, and downwards extrapolation from the ARs for adults applying a growth factor. For all children, ARs are derived from adult ARs using an allometric scaling and growth factors. For children of both sexes aged 1–14 years, ARs range between 0.5 and 1.2 mg/day. For children aged 15–17 years, the Panel derives the same ARs as for adults. PRIs for children aged 1–17 years range between 0.6 and 1.7 mg/day. Extrapolation of ARs by an allometric scaling considered differences in reference body weight. For pregnant and lactating women, additional requirements are considered, based on the uptake of vitamin B6 by the fetal and maternal tissues and the losses through breast milk, and PRIs of 1.8 and 1.7 mg/day, respectively, are derived.

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Keywords: vitamin B6, pyridoxine, pyridoxamine, pyridoxal, Average Requirement, Population Reference Intake, Dietary Reference Value

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Correspondence: nda@efsa.europa.eu

Panel members: Jean-Louis Bresson, Barbara Burlingame, Tara Dean, Susan Fairweather-Tait, Marina Heinonen, Karen-Ildico Hirsch-Ernst, Inge Mangelsdorf, Harry McArdle, Androniki Naska, Monika Neuhäuser-Berthold, Grażyna Nowicka, Kristina Pentieva, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Martin Stern, Daniel Tomé, Dominique Turck, Henk Van Loveren, Marco Vinceti and Peter Willatts

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Summary

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a Scientific Opinion on Dietary Reference Values (DRV) for the European population, including vitamin B6.

The term vitamin B6, which is used in the current Scientific Opinion, is a generic descriptor for a group of 2-methyl,3-hydroxy,5-hydroxymethylpyridine derivatives. Vitamin B6 includes pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), and their respective phosphorylated forms, pyridoxine 5′-phosphate (PNP), pyridoxal 5′-phosphate (PLP) and pyridoxamine 5′-phosphate (PMP). All these derivatives are present in food. The metabolically active forms, PLP and PMP, act as cofactors of enzymes involved in amino acid metabolism, one-carbon reactions, glycogenolysis and gluconeogenesis, haem synthesis, niacin formation, and also in lipid metabolism, neurotransmitter synthesis and hormone action. However, all six vitamin B6 derivatives have vitamin activity as they can be converted in the body to PLP and PMP, through enzyme-mediated reactions. The most typical features of vitamin B6 deficiency, although rare, are hypochromic microcytic anaemia and neurological abnormalities (convulsive seizures, abnormal electroencephalograms).

The bioavailability of PN, PL and PM is similar. The Panel considers that the bioavailability of pyridoxine-5′-β-D-glucoside (PNG) present in some plants is 50% lower than that of PN and, thus that the bioavailability of vitamin B6 from a mixed diet is around 75%. The Panel also considers that the bioavailability of PN from supplements is about 95%. The vitamin B6 derivatives can be converted to each other through enzyme-mediated reactions in the intestine, in the liver and in other tissues. After absorption, vitamin B6 derivatives are transferred via the portal circulation to the liver where they are metabolised, and are released back to the circulation, where PLP and PL, bound to albumin, are the main forms of the total plasma vitamin B6. Vitamin B6 derivatives are distributed to tissues, in which the predominant vitamin B6 derivative is PLP. The average vitamin B6 content of human body is about 15 nmol/g (assumed to be equivalent to 3.7 μg/g tissue). The majority (75–80%) of the total vitamin B6 is located in muscles (PLP bound to muscle glycogen phosphorylase) including heart, about 5–10% is in the liver and smaller amounts of vitamin B6 are contained in plasma, erythrocytes and other organs. Vitamin B6 is excreted mainly through the urine in the form of its catabolic product 4-pyridoxic acid (4-PA). The mechanism (active or passive) of vitamin B6 placental transfer is unclear.

The Panel notes limitations in biomarkers of vitamin B6 intake and status, i.e. plasma PLP concentration, the concentrations of total vitamin B6 in plasma, of PL and PMP in plasma or erythrocytes, of PLP in erythrocytes, and of total vitamin B6 or 4-PA in urine. The Panel also notes limitations in biomarkers of function, i.e. activation coefficients of erythrocyte aspartate aminotransferase and erythrocyte alanine aminotransferase, urinary excretion of tryptophan catabolites after the tryptophan loading test, ratios of tryptophan metabolites in plasma, urinary concentrations of cystathionine and plasma homocysteine concentration after a methionine load, plasma cystathionine concentration, and some immune-related factors.

The Panel considers that the most suitable biomarker for deriving DRVs for vitamin B6 is plasma PLP concentration: although it has some limitations, plasma PLP concentration is the only biomarker that reflects the tissue stores of vitamin B6 (biomarker of status) and has a defined cut-off value for an adequate vitamin B6 status. The Panel considers it suitable to be used for deriving the DRVs for vitamin B6 in children and adults. The Panel notes that mean values below 30 nmol/L are associated with a wide range of metabolic effects including perturbations of amino acid, lipid, and organic acid profiles in plasma. The Panel considers that a plasma PLP concentration of 30 nmol/L, as a population mean, is indicative of an adequate vitamin B6 status for all age and sex groups. The Panel notes that there is no consistent relationship between plasma PLP concentrations and protein intake, and considers that there is no conclusive evidence that vitamin B6 requirements change according to protein intake in the range of observed intake in Europe. Thus, the Panel considers not appropriate to standardise vitamin B6 requirements on protein intake. In view of the limited and/or inconsistent evidence on an association between vitamin B6 intake or plasma PLP concentration and health consequences, the Panel considers that the data available cannot be used for deriving the requirement for vitamin B6.

In the absence of information on the variability in the requirement, a coefficient of variation (CV) of 10% was used to calculate Population Reference Intakes (PRIs) from the Average Requirements (ARs) for all age groups in children and in adults, rounding to the nearest decimal place. When ARs were derived from one group to the other, an allometric scaling was applied on the assumption that vitamin B6 requirement is related to metabolically active body mass.

For adults, the Panel considers that ARs and PRIs for vitamin B6 can be derived from the vitamin B6 intake required to maintain a (mean) concentration of plasma PLP above 30 nmol/L. The Panel considers the inverse prediction examination of a linear regression analysis of plasma PLP concentration vs vitamin B6 intake (from food including supplements, which were adjusted for their difference in bioavailability), which combined data from five references on intervention studies in 44 young women. The Panel also considers data from two small intervention studies supported by results from three large cross-sectional observational studies, all in older adults. The Panel notes that the vitamin B6 intake required to maintain a (mean) concentration of plasma PLP above 30 nmol/L derived from the data in older women (1.3 mg/day) is slightly higher than the result obtained in younger women (1.2 mg/day). As a conservative approach, the Panel sets an AR for all women at 1.3 mg/day and a PRI at 1.6 mg/day. In the absence of reliable data to determine vitamin B6 requirement in men, the Panel sets an AR by an allometric scaling from the AR of women, and taking into account the difference in reference body weight. The Panel sets an AR for men at 1.5 mg/day and a PRI at 1.7 mg/day.

For infants aged 7–11 months and children aged 1–17 years, the Panel notes the absence of reliable data on which to base vitamin B6 requirements. The Panel also considers unnecessary to give sex-specific DRVs for infants and children up to 14 years of age, but chooses to set different PRIs for boys and girls aged 15–17 years as for adults.

For infants aged 7–11 months**,** the Panel proposes an Adequate Intake (AI) at 0.3 mg/day, combining the results of two extrapolation approaches based on an allometric scaling, both taking into account the differences in reference body weight. The proposed AI is the average of the results of upwards extrapolation from the estimated intake of vitamin B6 of exclusively breastfed infants from birth to 6 months, and of downwards extrapolation from the ARs for adults applying a growth factor.

For children aged 1–17 years, the Panel derives ARs by downwards extrapolation from adult ARs, by an allometric scaling, applying growth factors and taking into account the differences in reference body weight. For children of both sexes aged 1–14 years, the Panel sets ARs ranging between 0.5 and 1.2 mg/day, while for children aged 15–17 years, the Panel derives the same ARs as for adults. PRIs range from 0.6 to 1.4 mg/day for children aged 1–14 years, while for children aged 15–17 years, PRIs are 1.6 mg/day for girls and 1.7 mg/day for boys.

For pregnant and lactating women, the AR for non-pregnant non-lactating women is increased to account for the uptake of vitamin B6 by the fetal and maternal tissues, and the losses through breast milk, respectively. For pregnant women, the additional vitamin B6 intake (0.2 mg/day) is estimated, based on the mean gestational weight gain (12 kg) and the average vitamin B6 content of the human tissue (3.7 μg/g tissue), a pregnancy duration of 280 days and the vitamin B6 bioavailability from a mixed diet (75%). The Panel sets an AR for pregnant women at 1.5 mg/day and a PRI at 1.8 mg/day. For lactating women, the additional vitamin B6 intake (0.133 mg/day) is estimated, considering an average concentration of vitamin B6 in breast milk (0.130 mg/L), the mean milk transfer during the first 6 months of lactation in exclusively breastfeeding women (0.8 L/day), and the vitamin B6 bioavailability from a mixed diet (75%). The Panel sets an AR for lactating women at 1.4 mg/day and a PRI at 1.7 mg/day.

Based on data from 13 surveys in nine countries of the European Union, average total vitamin B6 intake ranges across countries from 0.4 to 0.8 mg/day in infants, from 0.9 to 1.3 mg/day in children aged 1 to < 3 years, from 1 to 1.6 mg/day in children aged 3 to < 10 years, and from 1.5 to 2.3 mg/day in children aged 11 to < 18 years. Average total vitamin B6 intake ranges between 1.4 and 3.1 mg/day in adults.

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Background as provided by the European Commission

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and if necessary to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on the nutrient and energy intakes for the European Community.[1] The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then, new scientific data have become available for some of the nutrients, and scientific advisory bodies in many European Union (EU) Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients, these newly established (national) recommendations differ from the Reference Intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish Reference Intakes for other (essential) substances with a physiological effect.

In this context, the European Food Safety Authority (EFSA) is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients, and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public, it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context, EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food-based recommendations intended for the population as a whole.

Terms of Reference as provided by the European Commission

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No 178/2002[2], the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on Population Reference Intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance, EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, polyunsaturated fatty acids and monounsaturated fatty acids, *trans* fatty acids;
- Protein
- Dietary fibre.

Following on from the first part of the task, EFSA is asked to advise on Population Reference Intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, EFSA is asked to provide guidance on the translation of nutrient-based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

[1] Scientific Committee for Food, Nutrient and energy intakes for the European Community, Reports of the Scientific Committee for Food 31st series, Office for Official Publication of the European Communities, Luxembourg, 1993.

[2] Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–24.

Assessment

1. Introduction

In 1993, the SCF adopted an opinion on nutrient and energy intakes for the European Community and derived for vitamin B6 a Lower Threshold Intake (LTI), an Average Requirement (AR) and a Population Reference Intake (PRI) for adults expressed in μg pyridoxine/g dietary protein (SCF, 1993). The PRI in μg/g protein for adults was used also for children as well as for pregnant or lactating women. Corresponding values in mg/day were calculated considering specific energy requirements of children and adults and an average protein intake of 15% of energy intake, and the extra protein intake recommended during pregnancy or lactation.

2. Definition/category

Vitamin B6 is a generic descriptor for a group of 2-methyl,3-hydroxy,5-hydroxymethylpyridine derivatives exhibiting the biological activity of pyridoxine (American Institute of Nutrition, 1990). Although, the term 'pyridoxine' is still in use to refer to the group of vitamin B6 derivatives in some publications, the International Union of Pure and Applied Chemistry–International Union of Biochemistry (IUPAC–IUB) Commission on Biochemical Nomenclature recommended 'vitamin B6' to be adopted as a generic name, and 'pyridoxine' not to be used as a synonym of 'vitamin B6' (IUPAC–IUB CBN, 1973). Therefore, the term 'vitamin B6' is used in the current Scientific Opinion.

2.1. Chemistry

Vitamin B6 includes three derivatives that differ by the one-carbon substitution at the fourth position of the pyridine ring, i.e. pyridoxine (PN[3]), an alcohol, pyridoxal (PL[4]), an aldehyde, and pyridoxamine (PM[5]), an amine, and their three respective forms with a phosphate ester at the 5′-position, i.e. pyridoxine 5′-phosphate (PNP[6]), pyridoxal 5′-phosphate (PLP[7]) and pyridoxamine 5′-phosphate (PMP[8]) (da Silva et al., 2013) (Figure 1).

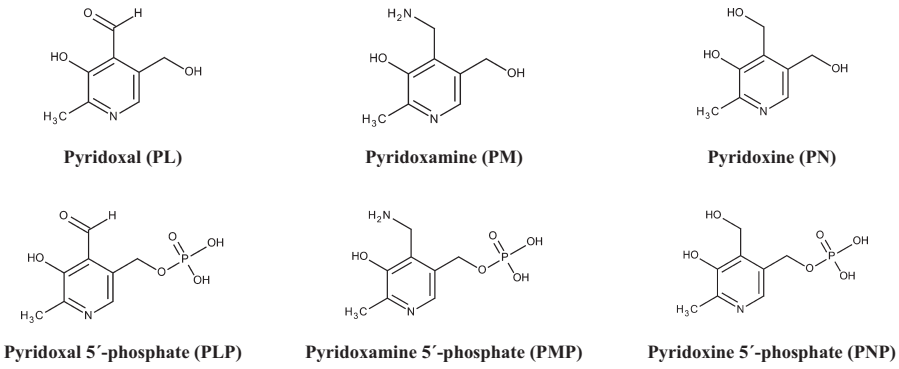

Figure 1: Structure of vitamin B6 derivatives: pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM), pyridoxine 5′-phosphate (PNP), pyridoxal 5′-phosphate (PLP) and pyridoxamine 5′- phosphate (PMP)

All these six vitamin B6 derivatives can be found in foods (Gregory and Feldstein, 1985; Gregory and Sartain, 1991; van Schoonhoven et al., 1994; Bognår and Ollilainen, 1997; IOM, 1998; Valls et al., 2001; Kall, 2003; Viñas et al., 2004; Gentili et al., 2008). Predominantly PLP, but also PMP, are the active forms that function as coenzymes in various metabolic reactions (Section 2.2.1). However, all six vitamin B6 derivatives are considered to have vitamin activity, as they can be converted in the body to PLP and PMP, through enzyme-mediated reactions (Gregory, 1997) (Section 2.3.5). The final catabolic product of the oxidation of all the derivatives is 4-pyridoxic acid (4-PA), which is metabolically inactive.

[3] PN: molecular mass: 169.2 g/mol.
[4] PL: molecular mass: 167.2 g/mol.
[5] PM: molecular mass: 168.2 g/mol.
[6] PNP: molecular mass: 249 g/mol.
[7] PLP: molecular mass: 247.1 g/mol.
[8] PMP: molecular mass: 248.2 g/mol.

Some plants contain glycosylated vitamin B6 in the form of pyridoxine-5′-β-D-glucoside (PNG), where glucose units are attached by a β-glycosidic bond to the 5′-hydroxymethyl group of PN (Gregory, 1997). Pyridoxine hydrochloride (PN-HCl) is the most commonly used synthetic form of vitamin B6 for food fortification and supplementation in available studies, although pyridoxine α-ketoglutarate is also reported in the literature to be administered as a supplement (Marconi et al., 1982; Linderman et al., 1992).

2.2. Function of vitamin B6

The metabolically active forms PLP and PMP act as cofactors for more than 100 enzymes involved primarily in amino acid metabolism, but also in one-carbon reactions, glycogenolysis and gluconeogenesis, haem synthesis, niacin formation and other functions (lipid metabolism, neurotransmitter synthesis and hormone action).

2.2.1. Biochemical functions

2.2.1.1. Amino acid metabolism

PLP and PMP are cofactors for enzymes participating in decarboxylation, transamination and racemisation reactions of amino acid metabolism (Bender, 2013). In PLP-dependent decarboxylation reactions, the carboxylic group (−COO) from one amino acid is removed and amines are formed. Some amines act as neurotransmitters or hormones (i.e. serotonin, taurine, dopamine, noradrenaline, histamine and γ-aminobutyric acid) and others, like diamines and polyamines, are involved in the regulation of DNA metabolism (Dakshinamurti et al., 1990; Bender, 2013). In transamination reactions, the amino group ($-NH_2$) from one amino acid is transferred to a α-keto acid. Transamination reactions are involved in the synthesis of dispensable amino acids and the interconversion and catabolism of all amino acids (except lysine). Racemisation reactions lead to the formation of racemic mixtures of D- and L-amino acids, which have a role in signalling during brain development (Bender, 2013).

2.2.1.2. One-carbon metabolism

PLP is essential for the normal functioning of several enzymes involved in one-carbon metabolism. It is a cofactor for both serine hydroxymethyltransferase and glycine decarboxylase. These enzymes are responsible for the transfer of one-carbon units to folate derivatives, which are used for the synthesis of purine and pyrimidine nucleotides, the remethylation of homocysteine (Hcy) to methionine and the production of the universal methyl donor *S*-adenosylmethionine (SAM). In turn, SAM is involved in transmethylation reactions activating a wide range of bioactive compounds (DNA, hormones, proteins, neurotransmitters and membrane phospholipids) (Chiang et al., 1996). PLP is also a cofactor of cystathionine β-synthase and cystathionine γ-lyase, enzymes involved in the transsulfuration pathway, where Hcy is metabolised to cysteine.

2.2.1.3. Glycogenolysis and gluconeogenesis

PLP is a cofactor for glycogen phosphorylase, which releases glucose-1-phosphate from glycogen in the muscle and liver (Sections 2.3.4 and 2.4.1.1). A study in young men showed that vitamin B6 concentration in the muscle is resistant to change, after 6 weeks of restriction of vitamin B6 intake (0.47 mg/day) followed by 6 weeks of supplementation with vitamin B6 (164 mg/day in addition to a self-selected diet) (Coburn et al., 1991). This suggests that PLP bound to glycogen phosphorylase in the muscle cannot be considered as a storage of vitamin B6 that can fulfil the metabolic needs of the body for the vitamin. However, PLP is released from the muscle under conditions of low energy intake when the glycogen reserves decrease (Black et al., 1978). Through its role of cofactor in transamination reactions, PLP is involved in the conversion of amino acids to α-keto acids (Section 2.2.1.1), which in turn can act as substrates for the generation of glucose (gluconeogenesis).

2.2.1.4. Haem synthesis

PLP is a cofactor for the δ-aminolevulinate synthase in the erythrocytes that is a key enzyme catalysing the first step in the haem biosynthesis. Symptoms associated with vitamin B6 deficiency include hypochromic microcytic anaemia (Section 2.2.2.1).

2.2.1.5. Niacin formation

The conversion of tryptophan to niacin involves several enzymes that are PLP-dependent (Section 2.3.7.1). However, the restriction of vitamin B6 intake to 0.2 mg/day for 28 days followed by vitamin B6 supplementation (0.8 and 2.0 mg/day) in young women did not show a marked change in the urinary excretion of niacin metabolites between pre-depletion, depletion and repletion phases or marked difference between supplementation groups (Leklem et al., 1975). This study suggests that the effect of vitamin B6 intake on the conversion of tryptophan to niacin (and the subsequent urinary excretion of niacin metabolites) was negligible, although niacin intake was not reported.

2.2.2. Health consequences of deficiency and excess

2.2.2.1. Deficiency

Symptoms associated with vitamin B6 deficiency include eczema, seborrhoeic dermatitis, cheilosis, glossitis, angular stomatitis, hypochromic microcytic anaemia (Section 2.2.1.4), hyperirritability, convulsive seizures and abnormal electroencephalograms (Sauberlich, 1981). The most typical features of vitamin B6 deficiency, however, are anaemia and neurological abnormalities. The anaemia caused by vitamin B6 deficiency is a consequence of defective haemoglobin biosynthesis, which allows the replication of erythrocytes to occur at a low haemoglobin level. Thus, the number of erythrocytes is high but the cells are small ('microcytic') and with reduced haemoglobin concentration (thus 'hypochromic') (da Silva et al., 2014). Neurological abnormalities in vitamin B6 deficiency are related to both the decrease in the synthesis of γ-aminobutyric acid (Section 2.2.1.1), a major inhibitory neurotransmitter in the brain, and to the increased concentration of tryptophan metabolites in the brain that have a proconvulsant effect (Dakshinamurti et al., 1990).

Vitamin B6 deficiency is rare. In the USA in the early 1950s, young infants, who consumed infant formula low in vitamin B6 (60 μg/L equivalent to an intake of 50 μg/day of vitamin B6) as a result of improper manufacturing procedure, developed hypochromic microcytic anaemia, failure to thrive, hyperirritability and convulsive seizures (Borschel, 1995). Plasma PLP concentration of 15 nmol/L was reported in one infant with convulsive seizures (Borschel and Kirksey, 1990). In a metabolic study,[9] women receiving a diet providing 50 μg/day of vitamin B6 showed abnormal electroencephalograms and plasma PLP concentrations of ~ 8.5 nmol/L (Kretsch et al., 1991).

2.2.2.2. Excess

SCF (2000) set a Tolerable Upper Intake Level (UL) based on adverse neurological effects of vitamin B6 in humans. For setting a UL for vitamin B6, SCF (2000) focused on a study in women attending a clinic for the treatment of premenstrual syndrome and who received supplemental vitamin B6, for less than 6 months to more than 5 years (Dalton and Dalton, 1987). In this study, out of 172 women, high supplemental doses of vitamin B6 taken for long periods (on average about 100 mg/day for 2.9 years) were related to the development of reversible peripheral sensory and motor neuropathy in 103 women. The symptoms included ataxia, muscle weakness and an impaired sense of touch of the limbs. However, the studies on adverse effects of vitamin B6 were not carried out under controlled conditions.

The SCF could not establish a No Observed Adverse Effect Level (NOAEL). The SCF considered that severe toxicity can be produced at doses of 500 mg/day or more, and that minor neurological symptoms may be apparent at doses of 100 mg/day or more if consumed for long periods. Using twice an uncertainty factor of 2 on the average intake (about 100 mg/day) observed in one study (Dalton and Dalton, 1987), a UL of 25 mg/day was derived for adults, including pregnant and lactating women. For children from 1 year of age onwards, the UL was extrapolated from the adult UL on a body weight basis, and was set at values between 5 mg/day (for 1–3 years) and 20 mg/day (for 15–17 years).

2.3. Physiology and metabolism

2.3.1. Intestinal absorption and bioavailability

The absorption of the ingested vitamin B6 occurs in the jejunum through unsaturable passive diffusion (Hamm et al., 1979). However, *in vitro* experiments with human intestinal epithelial Caco-2

[9] Well-controlled studies in which participants were housed in a metabolic unit are termed metabolic studies.

cells showed evidence that vitamin B6 absorption also occurs through a saturable pH-dependent carrier-mediated and proton-coupled process (Said et al., 2003). A pool of vitamin B6 synthesised by the intestinal microbiota is absorbed in the colon by the same carrier-mediated mechanism (Said et al., 2008). *In vitro* studies on colonocytes also showed that, under the conditions of 'low' vitamin B6 concentration in the growth media, adaptive upregulation of intestinal vitamin B6 uptake is observed (Said et al., 2008).

Before intestinal uptake, phosphorylated forms of vitamin B6 (PNP, PLP and PMP; Section 2.1) are subject to hydrolysis by the enzyme alkaline phosphatase, whereas dephosphorylated forms of vitamin B6 (PN, PL and PM) are absorbed without further modification (da Silva et al., 2014). After entering the enterocytes, dephosphorylated forms of vitamin B6 are converted back to their respective phosphorylated forms by the enzyme pyridoxal kinase (McCormick and Chen, 1999), a mechanism through which vitamin B6 is retained within the cell (i.e. metabolic trapping). However, in order to cross the enterocyte membrane and to move to the portal circulation, vitamin B6 phosphorylated forms have to be dephosphorylated again.

Bioavailability of vitamin B6 has been discussed in reviews (Gregory, 1990, 1993, 1997). It refers to the amount of ingested and absorbed vitamin that is utilised for normal physiological functions and storage (Jackson, 1997). Changes in vitamin B6 bioavailability may be estimated based on changes in the concentration of vitamin B6 biomarkers (Section 2.4). The various vitamin B6 derivatives are expected to have similar bioavailability, based on the fact that they interconvert to each other.

In an acute cross-over study, five men (mean ± SD: 27.2 ± 3.0 years) received, at weekly intervals and in a random order, a single equimolar dose (19.45 μmol) of one of three vitamin B6 forms PN, PL and PM, and the concentration of vitamin B6 biomarkers was monitored for 8 and 24 h in plasma and urine, respectively (Wozenski et al., 1980). There was a significantly lower ($p < 0.01$) urinary excretion of total vitamin B6, but no difference in the urinary excretion of 4-PA, after the ingestion of PL compared to PN and PM (Sections 2.3.6.1 and 2.4.1.3). The percentage rise in plasma PLP concentration was similar after the ingestion of PL, PN and PM. However, the area under the curve (AUC) for plasma PLP was significantly lower ($p < 0.01$) after the ingestion of PL compared to PN and PM. The authors attributed the differences in urinary excretion of total vitamin B6 and AUC for plasma PLP after the ingestion of equimolar doses of PL, PN and PM, to variability in their metabolic pathways, not to differences in bioavailability. The Panel agrees with this conclusion.

Pyridoxine-5′-β-D-glucoside (PNG) (Section 2.1) is hydrolysed enzymatically by the PNG hydrolase (Nakano et al., 1997) and the brush border lactase-phlorizin hydrolase (Mackey et al., 2002) before its absorption in the small intestine. However, PNG can also be absorbed unchanged (IOM, 1998).

In studies with stable isotopes in young men and women, the bioavailability of PNG was 50–58% of that of PN when the assessment was based on the urinary excretion of 4-PA (Gregory et al., 1991; Nakano et al., 1997). PNG also acted as inhibitor of the simultaneously ingested PN in a dose-dependent way (Nakano et al., 1997). Nine women (mean age ± SD, 29 ± 6 years), who consumed meals prepared and served in a metabolic kitchen, were divided into two groups who received, for 18 days in a cross-over design, diets containing either 1.52 or 1.44 mg/day vitamin B6, of which 27% and 9%, respectively, was PNG (Hansen et al., 1996b). After the consumption of the 27% PNG diet compared to the 9% PNG diet, there was a reduction by 10–18% of all the measured vitamin B6 biomarkers, i.e. urinary excretion of vitamin B6 and 4-PA, plasma PLP and vitamin B6 concentrations, and erythrocyte PLP concentration ($p < 0.05$ for all except plasma PLP). After the consumption of the 27% PNG diet compared to the 9% PNG diet, there was also a significant 50% increased faecal elimination of vitamin B6 ($p < 0.001$). The content of PNG in the diet varies based on the food selection, however, in the average diet, approximately 15% of total vitamin B6 intake is estimated to come from PNG (Andon et al., 1989).

In a cross-over study, 10 men (20–35 years) consumed a diet providing about 1.7 mg/day of vitamin B6 (background fibre intake not reported) with or without added wheat bran (15 g/day) for three periods of 18 days each (Lindberg et al., 1983). This study showed that the addition of 15 g/day of wheat bran to the diet, compared to no added bran, significantly decreased both plasma PLP ($p < 0.05$) and urinary 4-PA concentrations ($p < 0.01$) by 9–17%. It also significantly increased the faecal excretion of vitamin B6 ($p < 0.05$) (Section 2.3.6.2). The Panel considers that the effect of dietary fibre on vitamin B6 biomarkers of status was small and cannot influence the general vitamin B6 status under the conditions of an adequate diet.

Bioavailability of PN from supplements is considered to be almost complete and is estimated to be 95% (IOM, 1998). Bioavailability of vitamin B6 from a mixed diet was assessed in a controlled metabolic study with six healthy men (21–35 years) who went through three experimental periods

(Tarr et al., 1981). From day 1 to 35, they consumed a semipurified formula diet supplemented with PN-HCl (given once daily) and providing a total intake of 1.1 mg/day vitamin B6. From day 36 to 70, they consumed a diet based on natural food sources only (average US diet), providing a total intake of 2.3 mg/day vitamin B6. From day 71 to 91, they consumed a semipurified formula diet supplemented with PN-HCl, providing a total intake of 2.7 mg/day vitamin B6. The daily protein intake was 96 g throughout the study. Compared to PN-HCl, the bioavailability of vitamin B6 from a mixed diet was lower, and was 71% using a plasma PLP concentration, and 79% using a urinary vitamin B6 concentration. Thus, on average, the bioavailability of vitamin B6 from a mixed diet can be estimated to be around 75%. Based on this study, and assuming (as indicated above) 95% bioavailability of PN, which is the form of vitamin B6 most widely used as supplement in controlled (metabolic) studies, the Institute of Medicine (IOM, 1998) considered that vitamin B6 from food has 1.27 times lower bioavailability than PN from supplements (95% bioavailability of PN from supplements/75% bioavailability of vitamin B6 from food). This means that 1 mg vitamin B6 from food = 0.8 mg vitamin B6 (PN) from supplements.

The Panel considers that there are no major differences in the bioavailability of PN, PL and PM. The Panel notes that the bioavailability of PNG is 50% lower than that of PN, but it is unlikely that the consumption of PNG through the average diet would have implications on vitamin B6 biomarkers of status, as the contribution of PNG to the total vitamin B6 intake in the average diet is around 15%. The Panel considers that the bioavailability of vitamin B6 from a mixed diet is around 75%. Bioavailability of PN from supplements is considered to be almost complete and is estimated to be 95%.

2.3.2. Transport in blood

PLP and PL are the main forms of vitamin B6 in the circulation, PLP accounting for 70–90% of the total vitamin B6 in plasma (Leklem, 1990). Both PLP and PL in plasma are bound tightly to albumin (Dempsey and Christensen, 1962). Erythrocytes are able to take up all vitamin B6 derivatives and to convert them to PLP and PL that are bound to haemoglobin (Mehansho and Henderson, 1980). However, it is unknown whether the erythrocytes play a role in the transport of vitamin B6 to the tissues.

2.3.3. Distribution to tissues

After intestinal absorption, vitamin B6 derivatives are transferred via the portal circulation to the liver, where they are metabolised (Section 2.3.5) or from which they are released back in the circulation for distribution to other tissues. The phosphorylated vitamin B6 forms (PLP, PMP and PNP) are charged molecules that, in order to pass through the cellular membranes (Section 2.3.1), need to undergo dephosphorylation by tissue non-specific phosphatase (Van Hoof et al., 1990) or vitamin B6-specific alkaline phosphatase (Fonda, 1992). Labelled *in vitro* experiments with isolated hepatocytes showed that the cellular uptake of non-phosphorylated vitamin B6 forms (PL, PM and PN) is a saturable process (Kozik and McCormick, 1984).

Vitamin B6 is transferred through the blood brain barrier via facilitated diffusion, although the exact mechanism is not fully elucidated (Spector and Johanson, 2007). Studies in healthy adults demonstrated that, compared with plasma, the concentration of vitamin B6 in the cerebrospinal fluid is almost the same or slightly lower (Albersen et al., 2014), whereas animal experiments have shown that vitamin B6 content in the choroid plexus and in the brain is around 25–50 times higher (Spector and Greenwald, 1978). The homoeostasis of vitamin B6 in the central nervous system is not well maintained and low dietary intake of vitamin B6 can result in a disturbed brain function (i.e. abnormal electroencephalograms and seizures) (Borschel, 1995; Kretsch et al., 1995) (Section 2.2.2.1).

The mechanism of vitamin B6 placental transfer is unclear. Studies have reported up to five times higher plasma PLP concentration in the umbilical cord of the newborn or fetus than in maternal blood in pregnancy or at delivery, suggesting an active placental transfer of PLP from the mother to the fetus (Contractor and Shane, 1970; Shane and Contractor, 1980; Zempleni et al., 1992) (Section 5.1.4). However, in experiments with full-term human placentas perfused with physiological concentrations of some vitamin B6 derivatives (PL, PLP or PN), the placental transfer of PLP was negligible (Schenker et al., 1992), but this might be explained by the necessity of PLP to be dephosphorylated to PL before placental transfer. In contrast, the transfer of PL was effective in both directions (maternal–fetal and fetal–maternal), but significantly greater towards the fetus. This transfer was not inhibited by the

structural analogue 4-deoxypyridoxine, suggesting that it may not involve receptors or specific carriers but may be by passive diffusion.

2.3.4. Storage

In rats, about 75–80% of the total vitamin B6 is located in the muscle (including the heart) (Section 2.2.1.3), about 5–10% is in the liver and smaller amounts of vitamin B6 are contained in plasma, erythrocytes and other organs (Coburn et al., 1988a).

Studies using muscle biopsies in humans (considering that muscle is about 40% of the body weight) (Coburn et al., 1988b), as well as labelled (Coburn et al., 1985, 1988a) and non-labelled (Reithmayer et al., 1985) vitamin B6 derivatives in swine and rats, showed that the average vitamin B6 total body content is about 15 nmol/g (Coburn, 1990). Based on these data, it was estimated that the total body pool of vitamin B6 in a 70 kg person is approximately 1,000 μmol (Coburn et al., 1988b; Coburn, 1990). The main vitamin B6 derivative in the human tissues is PLP (with a molecular mass of 247.1 g/mol) (Krebs and Fischer, 1964; Coburn et al., 1988b) and, according to animal data, only the brain, heart and kidney have a higher amount of PMP compared with PLP (Coburn et al., 1988a). Thus, the total body content of 15 nmol/g would be equivalent to 3.7 μg/g tissue.

The pool of vitamin B6 in the circulation has a fast turnover as it responds quickly to changes in vitamin B6 intake and a steady state is reached by 7 days (Wozenski et al., 1980). In contrast, the pool of vitamin B6 in the muscle has a slow turnover and does not respond to a decrease in vitamin B6 intake, but it declines with a restriction of energy intake (Section 2.2.1.3).

The Panel considers that the average vitamin B6 content of human body is about 15 nmol/g (3.7 μg/g tissue) and that the main vitamin B6 derivative in the tissues is PLP.

2.3.5. Metabolism

Vitamin B6 derivatives can be converted to each other through enzyme-mediated reactions. PL, PM and PN are phosphorylated to PLP, PMP and PNP via the enzyme pyridoxal kinase, available in all tissues (e.g. the intestine, Section 2.3.1) (McCormick and Chen, 1999). PNP and PMP are converted to PLP via pyridoxine (pyridoxamine) phosphate oxidase, available only in the liver, kidney and brain (Kazarinoff and McCormick, 1975). PLP, PMP and PNP are dephosphorylated to PL, PM and PN via tissue non-specific phosphatase and B6-specific alkaline phosphatase (Sections 2.3.1 and 2.3.3).

Free PL in the liver is catabolised by aldehyde oxidase and aldehyde dehydrogenase to 4-PA, which is excreted through the urine (Sections 2.3.6 and 2.4.1.3). A study in liver biopsy samples from people without liver disease (Merrill et al., 1984) found that: (1) the rate of phosphorylation of vitamin B6 forms was higher than that of dephosphorylation; (2) the rate of catabolism of PL to 4-PA is comparable to the rate of phosphorylation of PL; (3) the rate of phosphorylation of PL to PLP via pyridoxal kinase is slower than the rate of the production of PLP via pyridoxine (pyridoxamine) phosphate oxidase; (4) pyridoxine (pyridoxamine) phosphate oxidase is inhibited by its product PLP thus is a regulating step in vitamin B6 metabolism. This study suggests that vitamin B6 metabolism is organised to ensure sufficient amount of the active metabolite PLP in the liver and other tissues and, at the same time, to prevent the accumulation of PLP within the cells.

2.3.6. Elimination

2.3.6.1. Urine

Vitamin B6 is excreted through the urine, mainly as its catabolic product 4-PA (Sections 2.3.5 and 2.4.1.3), but also the active forms of vitamin B6 can be found in the urine. In a study in humans, 85–90% of vitamin B6 ingested or administered intravenously could be recovered as urinary 4-PA (Lui et al., 1985), which suggests that urine is the main route for elimination of vitamin B6. Most of the excreted active forms of vitamin B6 are reabsorbed in the kidney tubules.

2.3.6.2. Faeces

In studies using labelled PN in humans (Tillotson et al., 1966) or rats (Cox et al., 1962), only about 3% of the ingested dose of vitamin B6 is excreted through the faeces. Using labelled isotopes, the excretion of 4-PA in urine is similar in either conventional and germ-free guinea pigs or rats (Coburn and Townsend, 1989). These animal data suggest that vitamin B6 synthesised by the intestinal microbiota may not be absorbed and metabolised (thus may be excreted through the faeces), but there is no data in humans to confirm this.

2.3.6.3. Breast milk

The concentration of vitamin B6 in breast milk is low during the first 1–2 weeks post-partum, but gradually increases with the progression of lactation (Moser-Veillon and Reynolds, 1990). This concentration also fluctuates with maternal dietary intake or supplementation (Styslinger and Kirksey, 1985; Borschel et al., 1986; Chang and Kirksey, 1990, 2002; Moser-Veillon and Reynolds, 1990; Lovelady et al., 2001). After maternal supplementation with 2.5, 4.0, 7.5 and 10 mg/day PN-HCl during the first 6 months of lactation, mean concentration of vitamin B6 in breast milk was significantly lower ($p < 0.05$) with the supplementation of 2.5 mg/day than with higher doses (Chang and Kirksey, 1990). This suggests that the incremental vitamin B6 intake of lactating women would transfer into the breast milk.

A comprehensive search of the literature published after January 2000 was performed as preparatory work to this Scientific Opinion, in order to identify data on vitamin B6 concentration in breast milk (LASER Analytica, 2014). This search was completed with additional literature published earlier or identified from the narrative review of Bates and Prentice (1994) or cited in SCF (2003).

Studies reporting either maternal vitamin B6 intake or vitamin B6 status were included in Appendix A, which contains 16 studies undertaken in the USA, on the mean concentration of vitamin B6 in breast milk from healthy lactating mothers. Data were reported for total vitamin B6, PL, PM, PN as well as their phosphorylated forms in breast milk samples collected between birth and about 8 months post-partum. Different analytical methods were used (high-performance liquid chromatography (HPLC), reversed-phase liquid chromatography (RPLC), ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) or microbiological methods).

In one study (Andon et al., 1989), no participants were supplemented. Five supplementation studies included a group with no supplementation (Thomas et al., 1979; Sneed et al., 1981; Morrison and Driskell, 1985; Styslinger and Kirksey, 1985; Hamaker et al., 1990). In the 10 remaining studies, the women were all supplemented, often with PN-HCl. Mean maternal total vitamin B6 intake (including diet and supplementation) ranged between < 2 and about 30 mg/day.

Data on biomarkers (plasma PLP, plasma vitamin B6 and erythrocyte alanine aminotransferase) of the mothers, and/or the infants and/or in cord blood were available for seven studies (Roepke and Kirksey, 1979; Morrison and Driskell, 1985; Borschel et al., 1986; Andon et al., 1989; Chang and Kirksey, 1990; Moser-Veillon and Reynolds, 1990; Lovelady et al., 2001).

In some studies, infants were full-term (Styslinger and Kirksey, 1985; Borschel et al., 1986; Kang-Yoon et al., 1992, 1995; Lovelady et al., 2001; Boylan et al., 2002). In the other studies, no information was provided on whether the infants were born at term or not, but a few of them gave some indications about the anthropometry of the infants (Andon et al., 1989; Chang and Kirksey, 2002).

For the estimation of the average concentration of vitamin B6 in breast milk, the Panel decided to consider only two studies. These studies were done in healthy unsupplemented lactating mothers with adequate vitamin B6 status (assessed as plasma PLP > 30 nmol/L) and information on their vitamin B6 intake, and providing mature milk analysed with the same method (microbiological assay, that measures all the different forms of vitamin B6) (Morrison and Driskell, 1985; Andon et al., 1989).

From the data collected from Andon et al. (1989) and the unsupplemented group of the study by Morrison and Driskell (1985) (n = 37 women in total), mean concentrations of vitamin B6 in mature breast milk ranged from 124 to 126 μg/L, and the average of this range was 125 rounded to 130 μg/L. Thus, the Panel considers that the average concentration of vitamin B6 in breast milk is 130 μg/L.

2.3.6.4. Conclusions on elimination

The Panel notes that urine is the main route for elimination of vitamin B6 (about 85–90% of the ingested vitamin B6). Based on data on mature milk from healthy unsupplemented lactating mothers, the Panel considers that the average concentration of vitamin B6 in breast milk is 130 μg/L.

2.3.7. Interaction with other nutrients

2.3.7.1. Riboflavin, niacin and zinc

The metabolism of vitamin B6 and the interconversion of different vitamin B6 forms to each other (Section 2.3.5) are dependent on riboflavin, niacin and zinc. Riboflavin is a cofactor for both pyridoxine (pyridoxamine) phosphate oxidase and aldehyde oxidase, whereas niacin is a cofactor for aldehyde dehydrogenase and zinc is a cofactor for pyridoxal kinase. A study in 41 older adults (mean age: about

77 years) showed that riboflavin supplementation at 1.6 mg/day for 12 weeks significantly increased ($p = 0.035$) mean plasma PLP concentration in those subjects who had plasma PLP < 20 nmol/L at baseline ($n = 4$) (Madigan et al., 1998). These findings are also supported by an *in vitro* investigation, which showed that the rate of conversion of PN to PLP in erythrocytes increased after a treatment with riboflavin (Perry et al., 1980).

2.3.7.2. Vitamin B6 and protein intake

Vitamin B6 is involved as a cofactor in non-proteogenic amino acid metabolism and four studies investigated whether protein intake can have an impact on the requirements for vitamin B6 intake.

In a cross-over study, Miller et al. (1985) fed eight young men (21–31 years; mean body weight of about 70 kg) with semipurified diets providing a constant intake of vitamin B6 at 1.6 mg/day and a protein intake at 0.5 ('low'), 1.0 ('medium') and 2.0 ('high') g/kg body weight (bw) per day. The volunteers received each diet for 15 days. Mean plasma PLP concentrations (Section 2.4) did not change significantly with the increase in protein content of the diet. Mean plasma PLP was lower than (but close to) 30 nmol/L with the 'high' protein intake, or above 30 nmol/L with the 'low' or 'medium' protein intake. These results suggest that vitamin B6 intake of 1.6 mg/day is sufficient to maintain mean plasma PLP close to or above 30 nmol/L, irrespective of the protein content of the diet. The Panel notes that, in this study in young men, mean plasma PLP concentrations did not change significantly with the increase in protein content of the diet.

In a cross-over study, Hansen et al. (1996a) fed nine healthy young women (mean ± SD: age of 26.8 ± 6.6 years, body weight 58.7 ± 4.6 kg) with diets providing a constant intake of vitamin B6 at 1.25 mg/day and a protein intake at 0.5 ('low'), 1.0 ('medium') and 2.0 ('high') g/kg bw per day. The volunteers received each diet for 15 days in a random order. Mean plasma PLP concentration was significantly higher ($p < 0.05$) with the 'low' protein intake than with the 'high' protein intake. Mean plasma PLP concentration with the 'medium' protein intake (below 30 nmol/L as reported in a figure) was not statistically different from that at 'low' or 'high' protein intake. Six and seven subjects had plasma PLP concentrations below 30 nmol/L after the periods of 'medium' and 'high' protein diets, respectively. These results suggest that, for 'high' protein intake, the requirements for vitamin B6 intake might be above 1.25 mg/day in young women. The Panel notes that, in this study in young women, mean plasma PLP concentrations were significantly higher with a daily protein intake of 0.5 g/kg bw compared to 2.0 g/kg bw.

In a depletion/repletion study (Ribaya-Mercado et al., 1991), six male and six female apparently 'healthy' older adults (61–71 years; mean body weight of about 95 kg (men) and 66 kg (women)) were split to receive a diet providing a daily protein intake of either 1.2 g/kg bw (four men, four women) or 0.8 g/kg bw (two men[10], two women) \two women). After a 20-day depletion period with an average vitamin B6 intake of 0.17 mg/day (men) and 0.1 mg/day (women), the volunteers went through three consecutive repletion periods of 21 days. During these repletion periods, vitamin B6 was provided at about 1.3, 2.0 and 2.9 mg/day (1.2 g protein/kg bw) or 1.2, 1.7, 2.5 mg/day (0.8 g protein/kg bw) for men and at about 0.9, 1.3 and 1.9 mg/day for women (for both protein intake). Mean plasma PLP concentration for both sexes dropped from around 33–42 nmol/L at baseline to 7.5–14 nmol/L at the end of the depletion period. For subjects receiving 1.2 g protein/kg bw per day, vitamin B6 intake of around 2.0 mg/day (men) and 1.9 mg/day (women) was required to achieve mean plasma PLP concentrations above 30 nmol/L, whereas PLP concentrations in all participants were less than 30 nmol/L with vitamin B6 intakes of 1.3 mg/day. For subjects receiving 0.8 g protein/kg bw per day, plasma PLP concentrations returned back to the baseline values (above 30 nmol/L) at vitamin B6 intake of about 1.3 mg/day for both men and women. The Panel notes that, in this study in older men and women, with a vitamin B6 intake of about 1.3 mg/day, plasma PLP concentrations were higher with a daily protein intake of 0.8 g/kg bw compared to 1.2 g/kg bw.

In a randomised cross-over study with a wash-out period of at least 3 weeks, Pannemans et al. (1994) compared the responses of vitamin B6 biomarkers to two different levels of protein and similar vitamin B6 intakes in healthy younger adults ($n = 29$, including 10 women, mean ± SEM: 29 ± 1 years) and older adults ($n = 26$, including nine women, mean ± SEM: 70 ± 1 years). Younger and older adults received diets containing 12% (Diet A, 0.9–1 g protein/kg bw per day) or 21% (Diet B, 1.5–1.8 g protein/kg bw per day) of total energy as protein for 3 weeks. The corresponding vitamin B6 intakes remained constant between 1.5 mg/day (Diet A) and 1.7 mg/day (Diet B). In younger adults, the level of protein intake did not have an effect on vitamin B6 biomarkers (plasma

[10] The paper, however, reports the results for one man and two women.

PLP, PL and total vitamin B6 concentrations, Section 2.4), whereas in older adults, mean plasma PLP concentration was significantly higher ($p < 0.01$) with Diet B (32 ± 3 nmol/L) compared to Diet A (27 ± 3 nmol/L). The Panel notes that, in the older adults of this study, mean plasma PLP concentration was significantly higher with a daily protein intake of 1.5–1.8 g/kg bw compared to 0.9–1 g/kg bw.

2.3.7.3. Conclusions on interactions with other nutrients

The Panel notes that there is evidence that riboflavin intake may have an impact on plasma PLP concentrations.

The four intervention studies available on vitamin B6 intake/status and protein intake were undertaken in young men or women, or in older adults, consuming controlled daily intakes that ranged between 0.5 and 2 g/kg bw for protein and mainly between 0.9 and 2.9 mg/day for vitamin B6 (constant intake of vitamin B6 or intake in the repletion phase). The Panel notes that there is no consistent evidence from these four intervention studies, undertaken in different age and sex groups, on the relationship between plasma PLP concentrations and protein intake. Therefore, given the inconsistent results, the Panel considers that there is no conclusive evidence that vitamin B6 requirements change according to protein intake in the range of observed intake in Europe (EFSA NDA Panel, 2012).

2.4. Biomarkers

2.4.1. Biomarkers of intake and status

2.4.1.1. Plasma pyridoxal 5′-phosphate (PLP)

Large cross-sectional studies conducted in different age groups among children and adults in Europe or the USA showed that vitamin B6 intake (estimated by food consumption data in combination with data from food composition databases) significantly correlates with plasma PLP concentrations (van der Wielen et al., 1996; Brussaard et al., 1997a,b; Bates et al., 1999a; Morris et al., 2008; Kerr et al., 2009). In a representative sample of the US population, an increase in total daily vitamin B6 intake of 1 mg corresponds to an increase of plasma PLP by about 12 nmol/L, after adjustments for potential confounders ($r = 0.32$, $p < 0.001$, least-square geometric mean concentration for 32 intake categories) (Morris et al., 2008). In this study, subjects were aged 1 year and above, and mean vitamin B6 intake was 1.86 ± 0.02 mg/day from foods and 1.94 ± 0.02 mg/day from foods and supplements (i.e. total vitamin B6 intake).

In an intervention study in adults, linear regression analysis also showed a significant positive relationship between plasma PLP concentration and vitamin B6 intake ($r = 0.56$, $p \leq 0.001$) (Huang et al., 1998). Combining data from tightly controlled intervention studies in adults who received graded amounts of vitamin B6, and accounting for the differences in the bioavailability (Section 2.3.1) of the vitamin in foods (which content was analytically determined) and supplements, there was a positive relationship ($r = 0.879$) between vitamin B6 intake[11] and plasma PLP concentration (Hansen et al., 2001) (Section 5.1.1.1).

Vitamin B6 intake much above the dietary range (i.e. 40 mg/day) increased more than 10-fold the mean plasma PLP concentration measured 3 days after supplementation (Bor et al., 2003). In contrast, for a vitamin B6 intake within the dietary range, it is widely accepted that a steady state of plasma PLP concentration is reached within 1–2 weeks (Tarr et al., 1981; Leklem, 1990), however, the Panel considers that this evidence is weak.

In intervention studies with controlled intakes in different age and sex groups, there is no conclusive evidence that vitamin B6 requirements change according to protein intake in the range of observed intake in Europe (Section 2.3.7.3).

Fasting plasma PLP concentrations in adults were found to relate to vitamin B6 body stores estimated by the difference between the influx of vitamin B6 in the circulation and its urinary excretion before or after vitamin B6 administration (Lui et al., 1985). In order to be able to calculate precisely the amount of vitamin B6 available for storage, this study used an intravenous route for the administration of vitamin B6 instead of the dietary route. However, direct evidence that plasma PLP correlates with the PLP in the tissue (skeletal muscle) was provided by animal studies (Lumeng et al., 1978).

[11] Range of vitamin B6 intake adjusted for bioavailability: about 0.5–3.5 mg/day (read on figure).

Adolescent or young adult males have higher plasma PLP concentration than adolescent or young adult females, but this is not always observed in children and older adults; this sex difference may be explained by hormonal reasons (Löwik et al., 1989; Bates et al., 1999a; Morris et al., 2008; Kerr et al., 2009).

Plasma PLP concentration is reported to decline with age. In cohort studies, in males (from teen years up to 90 years) not taking supplements, plasma PLP concentration decreases by approximately 4 nmol/L per decade (Rose et al., 1976a; Morris et al., 2008). Cross-sectional studies, including a large multicentre study in 11 European countries, found high prevalence (16–24%) of plasma PLP concentrations below 20 nmol/L in adults aged 50 years and over (Haller et al., 1991; van der Wielen et al., 1996; Brussaard et al., 1997a; Bates et al., 1999a). In contrast, cross-sectional studies showed low prevalence (0.5–5%) of plasma PLP concentrations below 20 nmol/L in adults aged less than 50 years and adolescents (Brussaard et al., 1997a; Bates et al., 1999b). Comparing two British national surveys in subjects aged 4–18 years (n = 1,006) or 65 years and over (n = 919), geometric mean plasma PLP concentration in children was significantly higher than in older adults (56.5 vs 34.0 nmol/L, $p < 0.0001$) (Bates et al., 1999b). The age-related decline in plasma PLP concentration in adults remained even after adjustments for confounders, such as dietary vitamin B6 intake (Morris et al., 2008). According to the available literature, this decline in plasma PLP concentration may be attributed to reasons including increased vitamin B6 catabolism and decreased protein binding capacity of plasma with advancing age that leads to increase in free PLP (unbound to albumin) in plasma and its subsequent destruction.

Pregnancy has been associated with 'low' plasma PLP concentrations. Studies reported up to 65–75% lower plasma PLP concentration in third-trimester pregnant women than in age-matched non-pregnant controls (Cleary et al., 1975; Trumbo and Wang, 1993). The 'low' plasma PLP concentration in the third trimester cannot be explained by the blood volume expansion and increased glomerular filtration rate, because these processes are intensive in the earlier stages of pregnancy and their rate is comparatively stable in the third trimester (Blackburn, 2013). Simultaneously with the depression of plasma PLP concentration during pregnancy, some studies indicated that the plasma PL concentration in pregnant women was significantly higher than in non-pregnant women, whereas the urinary concentration of the catabolic product 4-PA was not different (Barnard et al., 1987; Trumbo and Wang, 1993). However, other studies did not provide the same results (Contractor and Shane, 1970).

Lifestyle factors, such as smoking, alcohol consumption and physical activity, can also influence plasma PLP concentrations. Current smokers have been reported to have significantly lower plasma PLP concentrations than non-smokers (Vermaak et al., 1990; Ulvik et al., 2010), even at similar vitamin B6 intake (Giraud et al., 1995). In adults with a mean alcohol consumption of 19 (men) and 3 (women) g/day, alcohol consumption was associated with higher plasma PLP concentrations even after adjustment for vitamin B6 intake (van der Wielen et al., 1996). However, high prevalence of plasma PLP concentrations below 20 nmol/L has been found in chronic alcoholics with low vitamin B6 intake at the same time (Lumeng and Li, 1974; Bonjour, 1980). During intensive running and cycling in trained or untrained individuals, there was an increase in plasma PLP concentration by 10–35%, with a subsequent decrease and a corresponding increase in the urinary losses of vitamin B6 derivatives 30–60 min after the end of the exercise (Leklem and Shultz, 1983; Manore et al., 1987). The observed changes in plasma PLP concentration with exercise have been related to the mobilisation of PLP from the muscles where it is bound to glycogen phosphorylase (Section 2.2.1.3). Thus, exercise is considered to increase the turnover and losses of vitamin B6, but these losses are negligible (Woolf and Manore, 2006).

Studies show that inflammatory conditions (Friso et al., 2001; Gori et al., 2006; Morris et al., 2010) and increased concentration of inflammatory markers in the circulation (Bates et al., 1999b; Morris et al., 2010) are associated with 'low' plasma PLP concentration. However, the depressed plasma PLP concentrations in inflammatory conditions are not linked to insufficient intake of the vitamin, but rather to metabolic phenomenon inherent to inflammation with mobilisation of PLP in the sites of inflammation (Paul et al., 2013).

Analytical methods for measurement of plasma PLP concentrations include enzymatic (tyrosine decarboxylase), HPLC and LC–MS/MS based assays. An interlaboratory comparison of HPLC or enzymatic measurements of serum PLP concentration has shown a good agreement among methods, but some differences in laboratory proficiency (Rybak et al., 2005). Therefore, the plasma/serum PLP values produced by different laboratories should be compared with caution.

Different cut-off values for plasma PLP concentrations have been used to define an adequate vitamin B6 status. Lumeng and Li (1974) arbitrarily adopted a cut-off of 20 nmol/L, based on the

lowest plasma PLP concentration found in 94 unsupplemented men (18–68 years), medically confirmed to be free of chronic and acute illnesses and consuming self-selected diets. However, in 60 healthy unsupplemented women (19–50 years) consuming a self-selected diet, Hansen et al. (2001) determined a cut-off of 30 nmol/L by applying a previously developed statistical approach (Sauberlich, 1999). This approach defines an adequate status for a certain micronutrient as corresponding to values above the 30th percentile for the respective biomarker, in a reference population group. A study undertaken under controlled conditions showed that a mean PLP concentration below 30 nmol/L is associated with some unfavourable metabolic effects in adults (Gregory et al., 2013). These healthy young adults (n = 23; 12 men and 11 women with a mean age of about 25 years) received a diet with restricted vitamin B6 content (0.37 ± 0.04 mg/day) for 28 days. Plasma PLP concentration significantly decreased from (mean ± SD) 52 ± 14 nmol/L at baseline to 21 ± 5 nmol/L (range: 12.3–29.3 nmol/L) at the end of the intervention period ($p < 0.05$). This study also showed a wide range of metabolic effects including perturbations of amino acid, lipid and organic acid profiles in plasma (Gregory et al., 2013; da Silva et al., 2013). PLP concentrations in the range of 20–30 nmol/L were suggested to correspond to a marginal vitamin B6 status, whereas PLP concentrations above 30 nmol/L were considered indicative for an adequate status (da Silva et al., 2014).

The Panel considers that plasma PLP concentrations reflect vitamin B6 intake and status in younger and older adults and children. The Panel notes that plasma PLP concentrations decline with age, during pregnancy (compared to non-pregnant women) and inflammatory conditions. The Panel notes the lack of consensus in the criteria used to define adequate vitamin B6 status based on plasma PLP concentrations. However, the Panel takes into account that mean values below 30 nmol/L are associated with a wide range of metabolic effects including perturbations of amino acid, lipid, and organic acid profiles in plasma (Gregory et al., 2013). Thus, the Panel considers that plasma PLP concentration of 30 nmol/L as a population mean is indicative of an adequate vitamin B6 status. Although the evidence for the suitability of the cut-off value for plasma PLP concentration reflecting adequate vitamin B6 status was provided from a study in young adults, the Panel considers to use the same value also for older adults and children.

2.4.1.2. Other vitamin B6 derivatives in blood

The concentrations of total vitamin B6 in plasma (a combined measurement of all vitamin B6 derivatives), of individual vitamin B6 derivatives (PL and PMP) in plasma or erythrocytes, and of PLP in erythrocytes, as well as ratios of concentrations in plasma (PLP, PL and PA) have been assessed in adults (Miller et al., 1985; Pannemans et al., 1994; Hansen et al., 1997, 2001; Huang et al., 1998; Masse et al., 2004; Vasilaki et al., 2008; Ulvik et al., 2014). However, criteria for adequacy of these biomarkers have not been developed and their usefulness for assessment of vitamin B6 status is limited.

The Panel considers that the concentration of total vitamin B6 in plasma, the concentration of PL and PMP in plasma or erythrocytes, the concentration of PLP in erythrocytes, as well as ratios of concentrations of vitamin B6 forms in plasma, are not suitable biomarkers of vitamin B6 intake and status.

2.4.1.3. 4-pyridoxic acid (4-PA) and total vitamin B6 in urine

Urinary 4-PA provides a measure of the end product of vitamin B6 metabolism and accounts for 85% of all vitamin B6 derivatives excreted through the urine (Lui et al., 1985). With controlled diets with graded amounts of vitamin B6 (from 0.05 to 2.7 mg/day), 4-PA and total vitamin B6 concentrations in urine changed with change in vitamin B6 intake (Kretsch et al., 1995) and there was a significant correlation between these concentrations and vitamin B6 intake ($r = 0.673–0.858$ for urinary total vitamin B6, r of about 0.94 for urinary 4-PA, $p < 0.05$) (Hansen et al., 1997, 2001). A cross-sectional study in Dutch adults also reported a positive significant relationship ($r = 0.50$ in men, 0.40 in women, $p < 0.05$) between urinary 4-PA concentration and vitamin B6 intake (intake not reported as such, measured by a food frequency questionnaire (FFQ) and a 3-day dietary record) (Brussaard et al., 1997b).

Urinary 4-PA concentration significantly decreased with increased protein content of the diet in cross-over studies in younger men (Miller et al., 1985) or women (Hansen et al., 1996a) ($p < 0.01$) (Section 2.3.7.2). In young men with a constant intake of vitamin B6 (1.6 mg/day), the urinary excretion of 4-PA was about 46% of ingested vitamin B6 when the daily protein intake was 0.5 g/kg bw, but this was reduced by about 17% with the daily protein intake of 2.0 g/kg bw (Miller et al., 1985). However, studies in older adults failed to confirm the inverse relationship between urinary 4-PA

concentration and protein intake (Ribaya-Mercado et al., 1991; Pannemans et al., 1994) (Section 2.3.7.2).

Total vitamin B6 and 4-PA concentrations in urine respond rapidly to changes in vitamin B6 intake (2.3–10.3 mg/day) with a steady state achieved within 7 days (Lee and Leklem, 1985). In intervention studies, including some with a depletion/repletion design, with a wide range of vitamin B6 intake administered (0.05–2.7 mg/day), urinary 4-PA concentration paralleled well the changes in plasma PLP concentration (Brown et al., 1975; Kretsch et al., 1995; Hansen et al., 1997, 2001; Huang et al., 1998) (Section 5.1.1). In these studies, it significantly decreased during the depletion phase compared to baseline and significantly increased during the repletion phase. However, in four men (23–30 years) receiving daily intravenous injections of vitamin B6 (PN-HCl at 122 μmol/day, equal to 25 mg/day) for 4 weeks, urinary 4-PA concentration progressively increased and reached a steady-state 10 days after the initiation of the injections (Lui et al., 1985) (Section 2.4.1.3). Then, urinary 4-PA concentration declined sharply to the baseline level after 5 days of discontinuation of the injections, despite the high vitamin B6 status of the subjects, evident by the elevated plasma PLP concentrations (higher than baseline values) maintained in the course of the following 2 months. The discrepancy between these findings is probably a result of the greater length of the intervention with higher amounts of B6 administered, and the subsequent longer monitoring of both plasma PLP and urinary 4-PA concentrations in the study by Lui et al. (1985), in comparison with the other intervention studies. This suggests that urinary 4-PA concentration reflects well only the recent vitamin B6 intake, but it is not a good biomarker of status.

Urinary excretion of 4-PA is higher in males compared with females, after adjustment for dietary intake of vitamin B6 (Brussaard et al., 1997a). Urinary total vitamin B6 concentrations, but not 4-PA concentrations, decline with age in adults. In postmenopausal (mean ± SD: 55.3 ± 4.0 years) and young (24.4 ± 3.2 years) women consuming diets providing 2.3 or 10 mg/day of vitamin B6, at every sampling week, older women consistently had lower urinary concentration of total vitamin B6 (by 20%) (difference statistically significant only at 2.3 mg/day vitamin B6), but similar urinary concentration of 4-PA, compared with younger women (Lee and Leklem, 1985).

Based on studies with controlled vitamin B6 intake, and assuming that a vitamin B6 intake of 1.25–1.5 mg/day is nutritionally adequate as it corrects abnormal tryptophan metabolism, Shultz and Leklem (1981) determined that the urinary excretion of 4-PA > 5 μmol/day and of total vitamin B6 > 0.6 μmol/day in both males and females correspond to 'adequate' vitamin B6 status. However, the method (Shultz and Leklem, 1981; Leklem, 1990) for defining these criteria has been criticised, as it predetermines that the vitamin B6 intake required to reach the cut-off for the urinary excretion of 4-PA is also the one required for the achievement of an adequate vitamin B6 status (IOM, 1998). The Panel supports this criticism and does not agree that the previously determined cut-offs for urinary 4-PA and total vitamin B6 reflect adequate vitamin B6 status.

Urinary 4-PA concentration provides a measure of the end product of vitamin B6 metabolism. The Panel concludes that urinary total vitamin B6 and 4-PA concentrations are biomarkers that reflect the recent vitamin B6 intake (i.e. of the last 5–7 days). The Panel considers that total vitamin B6 and 4-PA concentrations in urine are not reliable biomarkers of vitamin B6 status, as there is a sharp decline in urinary 4-PA concentration to the baseline level after 5 days of discontinuation of daily vitamin B6 injections, while plasma PLP concentrations higher than baseline values were maintained in the course of the following 2 months (Lui et al., 1985). The Panel also notes that there are no well-accepted criteria for their adequacy, which limits their interpretation.

2.4.2. Biomarkers of function

2.4.2.1. Erythrocyte aminotransferase stimulated activities

Erythrocyte aminotransferase enzymes, such as erythrocyte aspartate aminotransferase (EAST) and erythrocyte alanine aminotransferase (EALT), require PLP as a cofactor. The degree of saturation of the enzyme with the cofactor PLP can provide indirect information on the vitamin B6 status. This can be determined by the respective activation coefficients of these enzymes (α-EAST and α-EALT), expressed as the ratio of the enzyme activity measured with and without the cofactor PLP. As the enzyme synthesis does not occur in mature erythrocytes and the erythrocytes' life span is around 120 days, α-EAST and α-EALT have been considered as long-term biomarkers of vitamin B6 function (Bitsch, 1993).

The value of α-EAST and α-EALT increases with the decline of vitamin B6 intake. Compared to EAST, the erythrocyte EALT activity is more sensitive to changes of vitamin B6 intake (70% increase reached in 5 weeks, compared to 46% increase reached in 3–7 weeks) and better parallels the

response of plasma PLP concentration to such changes in adults (Brown et al., 1975; Kretsch et al., 1995). However, EAST is more frequently used as a biomarker, because the activity of EALT is low, i.e. only 5% of that of EAST, and EALT is prone to destruction in stored frozen samples (Bitsch, 1993).

In observational studies in adults, the inverse correlation between α-EAST or α-EALT and vitamin B6 intake in mg/g of protein was statistically significant, but weak (r from −0.14 to −0.16, $p < 0.05$) (Löwik et al., 1989; Brussaard et al., 1997b).

Intervention studies in adults, with controlled vitamin B6 intake and using α-EAST and α-EALT as biomarkers of vitamin B6 function, showed inconsistent results despite the similarity of their design. Some of them showed that α-EAST and α-EALT responded to changes in vitamin B6 intake and status (plasma PLP concentration) (Kretsch et al., 1995; Huang et al., 1998), whereas others did not show any significant differences in α-EAST and α-EALT according to changes in vitamin B6 intake (Brown et al., 1975; Hansen et al., 1997, 2001). The reason for this discrepancy in results is unknown, but probably is related to the short durations of vitamin B6 interventions (2–4 weeks), which were insufficient to elicit stable responses of α-EAST and α-EALT.

α-EAST is inversely and significantly associated with alcohol consumption even after adjustment for vitamin B6 intake (in populations with mean alcohol consumption of 17 g/day and 8 g/day for men and women, respectively) (Löwik et al., 1990). It was suggested that alcohol affects directly the apoenzyme or the binding site of PLP to the apoenzyme (Bonjour, 1980). A lower mean EAST activity has also been reported in unsupplemented older adults (65–79 years) (Löwik et al., 1989), compared to published data on younger adults (18–65 years) (difference not statistically tested). α-EAST is not affected by protein intake (Ribaya-Mercado et al., 1991; Pannemans et al., 1994).

There are no standardised criteria for assessing the adequacy of α-EAST and α-EALT. The cut-off values used in different studies were determined in reference groups of healthy people. However, the selection of these reference groups was not based on strict and well-defined criteria, which resulted in a huge variability in the cut-off values. For example, for α-EAST, cut-off values from < 1.8 (Leklem, 1990) to 2.0 (Rose et al., 1976a; Vuilleumier et al., 1983) and 2.28 (Tolonen et al., 1988) have been applied. The lack of agreement for the criteria of interpretation limits the usefulness of these biomarkers as biomarkers of vitamin B6 function.

The Panel notes that the value of α-EAST and α-EALT increases with the decline of vitamin B6 intake, but considers that there are insufficient data to support the use of α-EAST and α-EALT as biomarkers of vitamin B6 function. In addition, the Panel notes the lack of agreement on the criteria for the assessment of their adequacy.

2.4.2.2. Tryptophan catabolites

Tryptophan catabolic pathway involves several PLP-dependent enzymes. The measurement of the urinary excretion of tryptophan metabolites after tryptophan load is a widely used test for assessment of vitamin B6 function. In case of vitamin B6 'insufficiency', the activities of the PLP-dependent enzymes are affected, which results in an increased urinary excretion of tryptophan metabolites such as xanthurenic and kynurenic acids.

Intervention studies in adults with controlled vitamin B6 intake found that urinary excretion of tryptophan metabolites after a tryptophan load were responsive to vitamin B6 intake (Baker et al., 1964; Yess et al., 1964; Miller and Linkswiler, 1967; Ribaya-Mercado et al., 1991; Kretsch et al., 1995; Hansen et al., 1997). In young women ($n = 9$), urinary concentration of xanthurenic acid was significantly correlated with vitamin B6 intake (0.84–2.39 mg/day) ($r = -0.583$, $p < 0.001$), contrary to urinary concentration of kynurenic acid (Hansen et al., 1997).

The tryptophan loading test has been administered by different protocols with tryptophan doses from 2 to 10 g, which creates difficulties in the comparison of results between studies and their interpretation. Moreover, various factors unrelated to vitamin B6 could interfere with the urinary excretion of tryptophan metabolites. As some of the enzymes in the tryptophan catabolic pathway are under the influence of steroid hormones, a transient increase in glucocorticoid hormone levels can provoke a higher urinary excretion of tryptophan catabolites and might be falsely diagnosed with vitamin B6 deficiency (Coon and Nagler, 1969). Elevated urinary concentrations of xanthurenic and kynurenic acids have been reported as a result of indirect influence of bacterial endotoxins and viral infections on tryptophan metabolism (Brown et al., 1987). Tryptophan catabolism can be inhibited by oestrogen metabolites even when vitamin B6 status is 'adequate', which makes the tryptophan loading test inappropriate for pregnant women and those taking oral contraceptives (Bender, 1987). Other factors that could affect the concentration of xanthurenic acid include protein intake, exercise and lean body mass (Bender, 1987).

Based on studies with controlled vitamin B6 intake and assuming that vitamin B6 intake of 1.25–1.5 mg/day is nutritionally adequate, Leklem (1990) considered that urinary xanthurenic acid excretion of less than 65 μmol/day after a load of 2 g L-tryptophan may correspond to adequate vitamin B6 status. However, the method for defining these criteria for urinary xanthurenic acid excretion has been criticised, since it predetermines that vitamin B6 intake of 1.25–1.5 mg/day is required for vitamin B6 adequacy (IOM, 1998). The Panel supports this criticism and does not agree that the previously determined cut-off for urinary xanthurenic acid reflects adequate vitamin B6 function.

Plasma concentrations and ratios of different tryptophan metabolites have been recently proposed as biomarkers for vitamin B6. In a large randomised clinical trial of 2,584 patients with coronary vascular diseases (stable angina pectoris and aortic stenosis), ratios of 3-hydroxykynurenine to xanthurenic acid, 3-hydroxylanthranilic acid and kynurenic acid were significantly correlated with plasma PLP concentrations (non-linear association) and these ratios were responsive to vitamin B6 supplementation (40 mg/day PN-HCl) (Ulvik et al., 2013). Although the results of a mathematical modelling approach using kinetic constants obtained from different species, including rats, mice, and humans, supported these findings (Rios-Avila et al., 2013), the validity of the ratios of tryptophan metabolites in plasma as reliable vitamin B6 biomarkers of function requires to be confirmed in healthy populations with a broad range of vitamin B6 intake.

The Panel notes that the urinary excretion of tryptophan catabolites after the tryptophan loading test is subject to various confounders, especially in pregnant women and those taking oral contraceptives. The Panel also notes the lack of a standardised protocol for the administration of the tryptophan loading test and that there are no well-accepted criteria for adequacy, which limits the interpretation of the results. Thus, the concentrations of tryptophan catabolites in urine after tryptophan loading test are not reliable biomarkers for the assessment of vitamin B6 function. The Panel considers that there are also insufficient data to support the use of the ratios of tryptophan metabolites in plasma as vitamin B6 biomarkers.

2.4.2.3. Metabolites of transsulfuration pathway

The transsulfuration pathway, which is part of the methionine cycle, involves two PLP-dependent enzyme steps converting Hcy to cystathionine and cysteine, and is activated by methionine intake or methionine load. In vitamin B6 insufficiency (plasma PLP < 30 nmol/L) or dietary depletion (0.16 mg/day), plasma total homocysteine (tHcy) concentration increased after a methionine loading test compared to baseline (Ubbink et al., 1996), with rise in the urinary excretion of homocystine (oxidative and stable product of Hcy) and cystathionine (Park and Linkswiler, 1970). Although folate is the main determinant of Hcy under fasting conditions, an epidemiological study showed significantly higher mean tHcy concentrations in the lowest decile of PLP concentration compared with the highest ($p < 0.01$) in non-fasting plasma samples (Selhub et al., 1993).

The monitoring of the concentrations of tHcy in plasma and cystathionine in urine after a methionine load has been used as biomarker of vitamin B6 function in a limited number of studies and with different protocols (Park and Linkswiler, 1970; Shin and Linkswiler, 1974; Linkswiler, 1981). Based on a depletion/repletion study in adults receiving a controlled vitamin B6 intake, Linkswiler (1981) found urinary cystathionine concentration of less than 350 μmol/day after a methionine loading dose of 3 g to be indicative of an adequate vitamin B6 function (confirmed by other biomarkers such as plasma PLP and urinary 4-PA concentrations). However, this cut-off is relevant only to the specific protocol of administration of the test and it is based on a single reference that has not been confirmed by others. No criteria for adequacy based on the increase in plasma Hcy concentrations after methionine load have been reported.

An intervention study with restricted vitamin B6 intake (< 0.5 mg/day for 4 weeks) in nine young men and women (20–30 years), which resulted in plasma PLP concentrations < 30 nmol/L, showed a simultaneous significant increase in plasma cystathionine concentration compared to baseline ($p < 0.001$) (Davis et al., 2006). The Panel notes that more data are required to determine whether plasma cystathionine concentration is a sensitive biomarker of vitamin B6 intake, status or function.

The Panel notes that urinary concentration of cystathionine and plasma Hcy concentration after a methionine load have been used only in a limited number of studies and that various versions of the protocol of the methionine loading test exist. Although a criterion for adequacy for urinary cystathionine excretion after a 3 g methionine loading dose was determined (Linkswiler, 1981), this is based on a single study and has not been confirmed by others. The Panel considers that there are insufficient data to support the use of urinary cystathionine excretion and plasma Hcy concentration after a methionine load as biomarkers of vitamin B6 function. There are insufficient data to support

the use of the plasma cystathionine concentration as a biomarker of vitamin B6 intake, status or function.

2.4.2.4. Other biomarkers of function

Vitamin B6 is involved in immune and inflammatory responses, and variations of vitamin B6 intake and the corresponding changes in plasma PLP concentration were reported to be associated with changes in some immune markers.

In a depletion/repletion intervention study with controlled vitamin B6 intake, 24 young healthy men (mean age: 23 ± 2.6 years) received a diet supplemented with vitamin B6 (4 mg PN-HCl) for 2 weeks and then followed a depletion phase for 11 weeks (n = 12) or continued with the supplemented diet (n = 12) (van den Berg et al., 1988). The depletion diet was 'adequate' in respect of energy and all other nutrients but providing a low vitamin B6 intake (0.67 mg/day). There was a drop in mean plasma PLP from 78 to 17 nmol/L in the depletion group, together with a significantly lower number of T-helper cells in the depletion group compared to the controls and a significantly lower (decreased) concentration of immunoglobulin D ($p < 0.05$). There was no significant change in the other parameters investigated (e.g. total lymphocytes, total T-cells, other immunoglobulins).

In a metabolic study, seven healthy young women (mean age: 28 ± 6 years) underwent a 7-day adjustment period with vitamin B6 intake of 1.0 mg/day, followed by three successive 14-day experimental periods providing total vitamin B6 intake of 1.5, 2.1 and 2.7 mg/day (Kwak et al., 2002). There was a significant positive correlation between plasma PLP concentration and lymphocyte proliferation (i.e. mitogenic response to three different phytohaemagglutinin concentrations, $r = 0.393–0.456$, $p < 0.01$). The maximum lymphocyte proliferation was achieved with vitamin B6 intake at 2.1 mg/day and mean plasma PLP concentration at 40 nmol/L, and no further increase in the lymphocyte response was observed with a vitamin B6 intake of 2.7 mg/day.

In a metabolic depletion/repletion study on eight apparently healthy older adults (four men and four women) aged ≥ 61 years, subjects received during the 20-day depletion phase a vitamin B6 intake adjusted for body weight and equivalent to a mean intake of 0.17 mg/day and 0.10 mg/day in men and women, respectively (Meydani et al., 1991). The depletion phase was followed by repletion phases (each of 21 days), at mean intakes of 1.34, 1.96 and 2.88 mg/day in men and 0.89, 1.29 and 1.90 mg/day in women. The depletion diet decreased lymphocyte proliferation (i.e. response to two T-cell mitogens and a B-cell mitogen) and interleukin-2 production, while vitamin B6 intake at 1.90 mg/day for women and 2.88 mg/day for men restored the impaired immune parameters to the baseline values.

The Panel acknowledges that vitamin B6 has a role in the immune responses. However, the studies so far have focused on the effect of vitamin B6 intake and status (assessed by plasma PLP concentration) on immune parameters, such as lymphocyte proliferation, number of T-helper cells, immunoglobulin D concentration and interleukin-2 production. These parameters are also well known to respond to other factors and to perturbations in the status of various other micronutrients and cannot be considered specific for the assessment of vitamin B6 function and status. Therefore, the Panel considers that immune factors are not specific to the effect of vitamin B6 and cannot be used as biomarkers for vitamin B6 function.

2.4.3. Conclusions on biomarkers

The Panel concludes that all biomarkers of vitamin B6 intake, status or function are subject to limitations (e.g. affected by confounders and lack of strict criteria for their adequacy). The Panel considers that the most suitable biomarker for deriving DRVs for vitamin B6 is plasma PLP concentration, as it is the only one that reflects the tissue stores of vitamin B6 (biomarker of status) and has a defined cut-off value for an adequate vitamin B6 status. The Panel considers a plasma PLP concentration of 30 nmol/L, as a population mean, to be indicative of an adequate vitamin B6 status in both adults and children.

2.5. Effects of genotypes

Mutations of the genes encoding vitamin B6 metabolising enzymes, such as pyridoxine (pyridoxamine) phosphate oxidase and pyridoxal kinase (Section 2.3.5), were related to defective enzyme activities (Mills et al., 2005). The phenotype is presented with neonatal onset seizures and the surviving children are severely mentally retarded. The seizures are resistant to the classical

anticonvulsive therapy, but are responsive to pharmacological doses of PLP (10–85 mg/kg bw per day) (Mills et al., 2005, 2014).

The Panel considers that mutations of genes encoding vitamin B6 metabolising enzymes are associated with severe clinical manifestations (seizures, mental retardation) and therefore cannot be used for setting DRVs for vitamin B6.

3. Dietary sources and intake data

3.1. Dietary sources

Foods rich in vitamin B6 include grains (whole grain corn/maize, brown rice, sorghum, quinoa, wheat germ), pulses, nuts, seeds, potatoes, some herbs and spices (e.g. garlic, curry, ginger), meat and meat products (e.g. poultry, pork, liver), fish (FAO/INFOODS, online).

Currently, PN-HCl and PNP may be added to both foods[12] and food supplements,[13] whereas PLP may be added to food supplements and pyridoxine dipalmitate to foods. The vitamin B6 content of infant and follow-on formulae and of processed cereal-based foods and baby foods for infants and children is regulated.[14]

3.2. Dietary intake

EFSA estimated dietary intake of total vitamin B6 from food consumption data from the EFSA Comprehensive European Food Consumption Database (EFSA, 2011b), classified according to the food classification and description system FoodEx2 (EFSA, 2011a). This assessment includes food consumption data from 13 dietary surveys (Appendix B–F) from nine countries (Finland, France, Germany, Ireland, Italy, Latvia, the Netherlands, Sweden and the UK). Individual data from these nationally representative (except for the Finnish surveys in children) surveys undertaken between 2000 and 2012 were available to EFSA, and classified according to the FoodEx2 food classification system (EFSA, 2011a). Total vitamin B6 intake calculations were performed only on subjects with at least two reporting days. The data covers all age groups from infants to adults.

Composition data for vitamin B6 were derived from the EFSA Nutrient Composition Database (Roe et al., 2013) involving several national food database compiler organisations that were allowed to borrow compatible data from other countries in case no original composition data were available. Food composition information from Finland, France, Germany, Italy, the Netherlands, Sweden and the UK, and the respective consumption data were used to calculate the intakes in these countries, assuming that the best intake estimate would be obtained when both the consumption data and the composition data are from the same country. The amount of borrowed vitamin B6 values in the seven composition databases varied between 13.6% and 94.3%, although, in six out of the seven databases, the percentage of borrowed values was higher than 60%. For countries not having any food composition database, i.e. Ireland and Latvia, food composition data from the UK and Germany, respectively, were used. The EFSA Food Composition Database does not contain information on the content of the individual vitamers, but presents the total vitamin B6 content of foods. EFSA estimates are based on consumption of foods that may be fortified or not (and without taking dietary supplements into account), although no information was available specifically on the consumption of vitamin B6 fortified foods.

Data on infants (1–11 months) were available from Finland, Germany, the UK and Italy. The contribution of human milk was taken into account if the amounts of human milk consumed (Italian INRAN–SCAI survey and the UK DNSIYC survey) or the number of breast milk consumption events (German VELS study) were reported. In case of the Italian INRAN–SCAI survey, human milk consumption had been estimated based on the number of eating occasions, using standard portions per eating occasion. In the Finnish Type 1 Diabetes Prediction and Prevention survey (DIPP) study,

[12] Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods, OJ L 404, 30.12.2006, p. 26.

[13] Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements, OJ L 183, 12.7.2002, p. 51.

[14] Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, OJ L 401, 30.12.2006, p.1 and Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children, OJ L 339, 06.12.2006, p. 16.

only the information 'breastfed infants' was available, but without any indication about the number of breast milk consumption events during 1 day or the amount of breast milk consumed per event. For the VELS study, the total amount of breast milk was calculated based on the observations by Paul et al. (1988) on breast milk consumption during one eating occasion at different ages, i.e. the amount of breast milk consumed on one eating occasion was set to 135 g/eating occasion for infants aged 6–7 months and to 100 g/eating occasion for infants aged 8–12 months. The Panel notes the limitations in the methods used for assessing breast milk consumption in infants (table footnotes of Appendices C–D) and related uncertainties in the vitamin B6 intake estimates for infants.

Average total vitamin B6 intake (approximately) ranged from 0.4 to 0.8 mg/day (0.1–0.3 mg/MJ) in infants, from 0.9 to 1.3 mg/day (0.2–0.3 mg/MJ) in children aged 1 to < 3 years, from 1 to 1.6 mg/day (0.2–0.3 mg/MJ) in children aged 3 to < 10 years, and from 1.5 to 2.3 mg/day (0.2–0.3 mg/MJ) in children aged 11 to < 18 years. Average total vitamin B6 intake ranged between 1.4 and 3.1 mg/day (0.2–0.3 mg/MJ) in adults (≥ 18 years old) (Appendices C and D). Average daily intake was slightly higher among males compared to females mainly due to larger quantities of food consumed per day.

The main food groups contributing to vitamin B6 intake were 'food products for young population' in infants and meat and meat products, milk and dairy products, grains and grain-based products, fruit and fruit products, starchy roots and tubers, and products thereof in all age groups. In addition, vegetables and vegetable products contributed to the vitamin B6 intake in all above 3 years of age. Differences in main contributors to vitamin B6 intake between genders were in most cases minor.

4. Overview of Dietary Reference Values and recommendations

4.1. Adults

The German-speaking countries (D-A-CH, 2015) considered that deficiency in healthy adults on a mixed diet is not observed when vitamin B6 intake is about 1.2–2 mg/day (Sauberlich, 1964; Selhub et al., 1993). They also considered that vitamin B6 requirement depends on protein intake due to the role of this vitamin in amino acid metabolism (Sauberlich, 1964; Miller et al., 1985; Hansen et al., 1997). A ratio of 0.02 mg vitamin B6 per gram of recommended protein intake was considered. A protein intake higher than the recommended intake for protein would thus imply a higher recommended vitamin B6 intake. Adjusting for variability, the PRIs were set for men at 1.5 mg/day (19–64 years) and 1.4 mg/day (≥ 65 years), and 1.2 mg/day for women whatever the age.

The Nordic countries (Nordic Council of Ministers, 2014)[15] considered depletion–repletion studies with controlled intakes of vitamin B6, which used plasma PLP concentration with the cut-off value of 20 nmol/L that can be reached at intakes of 0.6–1.0 mg/day or around 0.01 mg/g dietary protein (Brown et al., 1975; Coburn et al., 1991; Ribaya-Mercado et al., 1991; van der Beek et al., 1994; Kretsch et al., 1995; Huang et al., 1998). The Nordic countries also noted that clinical symptoms of deficiency have not been observed in adults at intakes above 0.5 mg/day and, according to controlled studies (Miller et al., 1985; Hansen et al., 1996a), plasma PLP concentrations are related to protein intake in men and women. A Recommended Intake (RI) of 0.015 mg/g dietary protein was set for all adults. The RIs for each sex and age group were calculated based on the reference value for energy intake and assuming a protein content of the diet of 15% of energy intake (15 E%) up to 60 years of age, and of 18 E% for older adults, thus were 1.6 mg/day for men whatever the age, and 1.3 or 1.2 mg/day for women, respectively, below or above 30 years. The AR and the Lower Intake Level (LI) were set at 0.013 and 0.01 mg/g dietary protein, respectively, corresponding to 1.0 (women) and 1.3 (men) mg/day for ARs and 0.8 (women) and 1.0 (men) mg/day for LIs, but the weak scientific basis for the LI was highlighted.

The World Health Organization/Food and Agriculture Organization (WHO/FAO, 2004) set a Recommended Nutrient Intake (RNI) of 1.3 mg/day for adults aged 19–50 years. WHO/FAO mentioned that a daily vitamin B6 intake of 1.6 mg in men with various protein intakes led to a plasma PLP concentration above 30 nmol/L (Miller et al., 1985), and that data on tryptophan load test suggested that the vitamin B6 requirement for men was between 1.0 and 1.5 mg/day (Linkswiler, 1976). WHO/FAO also mentioned four studies on vitamin B6 status in women (Brown et al., 1975; Kretsch et al., 1995; Hansen

[15] Further abbreviated into NCM in tables.

et al., 1996a, 1997), which suggested that vitamin B6 requirement may be about 1.0–1.2 mg/day. Regarding adults aged 51 years and over, WHO considered the same studies as IOM (1998) (Ribaya-Mercado et al., 1991; Selhub et al., 1993) to conclude that the requirement of older adults was higher than for younger adults for maintaining plasma PLP concentrations higher than 20 nmol/L. Thus, RNIs of 1.7 mg/day and 1.5 mg/day for, respectively, men and women were set.

The Health Council of the Netherlands (2003) set an Estimated Average Requirement (EAR) based on studies on vitamin B6 status assessed by plasma PLP concentrations, the tryptophan load test and α-EAST in men (Harding et al., 1959; Baker et al., 1964; Yess et al., 1964; Miller and Linkswiler, 1967; Canham et al., 1969; Park and Linkswiler, 1970; Miller et al., 1985) and in women (Donald et al., 1971; Shin and Linkswiler, 1974; Brown et al., 1975; Driskell et al., 1989; Kretsch et al., 1995; Hansen et al., 1996b, 1997, 2001; Huang et al., 1998). The Council considered a coefficient of variation (CV) of 20% for setting the Recommended Dietary Allowances (RDAs), due to uncertainty about the variation in the requirement (Hansen et al., 2001). The RDAs of 1.5 mg/day for adults aged 19–50 years apply for a daily protein intake up to the upper value of the Dutch intake range, i.e. 150 g/day, above which 0.01–0.02 mg of extra vitamin B6 were recommended per additional gram of protein. Based on data in older adults, and considering a plasma PLP concentration of at least 20 nmol/L as a criterion for adequate vitamin B6 status, the RDAs were 1.8 mg/day for men and 1.5 mg/day for women aged 51 years and older.

The French food safety agency Afssa (2001) did not set vitamin B6 reference values based on protein intakes. Percentiles of vitamin B6 intakes and corresponding plasma PLP concentrations in a subgroup of 616 men and 678 women from the SU.VI.MAX study were used for calculating the reference values, which were compared with the values obtained from depletion–repletion studies. For both sexes, Afssa considered that a plasma PLP concentration of 30 nmol/L would correspond to a low risk of vitamin B6 deficiency (Leklem, 1990), and used a CV of 10% to calculate PRIs from the ARs. For men aged 19–74 years, two depletion–repletion studies (Miller et al., 1985; Driskell et al., 1988) were reported. At the 25th percentile of vitamin B6 intake in men of the SU.VI.MAX study, i.e. 1.57 mg/day, the plasma PLP concentration was 36.2 nmol/L. An AR of 1.5 mg/day, and considering a SD of 0.15 mg/day, a PRI of 1.8 mg/day, were set. For women, six depletion–repletion studies (Brown et al., 1975; Driskell et al., 1989; Kretsch et al., 1995; Hansen et al., 1996b, 1997; Huang et al., 1998) were used. At the 25th percentile of vitamin B6 intake in women of the SU.VI.MAX study, i.e. 1.24 mg/day, the plasma PLP concentration was 30.5 nmol/L. An AR of 1.3 mg/day, and considering a SD of 0.13 mg/day, a (rounded) PRI of 1.5 mg/day were set. For older adults, data from a depletion–repletion study (Meydani et al., 1991) were considered that showed a restoration of interleukin-2 concentration and lymphocyte proliferation with a vitamin B6 intake higher than 2.0 mg/day in a small number of older subjects. It was concluded that older adults have a higher vitamin B6 requirement than younger ones. The PRI was set at 2.2 mg/day for ages 75 years and over.

IOM (1998) selected a cut-off for plasma PLP concentration of 20 nmol/L as the basis for deriving an EAR for vitamin B6, although its use may overestimate the B6 requirement for health maintenance of more than half of the study group (Lui et al., 1985). For men, the IOM also considered results from the tryptophan load test (Baker et al., 1964; Yess et al., 1964; Miller and Linkswiler, 1967; Linkswiler, 1976) or plasma concentrations of Hcy (Selhub et al., 1993). From the range of values for the EAR for food vitamin B6[16] suggested by these studies, the IOM set an EAR at 1.1 mg/day for men aged 19–50 years and an RDA at 1.3 mg/day. A CV of 10% was used to derive RDAs from EARs in adults, because of a lack of data on the variation in the vitamin B6 requirement. The IOM also used data on α-EAST and α-EALT. Studies considered were depletion–repletion studies (Brown et al., 1975; Kretsch et al., 1995; Hansen et al., 1997; Huang et al., 1998), and other intervention studies with various protein or pyridoxine glucoside intakes (Hansen et al., 1996a) or undertaken in obese and non-obese women (Driskell et al., 1989). The same EAR and RDA values as in men were set for women aged 19–50 years. The IOM noted that data on adults aged 51 years and over is limited to a depletion–repletion study (Ribaya-Mercado et al., 1991) and a study investigating the relationship between dietary vitamin B6 and plasma PLP and Hcy concentrations (Selhub et al., 1993). Thus, for adults above 50 years of age, the IOM set EARs and RDAs, respectively, at 1.4 and 1.7 mg/day for men, and 1.3 and 1.5 mg/day for women.

SCF (1993) stated that vitamin B6 requirement is related to protein intake (except in case of low protein intakes or restricted food intake). Data on changes in tryptophan and methionine metabolism

[16] IOM considered that the bioavailability of pyridoxine is 95%, whereas that of natural vitamin B6 in foods is only about 75%, thus that the bioavailability of synthetic vitamin B6 is 95/75 (i.e. 1.27) times higher (Tarr et al., 1981, Gregory, 1990).

and on the decline in blood concentrations of vitamin B6 during depletion–repletion studies (Miller and Linkswiler, 1967; Kelsay et al., 1968a,b; Canham et al., 1969) were used. Thus, the AR, the PRI and the LTI were, respectively, set at 13, 15 and 11 μg pyridoxine/g dietary protein, and the lack of experimental evidence to support the LTI was underlined. Considering energy requirements and an average protein intake of 15 E%, the ARs corresponded to 1.3 mg/day and 1.0 mg/day, and the PRIs to 1.5 mg/day and 1.1 mg/day, for men and women, respectively. The fall in plasma PLP concentration with age (contrary to erythrocyte aminotransferase activation coefficients), and age-related changes in vitamin B6 metabolism (Kelsay et al., 1968b) were also noted, but the SCF did not see evidence of an increase in vitamin B6 requirement with ageing.

The UK Committee on Medical Aspects of Food Policy (COMA) (DH, 1991) considered the same depletion–repletion studies as SCF (1993). The RNI, obtained by interpolation, the EAR and the Lower Reference Nutrient Intake (LRNI) were set, respectively, at 15, 13 and 11 μg/g dietary protein. At the UK EAR for energy and a protein intake of 14.7 E% observed at that time in British adults (Gregory, 1990), the RNI would correspond to 1.4 mg/day and 1.2 mg/day for men and women, respectively. The decrease in plasma PLP concentration with age (contrary to α-EAST), as well as the data on urinary concentration of 4-PA in middle-aged women compared to younger ones (Hamfelt, 1964; Rose et al., 1976b; Lee and Leklem, 1985), were noted. However, the UK COMA considered the evidence insufficient to increase references values for older adults.

An overview of DRVs for vitamin B6 for adults is presented in Table 1.

Table 1: Overview of Dietary Reference Values for vitamin B6 for adults

	D-A-CH (2015)	NCM (2014)	WHO/FAO (2004)	NL (2003)	Afssa (2001)	IOM (1998)	SCF (1993)	DH (1991)
Age (years)	19 to < 65	18–30	19–50	19–50	19–74	19–50	≥ 18	19 to ≥ 50
PRI Men (mg/day)	1.5	1.6	1.3	1.5	1.8	1.3	1.5	1.4
PRI Women (mg/day)	1.2	1.3	1.3	1.5	1.5	1.3	1.1	1.2
Age (years)	≥ 65	≥ 31	≥ 51	≥ 51	≥ 75	≥ 51		
PRI Men (mg/day)	1.4	1.6	1.7	1.8	2.2	1.7		
PRI Women (mg/day)	1.2	1.2	1.5	1.5	2.2	1.5		

Afssa: Agence française de sécurité sanitaire des aliments; D-A-CH: Deutschland–Austria–Confoederatio Helvetica; DH: Department of Health; FAO: Food and Agriculture Organization; IOM: US Institute of Medicine of the National Academy of Sciences; NCM: Nordic Council of Ministers; NL: Netherlands; PRI: Population Reference Intake; SCF: Scientific Committee for Food; WHO: World Health Organization.

4.2. Infants and children

D-A-CH (2015) set reference values for vitamin B6 for infants and children, ranging from 0.3 mg/day (4 to < 12 months) to 1.6 mg/day (boys 15 to < 19 years).

The Nordic countries (Nordic Council of Ministers, 2014) noted that symptoms of deficiency, such as convulsions, have been seen in infants consuming formulae containing 0.06 mg/L (Coursin, 1964). In the absence of scientific data, the NNR set RIs for infants and children based on the adult value, which corresponded to values ranging from 0.4 (6–11 months) to 1.6 (boys 14–17 years) mg/day.

WHO/FAO (2004) set an RNI of 0.1 mg/day for breastfed infants from birth to 6 months, based on a vitamin B6 breast milk concentration of 0.12–0.13 mg/L (West and Kirksey, 1976; Andon et al., 1989). For infants aged 7–12 months, the same approach as that of the IOM was used, and an RNI was set at 0.3 mg/day. For older children, WHO/FAO reported on one study (Heiskanen et al., 1995) in 198 healthy Finnish children followed between the age of 2 months and 11 years, which found an age-related decrease in erythrocyte PLP concentration, and an increase in aspartate aminotransferase activation. RNIs set for children ranged between 0.5 mg/day (1–3 years) and 1.3 mg/day (boys aged 10–18 years).

The Health Council of the Netherlands (2003) set Adequate Intakes (AIs) for children by interpolation between the AI for exclusively breastfed infants from birth to 5 months (which was based on their average vitamin B6 intake, i.e. 0.12 mg/day), and the RDA for adults. The AI for infants from

birth to 5 months was derived from a breast milk consumption of 800 mL/day and a mean vitamin B6 breast milk concentration of 0.15 mg/L observed in Western breastfeeding women not taking supplements and with an intake lower than 5 mg/day (Fomon and McCormick, 1993). The AIs ranged from 0.2 mg/day (6–11 months) to 1.5 mg/day (14–18 years) for vitamin B6 naturally present or added to foods.

Considering that data relating vitamin B6 intake and status in children and adolescents were limited (Bessey et al., 1957; Lewis and Nunn, 1977; Driskell and Moak, 1986), Afssa (2001) derived PRIs according to sex and age from adult values, adjusting for square height considered to be representative of lean body mass.

For breastfed infants from birth to 6 months, IOM (1998) set the AI at 0.1 mg/day, using an average breast milk consumption of 0.78 L/day (Hofvander et al., 1982; Butte et al., 1984; Chandra, 1984; Neville et al., 1988; Allen et al., 1991) and an average vitamin B6 concentration of 0.13 mg/L, which was reported for maternal vitamin B6 intakes of less than 2.5 mg/day (West and Kirksey, 1976). For infants aged 7–12 months, the IOM set an AI at 0.3 mg/day, as the mean between the value of 0.2 mg/day, obtained by upwards extrapolation from the AI for infants from birth to 6 months (using allometric scaling), and the value of 0.4 mg/day, obtained by downwards extrapolation from adult EARs (adjusting for metabolic body size and growth and adding a factor for variability). For children and adolescents aged 1–18 years EARs were extrapolated from adult values, and RDAs were calculated considering a CV of 10%. The IOM also estimated vitamin B6 requirements by assuming a direct relationship with protein intake, using protein intake data in children from the National Health and Nutrition Examination Survey (NHANES), but the values obtained were considered as too high. Thus, IOM concluded that, as for adults, there was no evidence to suggest that vitamin B6 requirements for children should be adjusted for protein intake.

As there were no data to suggest the need for a different vitamin B6 requirement for children compared to adults, SCF (1993) set the same PRI (15 μg/g dietary protein) for both populations. Considering energy requirements and an average protein intake of 15 E%, the PRI were set between 0.4 mg/day (6–11 months) and 1.1 mg/day (7–10 years), then differing according to sex (11–17 years).

The UK COMA (DH, 1991) applied the same LRNI, EAR and RNI as those set for adults (11, 13 and 15 μg/g dietary protein, respectively). At the UK EAR for energy and a protein intake of 14.7 E%, the RNI would correspond to values between 0.3 (7–9 months) and 1.0 mg/day (7–10 years), and then differing according to sex for 11–18 years.

An overview of DRVs for vitamin B6 for infants, children and adolescents is presented in Table 2.

Table 2: Overview of Dietary Reference Values for vitamin B6 for infants and children

	D-A-CH (2015)	NCM (2014)	WHO/ FAO (2004)	NL (2003)[a]	Afssa (2001)	IOM (1998)	SCF (1993)	DH (1991)
Age (months)	4 to < 12	6–11	7–12	6–11	0–12	7–12	6–11	7–9
PRI (mg/day)	0.3	0.4	0.3	0.2	0.3	0.3	0.4	0.3
Age (months)								10–12
PRI (mg/day)								0.4
Age (years)	1 to < 4	1 to < 2	1–3	1–3	1–3	1–3	1–3	1–3
PRI (mg/day)	0.4	0.5	0.5	0.4	0.6	0.5	0.7	0.7
Age (years)	4 to < 7	2–5	4–6	4–8	4–6	4–8	4–6	4–6
PRI (mg/day)	0.5	0.7	0.6	0.7	0.8	0.6	0.9	0.9
Age (years)	7 to < 10	6–9	7–9		7–9		7–10	7–10
PRI (mg/day)	0.7	1.0	1.0		1.0		1.1	1.0
Age (years)	10 to < 13	10–13	10–18	9–13	10–12	9–13	11–14	11–14
PRI Boys (mg/day)	1.0	1.3	1.3	1.1	1.3	1.0	1.3	1.2
PRI Girls (mg/day)	1.0	1.1	1.2	1.1	1.3	1.0	1.1	1.0
Age (years)	13 to < 15				13–15			
PRI Boys (mg/day)	1.4				1.6			
PRI Girls (mg/day)	1.4				1.5			
Age (years)	15 to < 19	14–17		14–18	16–18	14–18	15–17	15–18

	D-A-CH (2015)	NCM (2014)	WHO/ FAO (2004)	NL (2003)[a]	Afssa (2001)	IOM (1998)	SCF (1993)	DH (1991)
PRI Boys (mg/day)	1.6	1.6		1.5	1.8	1.3	1.5	1.5
PRI Girls (mg/day)	1.2	1.3		1.5	1.5	1.2	1.1	1.2

Afssa: Agence française de sécurité sanitaire des aliments; D-A-CH: Deutschland–Austria–Confoederatio Helvetica; DH: Department of Health; FAO: Food and Agriculture Organization; IOM: US Institute of Medicine of the National Academy of Sciences; NCM: Nordic Council of Ministers; NL: the Netherlands; PRI: Population Reference Intake; SCF: Scientific Committee for Food; WHO: World Health Organization.
(a): Adequate Intake.

4.3. Pregnancy

D-A-CH (2015) considered indications of a decrease in vitamin B6 status during the third trimester of pregnancy and proposed to increase the recommended intake by 0.7 mg/day, i.e. an intake of 1.9 mg/day was recommended from the fourth month of pregnancy.

Based on the increased energy requirement of pregnant women during the last two trimesters, the Nordic countries (Nordic Council of Ministers, 2014) recommended an additional intake of 0.2 mg/day of vitamin B6 (thus a RI of 1.5 mg/day) to cover the extra need of the fetus. The NNR cited one narrative review and two systematic reviews (Thaver et al., 2006; Simpson et al., 2010; Dror and Allen, 2012) that indicate that plasma PLP concentration decreases throughout pregnancy, but considered this data insufficient to support a higher reference value.

WHO/FAO (2004) noted a decrease in markers of vitamin B6 status during pregnancy especially in the third trimester, which might correspond to a normal physiological change (Cleary et al., 1975; Lumeng et al., 1976). An extra need of about 0.5 mg/day, i.e. an RNI of 1.9 mg/day, was derived for pregnancy.

The Health Council of the Netherlands (2003) considered the amount of vitamin B6 deposited in the fetus and the placenta, i.e. about 25 mg at the end of pregnancy (IOM, 1998), an incomplete vitamin B6 transfer to the fetus and increased metabolic needs of the mother. Based on this, the Council proposed for pregnancy an increment of 0.25 mg/day of the EAR, i.e. an EAR of 1.35 mg/day and, using a CV of 20%, an RDA of 1.9 mg/day.

Afssa (2001) reported on the same data cited by IOM (1998) on changes in markers of vitamin B6 status during pregnancy (Hamfelt and Tuvemo, 1972; Cleary et al., 1975; Lumeng et al., 1976; Shane and Contractor, 1980). Following the same approach, Afssa set the amount to be added to the PRI of non-pregnant women at 0.5 mg/day, i.e. a PRI of 2.0 mg/day for pregnancy.

IOM (1998) noted higher blood PLP concentrations in the fetus than in the mother, a significant fetal sequestration of vitamin B6, and a decrease in markers of vitamin B6 status (e.g. plasma PLP) during pregnancy particularly in the third trimester (Hamfelt and Tuvemo, 1972; Cleary et al., 1975; Lumeng et al., 1976; Shane and Contractor, 1980). The IOM, however, noted that it was unclear whether this decrease reflects normal physiological changes or poorer vitamin B6 status during pregnancy compared to non-pregnant women. Assuming a body store of 1,000 μmol (i.e. 169 mg) and a fetal, uterine, and placental accumulation of 15%, it was calculated that the fetus and placenta would accumulate approximately 25 mg of vitamin B6, i.e. an average amount of about 0.1 mg/day. Allowing for the increased metabolic needs and weight of the mother and assuming about 75% bioavailability of food vitamin B6, an additional average requirement of 0.25 mg in pregnancy was estimated, mainly for the second half of gestation. Considering that vitamin B6 cannot be stored at the beginning of pregnancy to compensate for this increased need afterwards, the IOM set an extra amount of 0.5 mg/day of vitamin B6 throughout pregnancy, and thus an EAR of 1.6 mg/day, and an RDA of 1.9 mg/day (considering a CV of 10%).

SCF (1993) mentioned the marked and progressive fall of plasma PLP concentrations during pregnancy, despite normal values for erythrocyte aminotransferase activation coefficients and excretion of 4-PA. It was noted that there were no data to suggest the need to maintain the same plasma PLP concentrations as in non-pregnant women. Thus, the same PRI as for non-pregnant women was set, i.e. 15 μg/g dietary protein. Considering the extra protein intake recommended during pregnancy, this corresponded to 1.3 mg/day.

The UK COMA (DH, 1991) and SCF (1993) had the same approach regarding vitamin B6 requirement during pregnancy. Thus, the UK derived a PRI of 1.2 mg/day for pregnant women.

4.4. Lactation

To compensate for the average secretion of 0.1 mg vitamin B6/day with mature breast milk by fully breastfeeding women, and to refill body stores depleted during pregnancy, D-A-CH (2015) proposed to increase the recommended vitamin B6 intake by 0.7 mg/day (Hansen et al., 1997) and set a recommended intake of 1.9 mg/day.

For lactation, the Nordic countries (Nordic Council of Ministers, 2014) recommended an additional intake of 0.3 mg/day to cover the needs for vitamin B6 related to its secretion in breast milk (thus a RI of 1.6 mg/day).

WHO/FAO (2004) stated that an addition of 0.6 mg/day to the RNI for non-lactating women may be prudent because low maternal intakes could lead to a compromised vitamin B6 status in the infant (Borschel, 1995), and set an RNI for lactating women at 2.0 mg/day.

The Health Council of the Netherlands (2003) considered that the average amount of vitamin B6 secreted in milk of exclusively breastfeeding women was 0.1 mg/day. Taking into account vitamin B6 bioavailability as well as a safety margin (Borschel et al., 1986), an increment of 0.25 mg/day was proposed, i.e. an EAR of 1.35 mg/day. Using a CV of 20%, an RDA of 1.9 mg/day was derived.

Afssa (2001) considered that vitamin B6 breast milk concentration reflects vitamin B6 status of the mother (West and Kirksey, 1976). Data on the relationship between vitamin B6 supplementation and breast milk concentration (Borschel et al., 1986) and on vitamin B6 status of lactating mothers and their infants (Andon et al., 1989) were taken into account. Afssa considered that a precise increment in vitamin B6 requirement during lactation could not be derived from the available data, but assumed it higher than 0.2 mg/day (needed for milk production), and set a value at 0.5 mg/day, in addition to the PRI of non-lactating women. Therefore, the PRI for lactating women was 2.0 mg/day.

IOM (1998) noted that the vitamin B6 concentration in human milk varies depending on the mother's vitamin B6 intake, and that the additional requirement for lactation exceeds considerably the amount that is secreted via breast milk (West and Kirksey, 1976; Borschel et al., 1986). In order to reach the vitamin B6 concentration in breast milk of 0.13 mg/L (West and Kirksey, 1976), the IOM estimated that an amount of vitamin B6 equal to five times this concentration should be consumed. The EAR for lactating adolescents and adult women was set at 1.7 mg/day, and the RDA at 2 mg/day, assuming a CV of 10%.

Considering that there were no data to suggest a change in vitamin B6 metabolism during lactation, SCF (1993) set the same PRI as for non-lactating women, i.e. 15 μg/g dietary protein. Considering the extra protein intake recommended during lactation, this amount corresponded to 1.4 mg/day.

For lactation, the UK COMA (DH, 1991) did not propose any increment to the vitamin B6 reference value of non-lactating women.

An overview of DRVs for vitamin B6 for pregnant or lactating women is presented in Table 3.

Table 3: Overview of Dietary Reference Values for vitamin B6 for pregnant and lactating women

	D-A-CH (2015)	NCM (2014)	WHO/FAO (2004)	NL (2003)	Afssa (2001)	IOM (1998)	SCF (1993)	DH (1991)
PRI Pregnancy (mg/day)	1.9[(a)]	1.5	1.9	1.9	2.0	1.9	1.3	1.2
PRI Lactation (mg/day)	1.9	1.6	2.0	1.9	2.0	2.0	1.4	1.2

Afssa: Agence française de sécurité sanitaire des aliments; D-A-CH: Deutschland–Austria–Confoederatio Helvetica; DH: Department of Health; FAO: Food and Agriculture Organization; IOM: US Institute of Medicine of the National Academy of Sciences; NCM: Nordic Council of Ministers; NL: the Netherlands; PRI: Population Reference Intake; SCF: Scientific Committee for Food; WHO: World Health Organization.
(a): From the fourth month.

5. Criteria (endpoints) on which to base Dietary Reference Values

5.1. Indicators of vitamin B6 requirement

The Panel considers that plasma PLP concentration is the most suitable biomarker for deriving the DRVs for vitamin B6 (Sections 2.4.1.1 and 2.4.3). The Panel also considers that plasma PLP concentration of 30 nmol/L as a population mean is indicative of an adequate vitamin B6 status for all age and sex groups (Section 2.4.3). The Panel notes that there is no consistent relationship between

plasma PLP concentrations and protein intake, and considers that there is no conclusive evidence that vitamin B6 requirements change according to protein intake in the range of observed intake in Europe (Section 2.3.7.3). Thus, the Panel considers that it is not appropriate to standardise vitamin B6 requirements on protein intake. The application of these criteria for defining vitamin B6 requirements in different population groups is discussed below.

5.1.1. Adults

Various intervention studies, including studies with depletion–repletion design, have been conducted in women in order to determine dietary requirements for vitamin B6 based on the changes in plasma PLP concentration (Brown et al., 1975; Miller et al., 1985; Kretsch et al., 1995; Hansen et al., 1996a, 1997, 2001; Huang et al., 1998). Although the participants in these studies were not always housed in a metabolic unit, they consumed only food provided by the investigators. All the meals included in the experimental diets were prepared in a metabolic kitchen and the vitamin B6 content of the diets was analysed and, in some of the studies, adjusted for bioavailability. The common principle of defining vitamin B6 requirements in these studies was based on the amount of dietary vitamin B6 necessary to reach plasma PLP concentration indicative of an adequate status or, in the case of depletion–repletion studies, to restore the baseline plasma PLP concentration after a period of vitamin B6 depletion. In addition, linear regression analysis has been conducted to quantify the mean amount of dietary vitamin B6 required for depleted plasma PLP concentration to be restored to 30 nmol/L (Hansen et al., 2001). As these studies have used a combination of vitamin B6 from supplements, together with vitamin B6 from food, the differences in bioavailability of supplemental versus food vitamin B6 (95% vs 75%) have been considered by the Panel (Section 2.3.1).

5.1.1.1. Women

Seven intervention studies have used plasma PLP concentration as a criterion for assessing the requirement for vitamin B6 in women. In these studies, the basal or depletion diet was providing between 0.05 and 1.25 mg/day vitamin B6, whereas vitamin B6 intakes during repletion periods ranged between 0.5 and 2.7 mg/day vitamin B6, and protein intake ranged between 0.5 and 2 g protein/kg bw per day.

Brown et al. (1975) carried out a depletion–repletion study in nine healthy women (mean ± SD: 22.3 ± 1.9 years, range: 20–30 years). The women underwent a depletion phase of 28 days by consuming a diet providing daily 78 g protein (i.e. about 1.3 g protein/kg bw per day) and 0.19 mg vitamin B6. After the depletion phase, in which mean plasma PLP fell from 47 to 13 nmol/L, the women were split into two groups to receive 0.66 mg/day (n = 6) or 1.65 mg/day (n = 3) pyridoxine in addition to the basal diet (0.19 mg/day) for another 28 days. At the end of the repletion phase, women who received the additional 0.66 mg/day as supplement[17] had a mean (± SD) plasma PLP concentration of 22.7 ± 13.8 nmol/L. By contrast, women who received the additional 1.65 mg/day as supplement[18] had a mean plasma PLP of 60.7 ± 20.2 nmol/L, which was higher than at baseline. These results suggest that vitamin B6 requirements are higher than 1 mg/day, but lower that 2.3 mg/day, in young women.

Kretsch et al. (1995) investigated, in a depletion–repletion study in eight healthy women (21–30 years), the effect of animal and plant protein on vitamin B6 requirements. The women underwent a depletion phase by consuming a 'high' protein diet (1.55 g protein/kg bw per day, equivalent to ~ 100 g/day of protein) providing 0.05 mg/day of vitamin B6 for 11–28 days. Two women exited the depletion phase on days 11 and 12 due to abnormal electroencephalograms. After the depletion phase, in which mean plasma PLP fell from 25 to 9 nmol/L, the women were randomly assigned to two groups to receive a diet based either on animal (n = 4) or plant (n = 4) protein. Then, they followed consecutive repletion periods with a total vitamin B6 intake at 0.5 mg/day (14 days), 1 mg/day (14 days), 1.5 mg/day (21 days) and 2 mg/day (14 days). Plasma PLP concentrations did not show significant differences between women on animal or plant protein diets and the results of the two groups were combined by the authors. The mean (± SD) plasma PLP concentration, achieved with a vitamin B6 intake of 1 mg/day (18.7 ± 8.1 nmol/L), was not statistically different from the baseline value of 25.4 ± 10.6 nmol/L. The mean plasma PLP concentrations, achieved with a vitamin B6 intake of 1.5 or 2 mg/day, were above 30 nmol/L (but were not statistically different from the baseline mean PLP concentration of 25.4 nmol/L).

[17] That is total vitamin B6 intake: 0.19 + 0.66 × 1.27 equals about 1 mg/day.
[18] That is total vitamin B6 intake: 0.19 + 1.65 × 1.27 equals about 2.3 mg/day.

Hansen et al. (1996a) (described in detail in Section 2.3.7.2) found that vitamin B6 intake of 1.25 mg/day (one dose of vitamin B6 was used) was not sufficient to maintain the mean plasma PLP concentration at 30 nmol/L in nine young healthy women.

Hansen et al. (1997) reported the results of two intervention studies on healthy women. In the first study, 10 women (mean age ± SD: 27.5 ± 6.8 years) were placed for 15 days on a basal diet providing 85 g protein (about 1.2 g/kg bw) and 1.03 mg vitamin B6 per day. After this initial period, in which mean plasma PLP was about 28 nmol/L, the women underwent three consecutive periods (each with a duration of 12 days) with a total vitamin B6 intake at 1.33, 1.73 and 2.39 mg/day. In the second study, six women (mean age ± SD: 28.2 ± 2.6 years) were placed for 12 days on a basal diet providing 85 g protein (about 1.2 g/kg bw) and 0.84 mg vitamin B6 per day (depletion period). After this initial period, in which mean plasma PLP was 26.5 nmol/L, the women underwent two successive repletion periods (each with duration of 10 days), with a vitamin B6 intake at 1.14 and 2.34 mg/day. Mean plasma PLP concentrations were above 30 nmol/L at an intake of 1.33 mg/day (mean ± SD: 32.4 ± 11.6 nmol/L, however, not statistically different from baseline mean PLP of about 28 nmol/L at 1.03 mg/day vitamin B6). Mean plasma PLP concentrations were also above 30 nmol/L at higher intake levels, i.e. 1.73, 2.34, 2.39 mg/day (all concentrations statistically different from their respective baseline value, $p < 0.05$). Plasma PLP concentrations were above the cut-off of 30 nmol/L in six women at a vitamin B6 intake of 1.33 mg/day (first study), while plasma PLP was less than 30 nmol/L in four women receiving 1.14 mg/day vitamin B6 (second study).

Huang et al. (1998) carried out a depletion–repletion study in eight healthy women (mean ± SD: 30.5 ± 2.1 years) who were fed a lacto-ovo-vegetarian basal diet providing 1.55 g protein/kg bw per day (96 g/day protein) and 0.45 mg/day vitamin B6 for a total of 92 days. The women underwent an adjustment period (1.6 mg/day vitamin B6; 9 days), a depletion phase with the basal diet as the only source of vitamin B6 (0.45 mg/day vitamin B6; 27 days) and three consecutive repletion periods with a total vitamin B6 intake at 1.26, 1.66 and 2.06 mg/day, respectively (each period of 14–21 days). Mean (± SD) plasma PLP concentration significantly decreased from 58.2 ± 16.3 nmol/L at baseline to 32.4 ± 10.5 nmol/L at the end of the depletion period ($p \leq 0.05$). Repletion with 1.26 mg/day vitamin B6 resulted in mean PLP concentrations of 38.3 ± 9.7 nmol/L (not statistically different from the mean PLP of 32.4 nmol/L during depletion). After the repletion with 1.66 mg/day, all women had PLP concentrations above 30 nmol/L.

Hansen et al. (2001) conducted an intervention study in seven healthy women (mean age ± SD: 28 ± 6 years), who received a basal diet providing 1.2 g protein/kg bw per day (mean intake of ~ 56 g protein/day). The women underwent a 7-day adjustment period (1.0 mg/day vitamin B6) followed by three consecutive 14-day experimental periods with a total vitamin B6 intake of 1.5, 2.1 and 2.7 mg/day, respectively. Mean (± SD) plasma PLP concentration significantly fell from 46.6 ± 13.9 nmol/L at baseline to 29.7 ± 7.1 nmol/L at the end of the adjustment period ($p \leq 0.05$), but four women out of seven had concentrations above 30 nmol/L. Mean (± SD) plasma PLP concentrations were above 30 nmol/L for the three consecutive experimental periods, i.e. 35.2 ± 6.0, 43.7 ± 7.2, and 56.1 ± 13.2 nmol/L, respectively (statistically different from the mean PLP of 29.7 nmol/L of the adjustment period only at 2.7 mg/day vitamin B6, $p \leq 0.05$). At the end of the three experimental periods, six out of seven women (with a vitamin B6 intake of 1.5 mg/day) or all of them (with a vitamin B6 intake of 2.1 and 2.7 mg/day) had PLP concentrations above the cut-off of 30 nmol/L for adequate vitamin B6 status.

Combining their own data with four other studies (Kretsch et al., 1995; Hansen et al., 1996a, 1997; Huang et al., 1998), Hansen et al. (2001) used inverse prediction from linear regression analysis of plasma PLP concentration vs vitamin B6 intake ($r = 0.879$) adjusted for bioavailability[19] and baseline PLP value. Hansen et al. (2001) calculated that the intake needed by 50% of the population to reach a PLP concentration of 30 nmol/L was 1.2 mg/day vitamin B6.

5.1.1.2. Men

In a cross-over study in eight young men (described in detail in Section 2.3.7.2), Miller et al. (1985) investigated the effect of diets providing different protein content on vitamin B6 status and found that vitamin B6 intake of 1.6 mg/day is sufficient to maintain mean plasma PLP concentration close to or above 30 nmol/L, irrespective of the protein content of the diet. The Panel notes that this study was designed to investigate the effect of protein intake on vitamin B6 status rather than to define the

[19] By converting supplemental vitamin B6 to dietary vitamin B6 equivalents according to the following formula: dietary vitamin B6 equivalents = food vitamin B6 + 1.27 × supplemental vitamin B6.

requirements for vitamin B6 intake (i.e. one dose of vitamin B6 was used) and it is unknown whether a lower intake of vitamin B6 would be also sufficient. The Panel considers than this study does not allow drawing conclusions on vitamin B6 requirement.

Another study conducted in men provided different levels of vitamin B6 intake and used plasma PLP concentrations as an assessment criterion. Driskell et al. (1988) randomly assigned 22 healthy young men (20–37 years) to three different isocaloric diets providing daily 81–84 g protein (about 1–1.1 g/kg bw) and vitamin B6 at 0.75 mg (n = 7), 0.88 mg (n = 7) or 0.98 mg (n = 8) for 8 weeks. At the end of the intervention, mean plasma PLP concentrations of these men were between about 70 and 100 nmol/L (depending on ethnicity and diet received), which were well above the cut-off of 30 nmol/L. The Panel notes that the baseline plasma PLP concentrations of the subjects in this study were not reported. The Panel also notes that the final results for high plasma PLP response to diets providing low vitamin B6 intake of less than 1 mg/day are in disagreement with the rest of available data in men (Miller et al., 1985) and women (Brown et al., 1975; Kretsch et al., 1995; Hansen et al., 1996a, 1997, 2001; Huang et al., 1998). The Panel considers that no conclusions on vitamin B6 requirement can be drawn from this study.

5.1.1.3. Older adults

In a depletion–repletion study (described in detail in Section 2.3.7.2), Ribaya-Mercado et al. (1991) investigated the requirements for vitamin B6 intake in 12 older adults (61–71 years). The participants went through a 20-day depletion period with an average vitamin B6 intake of 0.17 mg/day (males) and 0.1 mg/day (females) followed by three consecutive repletion periods of 21 days, when vitamin B6 was provided at about 1.2, 1.7 and 2.5 mg/day for a man and at 0.9, 1.3 and 1.9 mg/day for two women, for the group receiving 0.8 g protein/kg bw per day. Vitamin B6 intake of about 1.3 mg/day maintained plasma PLP concentrations at values above 30 nmol/L in the three subjects (one man, two women) who received 0.8 g protein/kg bw per day (close to the PRI for protein intake (EFSA NDA Panel, 2012)).

In a randomised cross-over study in older and younger adults (26 older adults, mean age ± SEM: 70 ± 1 years) (described in detail in Section 2.3.7.2), Pannemans et al. (1994) investigated the response of plasma PLP concentration to diets containing similar amounts of vitamin B6 and two different levels of protein (the lowest protein intake being with diet A: 0.9–1 g protein/kg bw per day, 1.5 mg/day vitamin B6). The Panel notes that older adults consistently showed significantly lower mean (± SEM) plasma PLP concentrations than younger adults (e.g. diet A: 27 ± 3 vs 47 ± 6 nmol/L, $p < 0.01$). The Panel also notes that this study was designed to investigate the effect of protein intake on vitamin B6 status rather than to define the requirements for vitamin B6 intake (i.e. one dose of vitamin B6 was used). The Panel notes that baseline plasma PLP concentrations of participants were not measured. The Panel, however, notes that the intervention of 3 weeks was in the range of the durations of the intervention periods of the other studies described (undertaken in younger women, younger men or older adults), and considers that it was sufficiently long to reach a stable concentration of plasma PLP (Section 2.4.1.1).

5.1.1.4. Conclusions on vitamin B6 requirements in adults

For women, the Panel notes that a vitamin B6 intake between 1 and 1.5 mg/day is sufficient to sustain mean plasma PLP concentration above the cut-off of 30 nmol/L (Section 5.1.1.1). The Panel notes that Hansen et al. (2001) determined by an inverse prediction analysis that the intake needed by 50% of a female population to reach a PLP concentration of 30 nmol/L was 1.2 mg/day vitamin B6. This analysis is based on 44 women in total, participating in strictly controlled interventions, carried out in different research centres, with diets providing predetermined vitamin B6 amounts covering a broad range of values (vitamin B6 intake adjusted for bioavailability (Sections 2.3.1 and 5.1.1.1): about 0.5–3.5 mg/day (read on figure)) (Kretsch et al., 1995; Hansen et al., 1996a, 1997, 2001; Huang et al., 1998).

For men, the Panel notes that the available data to assess vitamin B6 requirement are scarce. The Panel considers that the results of the two available studies in men (Miller et al., 1985; Driskell et al., 1988; Section 5.1.1.2) are not suitable for deriving the requirement for vitamin B6 in men.

For older adults, the Panel notes that the available data to assess vitamin B6 requirement are scarce (Section 5.1.1.3). The Panel notes that, in a carefully conducted depletion–repletion intervention study in older adults, a vitamin B6 intake of about 1.3 mg/day was sufficient to sustain plasma PLP concentrations above the cut-off of 30 nmol/L, in the only three participants receiving 0.8 g protein/kg bw per day (Ribaya-Mercado et al., 1991). The Panel notes that this result for older

adults (1.3 mg/day) is slightly higher than the result obtained for younger women (1.2 mg/day). In addition, the Panel acknowledges that the randomised cross-over intervention study by Pannemans et al. (1994) was not designed to define the requirements for vitamin B6 intake. However, the Panel considers that the higher vitamin B6 requirement for older adults suggested by Ribaya-Mercado et al. (1991) is in agreement with the study by Pannemans et al. (1994), which showed a consistently lower plasma PLP response to similar amounts of vitamin B6 in older adults compared with younger adults, and with the age-related decline of plasma PLP concentrations observed in large cross-sectional studies (Rose et al., 1976a; Bates et al., 1999b; Morris et al., 2008; Section 2.4.1.1).

5.1.2. Infants

At birth, newborns have high concentrations of plasma PLP (e.g. mean of 73 nmol/L in breastfed infants aged 1–5 days) (Borschel et al., 1986) (Appendix A), which gradually decrease with age. A study by Kang-Yoon et al. (1992) (Appendix A) has shown a sharp fall of mean plasma PLP concentrations from 114 nmol/L at birth to 32 nmol/L at 14 days of age, even in breastfed term neonates whose mothers were supplemented with pyridoxine at 2 mg/day. Plasma PLP concentration of 54 ± 44 nmol/L (mean ± SD) were found in 2 month-old breastfed infants whose mothers consumed a diet providing 1.46 mg/day of vitamin B6 (Andon et al., 1989) (Appendix A). The decline in the neonatal plasma PLP after birth has been related mainly to the fact that vitamin B6 supply *in utero* through the placenta is higher than the post-partum provision of vitamin B6 through breast milk (West and Kirksey, 1976; Kang-Yoon et al., 1992; Appendix A). This suggests that infants have limited capacity for storage of vitamin B6 and their plasma PLP is dependent on the intake of this vitamin. Information on vitamin B6 status and requirements in older infants is lacking.

The Panel considers that the available data on vitamin B6 intake and status in infants are unsuitable for deriving the requirement for vitamin B6 in infants. The Panel considers therefore that data on vitamin B6 intake of breastfed infants during the first 6 months of lactation can be used to derive a DRV for infants aged 7–11 months.

5.1.3. Children

There are no data for vitamin B6 requirements of children from intervention studies with carefully controlled vitamin B6 intake, and the only available information is coming from observational studies.

In a cross-sectional study in 35 children aged 3–4 years, Fries et al. (1981) estimated, using one 24-h recall and two dietary records conducted by the parents, that vitamin B6 intake of the children who were not on supplements ranged from 0.87 to 1.33 mg/day (mean = 1.2 mg/day). Plasma PLP concentration was above 30 nmol/L (range: 57.9–77.3 nmol/L) even in the 18 children not taking supplements.

In another cross-sectional study, Driskell and Moak (1986) investigated vitamin B6 dietary intake and status in 96 white and 90 black adolescent girls aged 12–16 years. Mean vitamin B6 intake, estimated using two non-sequential 24-h food recalls, was 1.25 mg/day for those who did not report to take supplements (n = 162). Plasma PLP concentrations ranged from 15 to 96 nmol/L (mean = 42 nmol/L) and 26% of the girls had a concentration below 34.4 nmol/L (accepted by these authors as a cut-off for adequate status). There was no significant correlation between vitamin B6 intake and plasma PLP concentrations. The Panel considers that no conclusion can be drawn from this study, since the lack of correlation between vitamin B6 intake and plasma PLP concentrations raises questions about the validity of the dietary intake data.

In the National Diet and Nutrient Survey (NDNS) representative for children aged 4–18 years in the UK (n = 1,006), vitamin B6 intake, estimated using 7-day dietary record, was significantly correlated ($r = 0.349$, $p < 0.01$) to plasma PLP concentration for the different age categories (median intake of 1.8 mg/day, mean PLP concentration above 30 nmol/L) (Bates et al., 1999b; Kerr et al., 2009). Six percent of the children had PLP values below the cut-off of 30 nmol/L and those were predominantly the girls aged 15–18 years. The boys aged 15–18 years had significantly higher plasma PLP concentrations compared with the girls.

A subsequent NDNS of British children aged 1.5–18 years (n = 902) reported similarly high mean vitamin B6 intakes and mean plasma PLP concentrations (Bates et al., 2014). Mean vitamin B6 intakes in different age and sex groups, estimated using 4-day dietary record, were 1.5 mg/day (children aged 1.5–3 years), 2.2 mg/day (boys aged 4–18 years) and 1.8–1.9 mg/day (girls 4–18 years). Mean plasma PLP concentrations, assessed in 'usually fasted' blood samples, ranged between 66 and 71 nmol/L according to sex and age groups (n = 34 for the age range 1.5–3 years, 218 for the age range 4–10 years, 498 for the age range 11–18 years).

The Panel notes that all the research on vitamin B6 intake and status in childhood is observational. The Panel notes the uncertainties in the food composition and consumption data and dietary assessment methods used to estimate dietary intakes. The Panel concludes that the available evidence on vitamin B6 intake and status in childhood does not provide reliable information to derive the requirement for vitamin B6 in children.

5.1.4. Pregnancy

Pregnancy has been associated with 'low' plasma PLP concentrations, which cannot be explained by the blood volume expansion and the increased glomerular filtration rate, as the most rapid decline of PLP occurs in the third trimester when both the blood volume and glomerular filtration rate are levelling off (Section 2.4.1.1). Some studies have found a compensatory increase in PL concentration in the circulation and unchanged urinary excretion of 4-PA during pregnancy, but others have failed to confirm these observations (Section 2.4.1.1). The higher concentrations of PLP in the umbilical cord blood of the newborn or fetus than that in maternal blood (Section 2.3.3) are considered to be the main reason for the low vitamin B6 status in pregnant women (compared to non-pregnant women).

Two vitamin B6 supplementation studies, which have not included a placebo group, were conducted in US pregnant women with various results (Cleary et al., 1975; Lumeng et al., 1976). In one study, plasma PLP concentration of 58 unsupplemented non-pregnant women were compared to 24 pregnant women at delivery (11 who had received 10 mg/day vitamin B6 during pregnancy, 13 who had received 2–2.5 mg/day during pregnancy; Cleary et al., 1975). Only women supplemented with 10 mg/day had a mean plasma PLP concentration of about 30 nmol/L (significantly lower than that of the non-pregnant women, $p < 0.05$). In the other study, pregnant women (n = 33, followed between 7 and 12 weeks of gestation to term) were randomised to a supplementation with 2.5 mg/day (n = 10 completers), 4 mg/day (n = 4 completers) or 10 mg/day vitamin B6 (n = 10 completers), in addition to the dietary intake (of around 2 mg/day for most of the study population) (Lumeng et al., 1976). Only women supplemented with 10 mg/day vitamin B6 had mean plasma PLP concentrations during the second and third trimesters of pregnancy that were not significantly different from that found initially, which was above 30 nmol/L.

However, in a Taiwanese study, 209 pregnant women were split into four groups receiving 0 mg/day (n = 83), 1 mg/day (n = 63), 2 mg/day (n = 43) or 3 mg/day (n = 20) vitamin B6, mean maternal vitamin B6 intake in pregnancy was of about 1 mg/day in all groups, and plasma PLP concentration was measured in umbilical cord and maternal plasma at delivery (Chang, 1999). A total vitamin B6 intake of 3 mg/day (2 mg/day from supplement and 1 mg/day from the diet) was sufficient to achieve a mean (± SD) plasma PLP concentrations above the cut-off of 30 nmol/L in both mothers (43 ± 10 nmol/L at delivery) and newborns (78 ± 2 nmol/L in umbilical cord).

Given the discrepancies in the results of the three available supplementation studies in pregnancy (Cleary et al., 1975; Lumeng et al., 1976; Chang, 1999), the Panel finds these data unsuitable for setting the requirement for vitamin B6 in pregnant women.

Although the mechanism of vitamin B6 passage through placenta is unclear (Section 2.3.3), the Panel considers that there is evidence for a high transfer of vitamin B6 from the mother to the fetus (Contractor and Shane, 1970; Shane and Contractor, 1980; Zempleni et al., 1992), suggesting that the requirement for vitamin B6 is increased in pregnant women compared to non-pregnant women. The Panel considers that the additional requirements for vitamin B6 during pregnancy can be estimated by the mean gestational weight gain, the content of vitamin B6 per gram body weight and correcting for the bioavailability of vitamin B6 from the diet. Analysis of muscle biopsies in humans and labelled and non-labelled studies in other (animal) species showed that the average vitamin B6 total body content is about 15 nmol/g (0.0037 mg/g tissue) (Section 2.3.4). The main vitamin B6 derivative in the human tissues is PLP (with a molecular mass of 247.1 g/mol) (Section 2.3.4). A mean gestational increase in body mass of 12 kg, for women with a singleton pregnancy and a pre-pregnancy BMIs in the range between 18.5 and 24.9 kg/m^2, has been previously considered (EFSA NDA Panel, 2013).

Assuming that vitamin B6 bioavailability from a mixed diet is 75% (Section 2.3.1) and that the duration of pregnancy is 280 days, the additional amount of vitamin B6 intake estimated to be required for pregnant women per day, after rounding to the nearest one decimal place, will be:

$$(0.0037 \text{ mg vitamin B6} \times 12,000 \text{ g}_{\text{gestational weight gain}} / 0.75_{\text{bioavailability}}) / 280 = 0.2 \text{ mg/day vitamin B6}$$

5.1.5. Lactation

Vitamin B6 concentration in breast milk is highly dependent on the dietary B6 intake (Section 2.3.6.3), but data on the effect of lactation on maternal vitamin B6 status are limited. Of the few available studies undertaken on healthy unsupplemented lactating mothers (Appendix A and Section 2.3.6.3), only two investigations reported data on mature milk and both maternal vitamin B6 intake and plasma PLP concentrations (Morrison and Driskell, 1985; Andon et al., 1989). The results of these two studies showed that a diet providing a mean of 1.16–1.46 mg/day vitamin B6 maintained the mean (± SD) plasma PLP concentrations within the adequate range in the mothers (34 ± 13 and 61.9 ± 23.9 nmol/L, respectively).

The Panel considers that an additional intake of vitamin B6 for lactating women is not required apart from an increment to compensate for the amount of vitamin B6 secreted through lactation.

5.2. Vitamin B6 intake/status and health consequences

A comprehensive search of the literature published between 1990 and 2012 was performed as a preparatory work to this opinion in order to identify relevant health outcomes possibly associated with vitamin B6 intake and which may inform the setting of DRVs for vitamin B6 (Eeuwijk et al., 2012). The main results of the preparatory work together with new evidence from subsequently published studies are summarised below.

The relationship between vitamin B6 intake and/or status and chronic disease outcomes has been investigated mostly in observational (prospective cohort, case-control, cross-sectional) studies, where a positive, an inverse, or a lack of an association between vitamin B6 intake/status and disease outcomes might be confounded by uncertainties inherent to the methodology used for the assessment of vitamin B6 intake and status, and by the effect of other dietary, lifestyle or undefined factors on the disease outcomes investigated. Of the available randomised controlled trials (RCTs) investigating the health effects of vitamin B6, the results only of those trials were considered that recorded total vitamin B6 intake, i.e. intake from diet and supplements. Studies on associations between plasma PLP concentrations, as a biomarker of vitamin B6 intake/status (Section 2.4.1.1), and health outcomes were considered as well.

5.2.1. Cardiovascular disease

Observational studies investigating the relationship between vitamin B6 intake and the risk of developing cardiovascular disease (CVD) have shown inconsistent results. In a prospective study in 80,000 US women followed for 14 years, each 2 mg/day increase in total vitamin B6 intake (i.e. from food and supplements) was associated with a 17% significant reduction in the relative risk (RR) (95% confidence interval (CI) = 0.74–0.93) of coronary heart disease (CHD) after controlling for major confounders (Rimm et al., 1998). However, prospective and case-control studies in other cohorts have not detected a significant relationship between total vitamin B6 intake and either CVD in general (Cui et al., 2010) or CHD (Ishihara et al., 2008; Cui et al., 2010), myocardial infarction (Ishihara et al., 2008) and stroke (He et al., 2004; Larsson et al., 2008).

Observational studies investigating the relationship between plasma PLP concentration and the risk of cardiovascular events have also shown similar inconsistent results. Some case-control studies reported a significant inverse association between plasma PLP concentrations and the incidence of CVD in men and women (Folsom et al., 1998; Robinson et al., 1998; Vanuzzo et al., 2007; Page et al., 2009). However, other prospective and case-control studies failed to find an association between plasma PLP concentration and risk of CVD (Chasan-Taber et al., 1996; de Bree et al., 2003; Kelly et al., 2004), myocardial infarction (Dierkes et al., 2007) or stroke (Weikert et al., 2007).

The Panel concludes that the data available on vitamin B6 intake or plasma PLP concentration and CVD-related health outcomes are inconsistent and cannot be used to derive DRVs for vitamin B6.

5.2.2. Cancer

Studies investigating the relationship between vitamin B6 intake or plasma PLP concentration and the risk of *colorectal cancer* have shown inconsistent results. A meta-analysis of nine prospective studies, including 435,000 participants with 6,064 cases of colorectal cancer, showed no significant difference in the risk of colorectal cancer when the highest (1.63 to ≥ 5.81 mg/day in the individual studies) and the lowest (1.02–1.90 mg/day in the individual studies) categories of vitamin B6 intake (dietary or total) were compared (RR = 0.90; 95% CI = 0.75–1.07; I^2 = 56%) (Larsson et al., 2010).

Prospective studies published subsequently also did not find a significant association between total vitamin B6 intake and risk of colorectal cancer (Schernhammer et al., 2011; Key et al., 2012), even when the association between morbidity and vitamin B6 intake in the remote past (12–16 years before diagnosis) or more recently (0–4 years before diagnosis) was examined separately (Zhang et al., 2012).

In the meta-analysis of Larsson et al. (2010) mentioned above, some cohorts provided data on both vitamin B6 intake and blood PLP concentration. In a meta-analysis of four nested case-control studies (I^2 = 0%), involving 883 cases of colorectal cancer and 1,424 controls, plasma/serum PLP concentration was inversely related to the risk of colorectal cancer, with a 50% significant decrease in risk for every 100 pmol/L increase in blood PLP concentrations (RR = 0.51; 95% CI = 0.38–0.69). A similar inverse association was observed in the European Prospective Investigation into Cancer and Nutrition (EPIC) study (1,365 cases and 2,319 healthy controls with a median follow-up of 3.6 years), that was not included in the meta-analysis of Larsson et al. (2010). In this prospective study, the RR for the highest (> 105.3 nmol/L) vs the lowest (< 45.4 nmol/L) quintile of plasma PLP was 0.68 (95% CI: 0.53–0.87; p for trend < 0.02) (Eussen et al., 2010b).

Studies investigating the relationship between vitamin B6 intake or blood PLP concentration and the risk of *breast cancer* also yielded inconsistent results, as shown by Wu et al. (2013). Meta-analysis of the results of six prospective and eight case-control studies including 14,260 breast cancer cases did not show a significant association between vitamin B6 intake (food and supplements) and risk of breast cancer in pre- and post-menopausal women (RR = 0.95; 95% CI = 0.83–1.08; I^2 = 56.2%). However, in a meta-analysis of five nested case-control studies involving 2,509 cases, a significant inverse association between serum PLP concentration and risk of breast cancer was found (RR = 0.80; 95% CI = 0.66–0.98; I^2 = 0.30%); stratified analyses showed that this association remained significant for post-menopausal women only (RR = 0.71; 95% CI = 0.57–0.88).

Studies investigating the relationship between vitamin B6 intake or blood PLP concentration and risk of *lung cancer* were also in disagreement. Prospective cohort studies (follow up of 11.2–15 years) did not find a significant association between food vitamin B6 intake and risk of lung cancer in both men and women (Bassett et al., 2012a; Takata et al., 2012). However, two large nested case-control studies with a follow-up of 5–6 years showed a significant inverse association (fourth vs first quartile odds ratio (OR) = 0.44; 95% CI = 0.33–0.60; p for trend < 0.000001) between plasma PLP concentration and risk of lung cancer (Johansson et al., 2010).

Studies investigating the relationship between vitamin B6 intake or blood PLP concentration and risk of *pancreatic cancer* have again shown inconsistent results. Both a prospective study, which followed 81,922 men and women (aged 45–83 years) for 7.2 years, and a nested case-control study (208 cases and 623 controls matched for age and smoking status, with time between baseline blood collection and diagnosis of up to 21 years), did not find a significant association between food vitamin B6 intake (Larsson et al., 2007) or plasma PLP concentration (Schernhammer et al., 2007) and risk of pancreatic cancer. In contrast, another nested case-control study from a cohort followed for 9.6 years and involving 463 incident pancreatic cancer cases and matched (age and sex) controls found a significant inverse association between plasma PLP concentration and risk of pancreatic cancer in women (Chuang et al., 2011). In this study, the OR was 0.42 (95% CI = 0.21–0.83) for the highest (> 54.8 nmol/L) vs the lowest (< 23.8 nmol/L) quintile of plasma PLP concentration in women, whereas the OR in men was 1.13 (95% CI = 0.55–2.32).

However, studies consistently showed no significant relationship between *prostate cancer* risk and vitamin B6 intake or blood PLP concentration. Two large prospective studies, which followed participants for 15–17 years, did not observe a significant association between risk of prostate cancer and vitamin B6 intake from food and supplements (Weinstein et al., 2006) or vitamin B6 intake from food only (Bassett et al., 2012b). Similarly, a case-control study involving 561 prostate cancer cases and 1,034 matched (age and recruitment date) controls failed to find a significant association between plasma PLP concentration and risk of prostate cancer (Johansson et al., 2009).

In addition, no association was shown between either vitamin B6 intake (from food only or food and supplements) and *endometrial cancer risk* (Liu et al., 2013) or plasma PLP concentration and risk of *gastric cancer* (Eussen et al., 2010a) or *renal cell carcinoma* (Gibson et al., 2010).

In one case-control study involving 1,910 women with *ovarian cancer* and 1,989 controls, vitamin B6 intake (food only), assessed via FFQ covering 1 year before the diagnosis, was inversely associated with the risk of ovarian cancer (Harris et al., 2012). The OR was 0.76 (95% CI = 0.64–0.92; p for trend = 0.002) when comparing the highest (> 2.1 mg/day) with the lowest (< 1.5 mg/day) quartiles of vitamin B6 intake.

The Panel notes that there is a disagreement between the different studies investigating the association between vitamin B6 intake or plasma/serum PLP concentrations and risk of various types of cancer. The Panel concludes that the data on vitamin B6 intake or serum/plasma PLP concentration and cancer are inconsistent and cannot be used to derive DRVs for vitamin B6.

5.2.3. Cognition and depression

The available four RCTs on cognition-related outcomes (Tolonen et al., 1988; Deijen et al., 1992; Bryan et al., 2002; Stott et al., 2005) used supplemental doses of vitamin B6 of 20–75 mg/day that were close to or above the UL for adults (Section 2.2.2.2), were of short duration (5–12 weeks) and showed inconsistent results.

One systematic review of prospective cohort and case-control studies (Raman et al., 2007) investigated the association between B-vitamins (including vitamin B6 intake or status assessed by plasma PLP concentration) and performance on cognitive tests or risk of Alzheimer's disease. The authors noted that no meta-analysis could be undertaken in particular due to the heterogeneity in the cognitive-testing methods applied (30 methods). Another systematic review (van de Rest et al., 2012) on B-vitamins or n-3 fatty acids and cognition-related outcomes or depression reported on reviews and individual observational and intervention studies on vitamin B6 intake or 'status' in healthy subjects or patients. Both systematic reviews, without meta-analyses, concluded on the limited/inconsistent evidence on the relationship between vitamin B6 intake or 'status' and these outcomes. The Panel notes that no quantitative data can be derived from these systematic reviews in order to set DRVs for vitamin B6.

The Panel concludes that the data available on vitamin B6 intake or plasma PLP concentration and cognitive outcomes or depression are limited/inconsistent and cannot be used for to derive DRVs for vitamin B6.

5.2.4. Risk of bone fracture

A cross-sectional study in men and women showed a significant inverse association between vitamin B6 intake (food only) (measured by a validated FFQ) and risk of fractures. There was a decrease in risk of non-vertebral fractures (hazard ratio (HR) = 0.77; 95% CI = 0.65–0.92; p = 0.005) and fragility fractures (HR = 0.55; 95% CI = 0.40–0.77; p = 0.0004) in the highest quartile of vitamin B6 intake (mean 2.03 mg/day) compared with quartiles 1–3 (range of mean intakes 1.30–1.67 mg/day), after adjustment for potential confounders (Yazdanpanah et al., 2007).

A prospective study in older men and women followed-up for 4 years did not find a significant association between plasma PLP concentration and risk of bone loss and hip fracture after adjustment for potential confounders, when participants with plasma PLP concentration < 20 nmol/L were compared with those with PLP concentrations ≥ 30 nmol/L (McLean et al., 2008).

The Panel concludes that the data available on vitamin B6 intake or plasma PLP concentration and bone fracture risk are limited and inconsistent and cannot be used for deriving DRVs for vitamin B6.

5.2.5. All-cause mortality

A cross-sectional study showed a significantly higher risk of total mortality in men and women who were in the lowest quartile of vitamin B6 intake (mean value of 1.30 mg/day) compared with those in the quartiles 2–4 (range of means: 1.50–2.03 mg/day) (HR = 1.24; 95% CI: 1.09–1.40; p = 0.001) (Yazdanpanah et al., 2007).

The Panel concludes that the available data on vitamin B6 intake and all-cause mortality are limited and cannot be used for deriving DRVs for vitamin B6.

5.2.6. Conclusions on vitamin B6 intake/status and health consequences

In view of the limited and/or inconsistent evidence on an association between vitamin B6 intake or plasma PLP concentration and health consequences, the Panel considers that the data available cannot be used for deriving DRVs for vitamin B6.

6. Data on which to base Dietary Reference Values

The Panel considers that, as the release of the DRVs for vitamin B6 intake for adults by SCF (1993), new data are available for some population groups (i.e. women). In addition, the Panel notes that there is no consistent relationship between plasma PLP concentrations and protein intake, and

considers that there is no conclusive evidence that vitamin B6 requirements change according to protein intake in the range of observed intake in Europe (Sections 2.3.7.3 and 5.1). Thus, the Panel considers not appropriate to standardise vitamin B6 requirements on protein intake (Section 5.1). Thus, the approach adopted in the current opinion takes into account only in general terms the total protein intake in relation to vitamin B6 requirements, in contrast to the method used by SCF (1993) for deriving the AR and PRI as ratios of vitamin B6 to dietary protein intake. In view of the limited and/or inconsistent evidence on an association between vitamin B6 intake or plasma PLP concentration and health consequences (Section 5.2), the Panel considers that the data available cannot be used for deriving DRVs for vitamin B6.

6.1. Adults

6.1.1. Women

For younger women, the Panel notes the results obtained from the inverse prediction examination of the linear regression analysis by Hansen et al. (2001) of plasma PLP concentration vs vitamin B6 intake adjusted for bioavailability (Section 5.1.1.4). These results are based on the combined data of 44 women (mean age about 20–30 years according to studies) participating in six intervention studies (five references) investigating the effect of diets providing a broad range of predetermined dietary vitamin B6 intake (adjusted vitamin B6 intake: about 0.5–3.5 mg/day (read on figure)) (Kretsch et al., 1995; Hansen et al., 1996a, 1997, 2001; Huang et al., 1998). This regression analysis showed that plasma PLP concentration was strongly correlated with vitamin B6 intake ($r = 0.879$) and that vitamin B6 intake of 1.2 mg/day is sufficient to maintain a plasma PLP concentration of 30 nmol/L for 50% of the population.

For older women, the Panel notes the results of the depletion/repletion intervention study by Ribaya-Mercado et al. (1991), which showed that intake of about 1.3 mg/day is sufficient to sustain plasma PLP concentrations above the cut-off for adequacy of 30 nmol/L in two women (61–71 years) who received 0.8 g protein/kg bw per day. The Panel notes that the higher vitamin B6 requirement in older adults than in younger women are in agreement with the results from a randomised cross-over intervention study by Pannemans et al. (1994), which demonstrated significantly lower mean plasma PLP response of older individuals (mean age ± SEM: 70 ± 1 years) compared with younger ones to two diets providing similar amounts of vitamin B6. The Panel notes that these results are also supported by the observed age-related decline in plasma PLP concentrations in several large cross-sectional observational studies (Rose CS et al., 1976; Bates et al., 1999b; Morris et al., 2008) (Sections 2.4.1.1 and 5.1.1.4).

As a conservative approach, the Panel concludes that an AR for vitamin B6 intake of all women can be set at 1.3 mg/day. Assuming a CV of 10% (in the absence of information on the variability in the requirement) and rounding to the nearest one decimal place, a PRI of 1.6 mg/day is derived.

6.1.2. Men

In the absence of reliable data to determine vitamin B6 requirement in men, the Panel proposes to extrapolate the AR for (all) men from the AR for (all) women (Section 6.1.1).

Allometric scaling is adopted, assuming that the requirement for vitamin B6 relates to metabolically active body mass and taking into account the differences in reference body weights. The reference body weights of 18–79 years old men (68.1 kg) and women (58.5 kg) were calculated by the measured body heights of 16,500 men and 19,969 women in 13 European Union (EU) Member States and assuming a BMI of 22 kg/m^2 (see Appendix 11 in EFSA NDA Panel (2013)).

$$AR_{men} = AR_{women} \times (weight_{men}/weight_{women})^{0.75}$$

Rounding to the nearest one decimal place, a vitamin B6 intake of 1.5 mg/day is set as an AR for men. The PRI was calculated based on the unrounded AR and assuming a CV of 10%, and rounding to the nearest one decimal place, a PRI of 1.7 mg/day for men is derived.

6.2. Infants

The Panel considers the limitations of the available studies on vitamin B6 intake and status in infants and concludes that these cannot be used to set an AR and a PRI (Section 5.1.2). The Panel considered two approaches to set DRVs for infants (7–11 months).

Vitamin B6 intake of infants from birth to 6 months is calculated by the average consumption of breast milk and its concentration of vitamin B6. Based on the two studies (Appendix A) on healthy unsupplemented mothers reporting data on mature milk and both maternal vitamin B6 intake and plasma PLP concentrations, the mean vitamin B6 concentration of breast milk is reported to be on average 0.125 mg/L (rounded to 0.130 mg/L, Section 2.3.6.3). For women exclusively breastfeeding, the mean milk transfer over the first 6 months post-partum is assumed to be 0.8 L/day (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009). Thus, the calculated vitamin B6 intake for infants aged 0–6 months is 0.1 mg/day.

In order to estimate AI of infants aged 7–11 months by upwards extrapolation from the calculated vitamin B6 intake for exclusively breastfed infants from birth to 6 months, allometric scaling was applied on the assumption that vitamin B6 requirement is related to metabolically active body mass. Averages of the median weight-for-age of male and female infants aged 3 months (6.1 kg) and 9 months (8.6 kg) according to the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006) were used, and a value of 0.13 mg/day was calculated for both boys and girls.

$$\text{AI}_{\text{infants 7–11 months}} = \text{vitamin B6 intake}_{\text{infants 0–6 months}} \times (\text{weight}_{\text{infants 9 months}}/\text{weight}_{\text{infants 3 months}})^{0.75}$$

Following this approach, the calculated AI for vitamin B6 for infants aged 7–11 months would be 0.13 mg/day.

The Panel also calculated the AR for infants by downwards extrapolation from the AR of adults (Sections 6.1.1 and 6.1.2). Allometric scaling was used on the assumption that vitamin B6 requirement is related to metabolically active body mass. For the calculation, averages of the median weight-for-age of male and female infants aged 9 months (8.6 kg) according to the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006), and reference body weights for men and women (Section 6.1.2) were used. The growth factor of 0.57 was applied for infant boys and girls (EFSA NDA Panel, 2014); the growth factor was calculated as the proportional increase in protein requirement for growth relative to the maintenance requirement (EFSA NDA Panel, 2012) (Section 6.3).

$$\text{AR}_{\text{infants 7–11 months}} = \text{AR}_{\text{adults}} \times (\text{weight}_{\text{infants 9 months}}/\text{weight}_{\text{adults}})^{0.75} \times (1 + \text{growth factor})$$

Following this approach, the calculated AR for vitamin B6 for infants aged 7–11 months would be 0.48 mg/day.

The Panel notes the methodological uncertainties of the EFSA intake estimates in infants (table footnotes of Appendices C and D and Section 3.2), due to limitations in the measurement of breast milk consumption in these surveys. Average total vitamin B6 intake ranged from 0.37 to 0.76 mg/day in infants (Section 3.2, Appendices C and D), with a midpoint of this range at about 0.6 mg/day. The Panel also notes that foods consumed by infants in the second half year of life are often fortified with vitamin B6.

Following the approach by the IOM (Section 4.2), an average of the upwards and downwards extrapolations described above would be 0.3 mg/day.

The Panel concludes that an AI of vitamin B6 can be set at 0.3 mg/day for infants aged 7–11 months (Table 4).

Table 4: Reference body weights and Adequate Intake (AI) of vitamin B6 for infants aged 7–11 months

Age	Reference body weight (kg)	AI (mg/day)
7–11 months	8.6[(a)]	0.3

(a): Average of the median weight-for-age of male or female infants, respectively, aged 9 months according to the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006).

6.3. Children

The Panel notes that there are no reliable data for children on which to base an AR for vitamin B6 (Section 5.1.3). Therefore, the ARs were calculated by downwards extrapolation from the AR of adults. Allometric scaling was used on the assumption that vitamin B6 requirement is related to metabolically active body mass:

$$AR_{child} = AR_{adults} \times (weight_{child}/weight_{adults})^{0.75} \times (1 + growth\ factor)$$

For the calculations (Table 5), median body weights of boys and girls (van Buuren et al., 2012) and median body weights of 18–79 years old men and women were used, based on measured body heights of 16,500 men and 19,969 women in 13 EU Member States and assuming a body mass index of 22 kg/m^2 (see Appendix 11 in EFSA NDA Panel (2013)). The following growth factors were applied: 0.25 for boys and girls aged 1–3 years, 0.06 for boys and girls aged 4–6 years, 0.13 for boys and girls aged 7–10 years, 0.11 for boys and 0.08 for girls aged 11–14 years and 0.08 for boys and 0.03 for girls aged 15–17 years. Growth factors were calculated as the proportional increase in protein requirement for growth relative to the maintenance requirement at the different ages (EFSA NDA Panel, 2012) (Section 6.2). The value for each age group corresponds to the mean of values for the years included (EFSA NDA Panel, 2014). For the calculation of the PRI, a CV of 10% was assumed (as the variability in the requirement is unknown) and the calculated values were rounded to the nearest one decimal place. The Panel considered unnecessary to set sex-specific PRIs for boys and girls aged 1–14 years, but chose to set different PRIs for boys and girls aged 15–17 years as for adults (Section 6.1). Although the calculations yielded a PRI for boys aged 15–17 years that was higher (i.e. 1.8 mg/day) than the value set for men (i.e. 1.7 mg/day, after rounding), the Panel considered that there was no reason for such a difference, and hence decided to set the same PRI for boys aged 15–17 years and men.

Table 5: Reference body weights (rounded), Average Requirements (ARs) and (rounded) Population Reference Intakes (PRIs) of vitamin B6 for children and adolescents

Age	Reference body weight (kg)		Calculated ARs (mg/day)		Calculated PRIs (mg/day)		Proposed PRIs (mg/day)
	Boys	Girls	Boys	Girls	Boys	Girls	Boys and girls
1–3 years	12.2[(a)]	11.5[(a)]	0.5	0.5	0.6	0.6	0.6
4–6 years	19.2[(b)]	18.7[(b)]	0.6	0.6	0.7	0.7	0.7
7–10 years	29.0[(c)]	28.4[(c)]	0.9	0.9	1.0	1.0	1.0
11–14 years	44.0[(d)]	45.1[(d)]	1.2	1.2	1.4	1.4	1.4
15–17 years	64.1[(e)]	56.4[(e)]	1.5	1.3	1.8	1.6	1.6 (girls) 1.7 (boys)[(f)]

(a): Average of the median weight-for-age of male or female children aged 24 months according to the WHO Growth Standards (WHO Multicentre Growth Reference Study Group, 2006).
(b): Average of the median weight of male or female children aged 5 years (van Buuren et al., 2012).
(c): Average of the median weight of male or female children aged 8.5 years (van Buuren et al., 2012).
(d): Average of the median weight of male or female children aged 12.5 years (van Buuren et al., 2012).
(e): Average of the median weight of male or female children aged 16 years (van Buuren et al., 2012).
(f): The Panel decided to set the same PRI for boys aged 15–17 years and for men.
Adult body weight used for calculations: 63.3 kg (average of 68.1 kg for men and 58.5 kg for women). In Table 5, values for ARs and PRIs were rounded to the nearest one decimal place, but PRIs were calculated based on the unrounded ARs.

6.4. Pregnancy

The Panel finds the available data on vitamin B6 supplementation in pregnant women unsuitable for setting the requirement for vitamin B6 for this population (Section 5.1.4). For pregnant women, a vitamin B6 intake in addition to that required for non-pregnant women is estimated based on the mean gestational weight gain and the average vitamin B6 content of the human tissue and by considering vitamin B6 bioavailability[20] from a mixed diet. The Panel assumed a total body content of vitamin B6 of 15 nmol/g (3.7 μg/g tissue) (Section 2.3.4), and considered a mean gestational increase in body weight of 12 kg, a pregnancy duration of 280 days and a bioavailability of vitamin B6 from a mixed diet of 75% (Section 2.3.1). The Panel thus estimated an additional amount of vitamin B6 intake for pregnant women to be 0.2 mg/day vitamin B6, after rounding to the nearest one decimal place (Section 5.1.4). Thus, as a conservative approach, a value of 0.2 mg/day is added to the AR of

[20] Bioavailability of vitamin B6 refers to the amount of ingested vitamin that is utilised for normal physiological functions and storage (Section 2.3.1).

non-pregnant women (1.3 mg/day), resulting in an AR of 1.5 mg/day. Assuming a CV of 10%, and rounding to the nearest one decimal place, a PRI of 1.8 mg/day vitamin B6 is derived.

6.5. Lactation

For lactating women, an additional intake of vitamin B6 is proposed to balance vitamin B6 losses in human milk. For women who are exclusively breastfeeding, the milk transfer over the first 6 months post-partum is assumed to be 0.8 L/day (Butte et al., 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009). Thus, considering an average concentration of vitamin B6 in breast milk of 0.125 mg/L (rounded to 0.130 mg/L, Section 2.3.6.3 and Appendix A) and a volume of secreted breast milk of 0.8 L/day, an average amount of 0.1 mg/day of vitamin B6 is estimated to be lost with milk over the first 6 months post-partum (Section 6.2). Assuming a bioavailability of vitamin B6 of 75%[20] (Section 2.3.1), a mean vitamin B6 intake of 0.133 mg/day is required to balance the amount of vitamin B6 secreted in milk for exclusively breastfeeding women during the first 6 months of lactation. This intake, added to the AR of non-lactating women (1.3 mg/day), results in an AR of 1.4 mg/day vitamin B6. The Panel notes that this corresponds to the vitamin B6 intake assessed by duplicate diet analysis in a study in healthy breastfeeding women (Andon et al., 1989) (Appendix A), whose mean plasma PLP concentration was above 30 nmol/L. Assuming a CV of 10%, and rounding to the nearest one decimal place, a PRI of 1.7 mg/day vitamin B6 is derived for exclusively breastfeeding women.

Conclusions

The Panel concludes that ARs and PRIs for vitamin B6 for adults can be derived from the vitamin B6 intake required to maintain a (mean) concentration of plasma PLP above 30 nmol/L. Based on new available intervention studies in young women, and also considering, as a conservative approach, data from (small) intervention studies supported by results from (large) cross-sectional observational studies in older adults, the Panel derives an AR for (all) women. In the absence of reliable data to determine vitamin B6 requirement in men, the Panel sets an AR for (all) men by allometric scaling from the AR for (all) women, taking into account the difference in reference body weights. For pregnant and lactating women, the AR for non-pregnant non-lactating women is increased to account for the uptake of vitamin B6 by the fetal and maternal tissues, and the losses through breast milk, respectively. For children aged 1–17 years, the Panel derives ARs by downwards extrapolation from adult values, by allometric scaling, applying growth factors and taking into account the differences in reference body weights. In the absence of information on the variability in the requirement, a CV of 10% is used to calculate PRIs from the ARs for all age groups in children and in adults. For infants aged 7–11 months, the Panel proposes an AI, combining the results of two extrapolation approaches by allometric scaling (both taking into account the differences in reference body weights). The proposed AI is the average of the results of upwards extrapolation from the estimated intake of vitamin B6 of exclusively breastfed infants from birth to 6 months, and of downwards extrapolation from the ARs for adults applying a growth factor. The Panel considers unnecessary to give sex-specific DRVs for infants and children up to 14 years of age (Table 6).

Table 6: Summary of Dietary Reference Values for vitamin B6

Age	Average Requirement (mg/day)	Population Reference Intake (mg/day)
7–11 months	–	0.3[(a)]
1–3 years	0.5	0.6
4–6 years	0.6	0.7
7–10 years	0.9	1.0
11–14 years	1.2	1.4
15–17 years (M)	1.5	1.7
15–17 years (F)	1.3	1.6
Adults (M)	1.5	1.7
Adults (F)	1.3	1.6
Pregnancy	1.5	1.8
Lactation	1.4	1.7

F: females; M: males.
(a): Adequate Intake.

Recommendations for research

The Panel suggests:

- to generate data from studies specifically designed to assess vitamin B6 requirements in men, older adults, infants, children and pregnant and lactating women,
- to generate data for developing criteria for adequacy for vitamin B6 biomarkers of intake, status and function in healthy populations,
- to conduct studies for clarifying the relationships between vitamin B6 intake, status and health outcome.

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Abbreviations

α-EALT	activation coefficient of erythrocyte alanine aminotransferase
α-EAST	activation coefficient of erythrocyte aspartate aminotransferase
4-PA	4-pyridoxic acid
Afssa	Agence française de sécurité sanitaire des aliments
AI	Adequate Intake
AR	Average Requirement
AUC	area under the curve
CHD	coronary heart disease
CI	confidence interval
COMA	Committee on Medical Aspects of Food Policy
CV	coefficient of variation
CVD	cardiovascular disease
D-A-CH	Deutschland–Austria–Confoederatio Helvetica
DH	Department of Health
DIPP	Type 1 Diabetes Prediction and Prevention survey
DNA	Deoxyribonucleic acid
DNFCS	Dutch National Food Consumption Survey
DNSIYC	Diet and Nutrition Survey of Infants and Young Children
DRV	Dietary Reference Value
EALT	erythrocyte alanine aminotransferase
EAR	Estimated Average Requirement
EAST	erythrocyte aspartate aminotransferase
EPIC	European Prospective Investigation into Cancer and Nutrition
EsKiMo	Ernährungsstudie als KIGGS-Modul
FAO	Food and Agriculture Organization
FFQ	Food Frequency Questionnaire
FINDIET	National dietary survey of Finland
Hcy	homocysteine
HPLC	high-performance liquid chromatography
HR	hazard ratio
INCA	Etude Individuelle Nationale de Consommations Alimentaires
INRAN-SCAI	Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia
IOM	US Institute of Medicine of the National Academy of Sciences
IUPAC–IUB	International Union of Pure and Applied Chemistry–International Union of Biochemistry
LC	liquid chromatography
LI	Lower Intake Level
LRNI	Lower Reference Nutrient Intake
LTI	Lower Threshold Intake
MM	molecular mass
MS	mass spectrometry
NANS	National Adult Nutrition Survey
NCM	Nordic Council of Ministers
NDNS	National Diet and Nutrient Survey
NHANES	National Health and Nutrition Examination Survey
NNR	Nordic Nutrition Recommendations
NWSSP	Nutrition and Wellbeing of Secondary School Pupils
NOAEL	No Observed Adverse Effect Level
OR	odds ratio
PL	pyridoxal
PLP	pyridoxal–5′–phosphate
PM	pyridoxamine

PMP	pyridoxamine 5′-phosphate
PN	pyridoxine
PNG	pyridoxine-5′-β-d-glucoside
PNP	pyridoxine 5′-phosphate
PN-HCl	pyridoxine hydrochloride
PRI	Population Reference Intake
RCT	randomised controlled trial
RDA	Recommended Dietary Allowance
RI	Recommended Intake
RNI	Recommended Nutrient Intake
RPLC	reversed-phase liquid chromatography
RR	relative risk
SAM	*s*-adenosylmethionine
SCF	Scientific Committee for Food
SD	standard deviation
SE	standard error
SEM	standard error of the mean
SU.VI.MAX	Supplémentation en vitamines et minéraux antioxydants
UL	Tolerable Upper Intake Level
UNU	United Nations University
UPLC	ultra-performance liquid chromatography
VELS	Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln
WHO	World Health Organization

Appendix A – Concentrations of vitamin B6 in breast milk of healthy mothers

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
Andon et al. (1989)	30	USA	Dietary total (glycosylated + non-glycosylated) vitamin B6 intakes (expressed in pyridoxine equivalents) 1.46 Women discontinued the consumption of vitamin B6 supplements within the first week post-partum	***Maternal status** Mean ± SD (range) 34 ± 13 (12–54)	~ 60 days post-partum	Vitamin B6 Mean ± SD (range) 124 ± 33 (59–195)	Microbiological assay (*Saccharomyces uvarum*)	Collection by the mothers of duplicate-plate diet (composites of food and beverage consumed during three 24-h periods); vitamin B6 content was analysed Milk sampled during each of three consecutive days Foremilk from each feeding or milk expression No information on whether the infants were born at term or not. However, infants were in the 10th–95th percentiles for weight and for length ≥ infants assumed to be born at term Concentration of plasma PLP in infants also reported in the paper
Borschel et al. (1986)	51 (initial sample) 39 (after 12 drop-outs) 8 (8)	USA	Dietary intake of vitamin B6 for both Group 1 and Group 2: 1.53 ± 0.08 **Group 1** Supplementation with vitamin B6, expressed in mg PN-HCl/day: 2.5	***Cord blood PLP** at delivery (n = 42): 223 ± 19	0–6 months post-partum	Vitamin B6 (range of means ± SD) **Group 1** 148 ± 24–212 ± 31	Not reported	Data on group 3 (n = 8) which was formula-fed are not reported in this Appendix One-day food record on the day of milk sampling Milk sampling (24 h period) was made at 1, 2, 4 and 6 months post-partum

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
	9 (9)		**Group 2** Supplementation with vitamin B6, expressed in mg PN-HCl/day: 15.0			**Group 2** 374 ± 36–534 ± 43		Foremilk Term infants Plasma PLP in cord blood at delivery and in neonates reported in the paper, but not in breastfeeding mothers Mean concentration of PLP in cord plasma at delivery correlated with the level of vitamin supplementation of the mothers
Boylan et al. (2002)	25 (25) 21 (21) with detectable pyridoxamine content in their milk 3 (3) with detectable pyridoxine content in their milk	USA (16 white, 2 African-American, 6 Hispanic, 1 other)	Vitamin B6 intake from food and supplements: 3.47 ± 1.36	–	8–11 days post partum	Mean ± SD (median; range) Pyridoxal 70 ± 50 (55; 20–216) Pyridoxamine 10 ± 10 (8; 2–40) Pyridoxine 10 ± 3 (10; 7–14) Phosphorylated form of vitamin B6: Not detected in any sample Vitamin B6[(a)] 90	High-performance liquid chromatographic with fluorescence detector	24-h dietary recall Eight samples collected on the 8th day post-partum Nine samples on the 9th day post-partum Six samples on the 10th day post-partum Two samples on the 11th day post-partum Out the 25 women, two were not taking vitamins supplements Term infants Maternal plasma PLP concentration not assessed

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
Chang and Kirksey (1990)	47 (35 studied longitudinally)	USA	Dietary intake: Range of mean in the four groups: 1.0–1.8 Supplementation with PN-HCl: 2.5, 4.0, 7.5 or 10 (four groups)	***Maternal status** (mean ± SEM) 93 ± 7 103 ± 8 155 ± 9 320 ± 12		Vitamin B6	Microbiological assay (*Saccharomyces uvarum*)	24-h records of food intakes during days of milk sampling Multivitamin and multimineral supplementation Vitamin B6 concentration in breast milk determined graphically No information on whether the infants were born at term or not Maternal plasma at 1, 4 and 6 months post-partum and cord blood concentrations of PLP were assessed Only maternal values at 6 months post-partum are reported here
	6 (6)		2.5		1 month post-partum	152		
	13 (13)		4.0			203		
	9 (9)		7.5			305		
	7 (7)		10.0			288		
	6 (6)		2.5		2 months post-partum	203		
	13 (13)		4.0			288		
	9 (9)		7.5			338		
	7 (7)		10.0			372		
	6 (6)		2.5		3 months post-partum	203		
	13 (13)		4.0			271		
	9 (9)		7.5			355		
	7 (7)		10.0			389		
	6 (6)		2.5		4 months post-partum	203		
	13 (13)		4.0			321		
	9 (9)		7.5			406		
	7 (7)		10.0			508		
	6 (6)		2.5		5 months post-partum	203		
	13 (13)		4.0			321		
	9 (9)		7.5			406		
	7 (7)		10.0			474		
	6 (6)		2.5		6 months post-partum	220		
	13 (13)		4.0			321		
	9 (9)		7.5			355		
	7 (7)		10.0			389		

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
Chang and Kirksey (2002)	47	USA	(Mean ± SE): four groups (a) Supplementation (PN-HCl) (b) Dietary vitamin B6 intakes (c) Total vitamin B6 intakes	–		Vitamin B6 Mean ± SE	Microbiological assay (*Saccharomyces uvarum*)	Method of dietary assessment of maternal vitamin B6 intake not indicated Multivitamin and multimineral supplementation No information on whether the infants were born at term or not. However, weight and length of infants were reported from birth to 6 months Maternal PLP concentrations measured but not reported in the paper Plasma PLP concentration of infants (nmol/L) measured at 1, 4 and 6 months, according to the level of maternal supplementation (presented graphically)
	11 (11)		(a) 2.5 (b) 1.6 ± 0.2 (SE) (c) 4.1 ± 0.2 (SE)		1 month post-partum	150.7 ± 5.1 (SE)		
	15 (15)		(a) 4.0 (b) 1.7 ± 0.2 (SE) (c) 6.2 ± 0.2 (SE)			200.3 ± 8.6 (SE)		
	10 (10)		(a) 7.5 (b) 1.6 ± 0.2 (SE) (c) 9.1 ± 0.2 (SE)			296.4 ± 14.7 (SE)		
	10 (10)		(a) 10.0 (b) 1.5 ± 0.4 (SE) (c) 11.5 ± 0.4 (SE)			288.3 ± 6.6 (SE)		
	9 (9)		(a) 2.5 (b) 1.4 ± 0.2 (SE) (c) 3.9 ± 0.2 (SE)		2 months post-partum	200.3 ± 5.6 (SE)		
	16 (16)		(a) 4.0 (b) 1.8 ± 0.3 (SE) (c) 6.3 ± 0.3 (SE)			293.4 ± 10.6 (SE)		
	10 (10)		(a) 7.5 (b) 1.2 ± 0.2 (SE) (c) 8.7 ± 0.2 (SE)			336.9 ± 13.7 (SE)		
	11 (11)		(a) 10.0 (b) 1.3 ± 0.2 (SE) (c) 11.3 ± 0.2 (SE)			375.3 ± 16.7 (SE)		

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
	10 (10)		(a) 2.5 (b) 1.9 ± 0.5 (SE) (c) 4.4 ± 0.5 (SE)		3 months post-partum	212.5 ± 9.6 (SE)		
	16 (16)		(a) 4.0 (b) 1.4 ± 0.1 (c) 5.9 ± 0.1			271.1 ± 9.1 (SE)		
	10 (10)		(a) 7.5 (b) 1.4 ± 0.2 (c) 8.9 ± 0.2			357.1 ± 8.1 (SE)		
	10 (10)		(a) 10.0 (b) 1.3 ± 0.2 (c) 11.3 ± 0.2			384.4 ± 0.2 (SE)		
	7 (7)		(a) 2.5 (b) 1.2 ± 0.1 (c) 3.7 ± 0.1		4 months post-partum	205.4 ± 8.1 (SE)		
	16 (16)		(a) 4.0 (b) 1.4 ± 0.2 (c) 5.9 ± 0.2			321.7 ± 10.1 (SE)		
	10 (10)		(a) 7.5 (b) 1.0 ± 0.1 (c) 8.5 ± 0.1			407.7 ± 15.7 (SE)		
	10 (10)		(a) 10.0 (b) 1.2 ± 0.2 (c) 11.2 ± 0.2			487.6 ± 27.8 (SE)		
	5 (5)		(a) 2.5 (b) 1.1 ± 0.2 (c) 3.6 ± 0.2		5 months post-partum	201.3 ± 8.6 (SE)		
	13 (13)		(a) 4.0 (b) 1.4 ± 0.2 (c) 5.9 ± 0.2			330.8 ± 13.1 (SE)		
	10 (10)		(a) 7.5 (b) 1.3 ± 0.2 (c) 8.8 ± 0.2			401.7 ± 15.2 (SE)		

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
	8 (8)		(a) 10.0 (b) 1.2 ± 0.2 (c) 11.2 ± 0.2			465.4 ± 21.8 (SE)		
	4 (4)		(a) 2.5 (b) 1.0 ± 0.3 (c) 3.5 ± 0.3		6 months post-partum	222.6 ± 12.7 (SE)		
	12 (12)		(a) 4.0 (b) 1.5 ± 0.2 (c) 6.0 ± 0.2			328.8 ± 12.7 (SE)		
	10 (10)		(a) 7.5 (b) 1.5 ± 0.2 (c) 9.0 ± 0.2			385.5 ± 14.7 (SE)		
	6 (6)		(a) 10.0 (b) 1.0 ± 0.1 (c) 11.0 ± 0.1			395.6 ± 17.7 (SE)		
Hamaker et al. (1990)	19	USA		–	1 month post-partum	(Period 1) Before supplementation (Period 2) 3–8 h post-supplementation (Period 3) 2–5 h after period 2 (Period 4) 5–15 h after period 2	RPLC Microbiological assay (*Saccharomyces uvarum*) for result comparison	Milk collection: foremilk at each infant feeding during one 24-h period No information on whether the infants were born at term or not Plasma PLP not assessed No information on the vitamin B6 dietary intake
	8		(a) Supplementation with 2.5 mg/day PN-HCl			(Period 1) 123 ± 44 (Period 2) 219 ± 102 (Period 3) 136 ± 42 (Period 4) 148 ± 87		
	9		(b) Supplementation with 27 mg/day PN-HCl			(Period 1) 271 ± 57 (Period 2) 693 ± 118 (Period 3) 406 ± 115 (Period 4) 291 ± 101		
	2		(c) No supplementation			Mean of the 4 periods for each subject: 140 and 130		

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
Kang-Yoon et al. (1992)	20 14 6	USA	Estimated dietary intake: 1.8 ± 0.2 (SE) (a) Supplementation with 2 mg/day PN-HCl (1.7 mg/day PN equivalents) (b) Supplementation with 27 mg/day PN-HCl (22.3 mg/day PN equivalents)	***Cord blood PLP** (a) 114 ± 14 (b) 171 ± 32	First 28 days of lactation for term infants	**Read on figures** (range of means according to days of lactation between 7 and 28 days) (a) 80–130 (b) 390–540	HPLC	24-h dietary recalls obtained on the days of sampling Milk collection: foremilk at each infant feeding during one 24-h period at 7, 14 and 28 days of lactation Vitamin B6 concentration in breast milk determined graphically Term infants Maternal blood samples were collected at delivery, and at 7, 14 and 28 days. Maternal PLP values not reported in the article
Kang-Yoon et al. (1995)	20 (term)	USA	Mean ± SE Dietary intake: Measured but not reported. (a) Supplementation with 2 mg/day PN-HCl (1.7 mg/day PN equivalents) (b) Supplementation with 27 mg/day PN-HCl (22.3 mg/day PN equivalents)	***Cord blood PLP** Term infants: (a) 114 ± 14 (b) 171 ± 32	**Term infants**: first 28 days of lactation	**Read on figures** (range of means according to days of lactation between 7 and 28 days) (a) 80–130 (b) 390–540	HPLC	24-h dietary recall collected weekly Milk collection at each infant feeding during a 24-h period on days 7, 14, 21, and 28 Vitamin B6 concentration in breast milk determined graphically Term (n = 20) and preterm (n = 13) infants. Only data on term infants are reported here

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
Lovelady et al. (2001)		USA	Mean ± SEM (a) Dietary vitamin B6 intake (b) Total vitamin B6 intake	Mean ± SEM		Mean ± SEM	Microbiological assay (*Saccharomyces uvarum*)	Three days dietary record Term infants
	11		Weight loss group: women with restricted intakes (500 kcal/day) + exercise	Weight loss group		Vitamin B6		Plasma concentrations of PLP, plasma total vitamin B6, and erythrocyte alanine aminotransferase (EALT) activity were assessed at 4–6 weeks, 9–11 weeks and 14–16 weeks post-partum
			4–6 weeks post-partum: (a) 2.7 ± 0.6 (b) 4.5 ± 0.6	*4–6 weeks post-partum:* 55.5 ± 6.9 (SEM)	4–6 weeks post-partum	147 ± 15		
			9–11 weeks post-partum: (a) 1.9 ± 0.2 (b) 3.8 ± 0.2	*9–11 weeks post-partum:* 75.3 ± 16 (SEM)	9–11 weeks post-partum	180 ± 19		
			14–16 weeks post-partum: (a) 1.8 ± 0.3 (SEM) (b) 3.8 ± 0.3 (SEM)	*14–16 weeks post-USApartum:* 63.3 ± 9.8 (SEM)	14–16 weeks post-partum	174 ± 19		
	10		Control group: Women with usual diet + no exercise	Control group:				
			4–6 weeks post-partum: (a) 2.2 ± 0.1 (SEM) (b) 4.0 ± 0.3 (SEM)	*4–6 weeks post-partum* 73.7 ± 12.8 (SEM)	4–6 weeks post-partum	141 ± 13		
			9–11 weeks post-partum: (a) 1.9 ± 0.2 (SEM) (b) 3.9 ± 0.2 (SEM)	*9–11 weeks post-partum* 81.2 ± 14.8 (SEM)	9–11 weeks post-partum	161 ± 19		

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
			14–16 weeks post-partum: (a) 1.8 ± 0.2 (SEM) (b) 3.8 ± 0.2 (SEM) Both groups of women received a supplement of PN-HCl: 2.0 mg/day (from 4 to 16 weeks *post-partum*)	*14–16 weeks post-partum* 70.0 ± 11.6 (SEM)	14–16 weeks post-partum	173 ± 19		
Morrison and Driskell (1985)	21	USA 7 14	(a) Dietary vitamin B6 intake (b) Total vitamin B6 (dietary intake and supplementation) Mothers grouped by the authors based on maternal EALT activities: 'adequate status' (< 16%) or 'inadequate' status (> 25%). 'Inadequate' status of vitamin B6 (no supplement users) *1.16 ± 0.24 'Adequate' status of vitamin B6 (supplement users) *1.52 ± 0.34 *11.23 ± 16.23	Inadequate' status of vitamin B6 (no supplement users, n = 7) 61.9 ± 23.9 'Adequate' status of vitamin B6 (supplement users, n = 7) 159.8 ± 73.2	3–7 months post-partum	'Total B6 vitamers' 126 160	Microbiological assay (*Saccharomyces uvarum*)	One 24-h recall and four days of food records Vitamin B6 content in breast milk measured by two analytical methods (microbiological assay or HPLC) Fore milk samples No information on whether the infants were born at term or not Blood for PLP and EALT measurements was obtained on the morning following the final morning of milk collection and the 5 days of food intake records

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
		7 14	'Inadequate' status of vitamin B6 (no supplement users) *1.16 ± 0.24 'Adequate' status of vitamin B6 (supplement users) *1.52 ± 0.34 *11.23 ± 16.23			53 129	HPLC	
Moser-Veillon and Reynolds (1990)	40 10 10 10	USA	Mean ± SEM (a) Daily supplementation: zinc and vitamin B6 (PN-HCl) (b) Dietary vitamin B6 intake **(a) 0 mg Zn, 0.5 mg vitamin B6** (b) 1.4 ± 0.6 (SEM) (n = 10) **(a) 0 mg Zn, 4.0 mg vitamin B6** (b) 2.2 ± 0.9 (SEM) (n = 10) **(a) 0 mg Zn, 0.5 mg vitamin B6** (b) 1.5 ± 0.5 (SEM) (n = 10)	Range of means: **0 mg Zn, 0.5 mg vitamin B6** 1–2 weeks post-partum 15–19 **0 mg Zn, 4.0 mg vitamin B6** 1–2 weeks post-partum 38–59	 1 week post-partum 2 weeks post-partum	Total vitamin B6 Mean ± SEM **0 mg Zn, 0.5 mg vitamin B6** 84 ± 15 (n = 8) **0 mg Zn, 4.0 mg vitamin B6** 185 ± 20 (n = 9) **0 mg Zn, 0.5 mg vitamin B6** 139 ± 16 (n = 8)	Microbiological assay (*Saccharomyces uvarum*)	Three-day dietary records Multivitamin and multiminerals supplementation of lactating women, but only vitamin B6 and zinc content differed between the four groups (n = 10 per group initially) The intake of the two groups supplemented with both zinc (25 mg/day) and vitamin B6 (0.5 or 4.0 mg/day) were not presented here Women took a supplement of 4.0 mg/day of pyridoxine during pregnancy (but no zinc supplementation)

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
	10		**(a) 0 mg Zn, 4.0 mg vitamin B6** (b) 1.9 ± 1.0 (SEM) (n = 10)			**0 mg Zn, 4.0 mg vitamin B6** 249 ± 26 (n = 10)		Supplementation of vitamin B6 and zinc was given to lactating women from the day after the delivery to nine months post-partum
	10		**(a) 0 mg Zn, 0.5 mg vitamin B6** (b) 1.5 ± 0.4 (SEM) (n = 10)	**0 mg Zn, 0.5 mg vitamin B6** 4–36 weeks post-partum 35–47	4 weeks post-partum	**0 mg Zn, 0.5 mg vitamin B6** 173 ± 24 (n = 7)		No information on whether the infants were born at term or not Concentration of plasma PLP and total vitamin B6 at 1, 2, 4, 12, 24 and 36 weeks post-partum reported in the article (range of means are reported here)
	10		**(a) 0 mg Zn, 4.0 mg vitamin B6** (b) 1.9 ± 0.5 (SEM) (n = 10)	**0 mg Zn, 4.0 mg vitamin B6** 4–36 weeks post-partum 87–125		**0 mg Zn, 4.0 mg vitamin B6** 410 ± 49 (SEM) (n = 10)		
	10		**(a) 0 mg Zn, 0.5 mg vitamin B6** (b) 1.5 ± 0.4 (SEM) (n = 8)		12 weeks post-partum	**0 mg Zn, 0.5 mg vitamin B6** 239 ± 47 (n = 8)		
	10		**(a) 0 mg Zn, 4.0 mg vitamin B6** (b) 1.7 ± 0.5 (SEM) (n = 9)			**0 mg Zn, 4.0 mg vitamin B6** 432 ± 57 (n = 9)		
	10		**(a) 0 mg Zn, 0.5 mg vitamin B6** (b) 1.4 ± 0.7 (SEM) (n = 7)		24 weeks post-partum	**0 mg Zn, 0.5 mg vitamin B6** 223 ± 31 (n = 7)		
	10		**(a) 0 mg Zn, 4.0 mg vitamin B6** (b) 2.3 ± 1.2 (SEM) (n = 9)			**0 mg Zn, 4.0 mg vitamin B6** 451 ± 69 (n = 9)		

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
	10 10		**(a) 0 mg Zn, 0.5 mg vitamin B6** (b) 1.3 ± 0.5 (SEM) (n = 4) **(a) 0 mg Zn, 4.0 mg vitamin B6** (b) 1.7 ± 0.3 (SEM) (n = 7)		36 weeks post-partum	**0 mg Zn, 0.5 mg vitamin B6** 238 ± 49 (n = 4) **0 mg Zn, 4.0 mg vitamin B6** 524 ± 80 (n = 7)		
Roepke and Kirksey (1979)	61	USA	All subjects: dietary intake: 1.24 total (food + supplements): 6.2 Mean ± SE Maternal age: 18–22 years 5.2 ± 0.5 (n = 53) Maternal age: 23–37 years 6.4 ± 1.2 (n = 44) Maternal age: 18–22 years Maternal age: 23–37 years	–	3 days post-partum 14 days post-partum	Vitamin B6 Mean ± SE 12.2 ± 3.0 (n = 22) 15.6 ± 3.5 (n = 32) 43.5 ± 8.0 (n = 21) 48.4 ± 8.4 (n = 29) No significant differences between age groups.	Microbiological assay (*Saccharomyces uvarum*)	102 pregnant women studied at delivery, including 86 studied at 5 and 7 months of pregnancy, 40 of which were followed during lactation in addition to 26 mothers. Among these 66 mothers followed post-partum, 61 were breastfeeding All subjects were asked to complete a 24-h recall and 3-day diet record. Most 3-day records were completed between 5 and 7 months of pregnancy. Milk samples collected before taking supplementation (if any). Detail on supplementation (e.g. type, dose) not reported No information on whether the infants were born at term or not

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
								Plasma concentrations of vitamin B6 at five and seven months of pregnancy and at delivery were reported in the article
Sneed et al. (1981)	16 9 7	USA	Mean ± SE (a) Supplementation with vitamin B6 (b) Total vitamin B6 intake **Supplemented group** 5–7 days post-partum (a) 4 (b) 5.33 ± 0.29 (SE) 43–45 days post-partum (a) 4 (b) 5.12 ± 0.31 (SE) **Unsupplemented group** 5–7 days post-partum (a) 0 (b) 1.52 ± 0.40 (SE) 43–45 days post-partum (a) 0 (b) 1.41 ± 0.56 (SE)	–	 5–7 days post-partum 43–45 days post-partum 5–7 days post-partum 43–45 days post-partum	Vitamin B6 Mean ± SD 248 ± 60 240 ± 57 123 ± 34 120 ± 33	Microbiological assay (*Saccharomyces uvarum*)	4-day dietary records (from 4 to 7 days post-partum and 42 to 45 days post-partum) Multivitamin and mineral supplementation compared with no supplementation Expressed milk before taking supplementation or placebo No information on whether the infants were born at term or not Plasma PLP not assessed

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
Styslinger and Kirksey (1985)	24 6 6 6 6	USA	Mean ± SEM: (a) Supplemental intake: four doses of PN-HCl (b) Dietary vitamin B6 intake (c) Total vitamin B6 intake (a) 0 (b) 2.0 ± 0.1 (c) 2.0 ± 0.1 (a) 2.5 (b) 1.9 ± 0.1 (c) 4.4 ± 0.1 (a) 10.0 (b) 1.6 ± 0.1 (c) 11.3 ± 0.2 (a) 20.0 (b) 1.7 ± 0.5 (c) 21.1 ± 0.4	–	2–3 months post-partum (mean stage of lactation: 11 weeks post-partum)	Vitamin B6 (Mean ± SEM) 93 ± 8 192 ± 16 247 ± 25 413 ± 45	Microbiological assay (*Saccharomyces uvarum*)	Maternal intake estimated by a three-day dietary record for 18 women, and a two-day dietary record for three women Dietary records not available for three women Multivitamin and multimineral supplementation Supplementation was taken for three consecutive days Full-term infants Plasma PLP concentration not assessed
Thomas et al. (1979)	17 10	USA	Mean ± SE (a) Supplementation with vitamin B6 (b) Total vitamin B6 intake **Supplemented group** 5–7 days post-partum (a) 4.0 (b) 5.69 ± 0.65 (SE)	–	5–7 days post-partum	Vitamin B6 Mean ± SE 225 ± 87	Microbiological assay (*Saccharomyces uvarum*)	Four-day dietary records (from 4 to 7 days post partum and 42 to 45 days post partum) Multivitamin and multimineral supplementation Milk collection: 3-day periods at I and 6 weeks post partum

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
	7		43–45 days post-partum (a) 4.0 (b) 5.11 ± 0.35 (SE) **Unsupplemented group** 5–7 days post-partum (a) 0 (b) 1.45 ± 0.62 (SE) 43–45 days post-partum (a) 0 (b) 0.84 ± 0.22 (SE)		43–45 days post-partum 5–7 days post-partum 43–45 days post-partum	237 ± 57 128 ± 59 204 ± 53		Expressed milk before taking supplementation For the unsupplemented group, expressed milk before the nursing of the infants No information on whether the infants were born at term or not Plasma PLP not assessed
West and Kirksey (1976)	19 13 (≥ 5 samples per subject) *5* *5* *3* 19 (≥ 5 samples per subject) 6	USA	 ≥ 2.5 (range: 2.5–12.5) Total vitamin B6 (diet + supplements): Mean ± SD (range) **intake < 2.5:* 1.8 ± 0.4 (1.3–2.2)	–	From < 3 to > 7 months post partum *< 3 months* *3–7 months* *> 7 months* Not specified	Mean ± SD (range) 257 ± 31 (212–298) 294 ± 105 (214–454) 248 ± 59 (189–307) 129 ± 39 (67–148)	Microbiological assay (*Saccharomyces carlsbergensis*)	Dietary record: three-day records Foremilk collection: prior to the early morning feeding on three consecutive days during 1 week, and on 1 day during each of two following weeks No information on whether the infants were born at term or not PN-HCI supplementation Plasma PLP concentration not assessed Vitamin B6 concentration in milk at different stages of lactation (< 3, 3–7, and > 7 months)

Reference	Number of women (number of samples)	Country	Maternal dietary intake (mg/day) (mean ± SD)	Maternal status or cord blood: plasma PLP concentration (nmol/L) (mean ± SD)	Stage of lactation	Vitamin B6 concentration in breast milk (μg/L) (mean ± SD)	Analytical method for breast milk concentration	Comments
	8 5 13 (63) *8* *(supplemented)* *5* *(unsupplemented)* 5 (50)		**intake 2.5–5.0:* 2.9 ± 0.6 (2.5–4.2) **intake > 5.0:* 11.1 ± 2.0 (7.1–12.5) Total vitamin B6 intake (diet + supplements) 7.5 ± 4.5 (2.2–12.5) Total vitamin B6 intake 2.2 ± 0.5 (1.5–2.7) Not reported Two subjects were not taking supplements		 Not specified 3 weeks to 3.5 months	239 ± 51 (189–348) 314 ± 52 (256–454) 257 ± 115 (115–464) (mean of days 1–3) 288 ± 127 (159–466) (mean of weeks 1–3) 278 ± 121(148–454) (total mean) 168 ± 64 (97–262) (mean of days 1–3) 166 ± 61 (94–230) (mean of weeks 1–3) 170 ± 59 (101–247) (total mean) 207 ± 99		Vitamin B6 intake and concentration in milk at different vitamin B6 intake: < 2.5; 2.5–5.0; > 5.0 mg/day) Vitamin B6 concentration in milk (early morning feeding) on different days and weeks, in supplemented versus unsupplemented women Variation in vitamin B6 concentration in milk in 24-h Foremilk collection five or six 4-h intervals during about 18 h on two consecutive days Subjects in this part of the study are different from those included in the experiment on the variation in vitamin B6 content in milk on different days and weeks

EALT: erythrocyte alanine aminotransferase; HPLC: High Performance Liquid Chromatography; PLP: Pyridoxal Phosphate; PN-HCl: Pyridoxine Hydrochloride; RPLC: Reversed-phase liquid chromatography; SD: Standard Deviation; SE: Standard Error; SEM: Standard Error of the Mean.

(a): Total vitamin B6, value not given in the article, calculated by adding the different forms (pyridoxal, pyridoxamine, pyridoxine) For the concentration of vitamin B6 in breast milk, the following molecular masses (MM) were used to convert the values reported in nmol/L (or μmol/L) to μg/L: MM (PN) = 169.18 g/mol; MM (PM) = 168.19 g/mol; MM (PL) = 167.16 g/mol For plasma PLP concentration, the MM of 247.14 g/mol was used to convert the values reported in the articles in ng/mL (or ng/L) to nmol/L.

Appendix B – Dietary surveys in the EFSA Comprehensive European Food Consumption Database included in EFSA's nutrient intake calculation for vitamin B6

Country	Dietary survey (year)	Year	Method	Days	Age (years)	Number of subjects						
						Infants[a] < 1 year	Children 1 to < 3 years	Children 3 to < 10 years	Children 10 to < 18 years	Adults 18 to < 65 years	Adults 65 to <75 years	Adults ≥ 75 years
Finland/1	NWSSP	2007–2008	48-h dietary recall[b]	2 × 2[b]	13–15				306			
Finland/2	FINDIET2012	2012	48-h dietary recall[b]	2[b]	25–74					1,295	413	
Finland/3	DIPP	2000–2010	Dietary record	3	0.5–6	499	500	750				
France	INCA2	2006–2007	Dietary record	7	3–79			482	973	2,276	264	84
Germany/1	EsKiMo	2006	Dietary record	3	6–11			835	393			
Germany/2	VELS	2001–2002	Dietary record	6	< 1–4	158	348[c]	296[c]				
Ireland	NANS	2008–2010	Dietary record	4	18–90					1,274	149	77
Italy	INRAN–SCAI 2005–06	2005–2006	Dietary record	3	< 1–98	16[d]	36[d]	193	247	2,313	290	228
Latvia	FC_PREGNANTWOMEN 2011	2011	24-h dietary recall	2	15–45				12[d]	991[c]		
Netherlands	DNFCS 2007–2010	2007–2010	24-h dietary recall	2	7–69			447	1,142	2,057	173	
Sweden	RISKMATEN	2010–2011	Dietary records (Web)[e]	4	18–80					1,430	295	72

Country	Dietary survey (year)	Year	Method	Days	Age (years)	Number of subjects						
						Infants[(a)] < 1 year	Children 1 to < 3 years	Children 3 to < 10 years	Children 10 to < 18 years	Adults 18 to < 65 years	Adults 65 to <75 years	Adults ≥ 75 years
United Kingdom/1	DNSIYC–2011	2011	Dietary record	4	0.3–1.5	1,369	1,314					
United Kingdom/2	NDNS–Rolling Programme (1–3 years)	2008–2011	Dietary record	4	1–94		185	651	666	1,266	166	139

DIPP: type 1 Diabetes Prediction and Prevention survey; DNFCS: Dutch National Food Consumption Survey; DNSIYC: Diet and Nutrition Survey of Infants and Young Children; EsKiMo: Ernährungsstudie als KIGGS-Modul; FC_PREGNANTWOMEN: food consumption of pregnant women in Latvia; FINDIET: the national dietary survey of Finland; INCA: étude Individuelle Nationale des Consommations Alimentaires; INRAN-SCAI: Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione – Studio sui Consumi Alimentari in Italia; NANS: National Adult Nutrition Survey; NDNS: National Diet and Nutrition Survey; NWSSP: Nutrition and Wellbeing of Secondary School Pupils; VELS: Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): Infants 1–11 months of age.

(b): A 48-h dietary recall comprises of two consecutive days.

(c): Four subjects from VELS study (one toddler and three other children) and one subject from Latvian study (one adult) were not considered in the assessment due to the fact that only one 24-h dietary recall day was available.

(d): 5th or 95th percentile intakes calculated from fewer than 60 subjects require cautious interpretation as the results may not be statistically robust (EFSA, 2011b) and, therefore, for these dietary surveys/age classes, the 5th and 95th percentile estimates are not presented in the intake results. The two infants from the VELS study were excluded from the assessment.

(e): The Swedish dietary records were introduced through the Internet.

Appendix C – Vitamin B6 intakes in males in different surveys, estimated by EFSA according to age class and country

Age class	Country	Survey	Intakes expressed in mg/day					Intakes expressed in mg/MJ				
			n[(a)]	Average	Median	P5	P95	n[(a)]	Average	Median	P5	P95
< 1 year[(b)]	Finland	DIPP_2001_2009	247	0.46	0.50	0.07	0.82	245	0.25	0.22	0.16	0.46
	Germany	VELS	84	0.75	0.71	0.31	1.19	84	0.23	0.22	0.14	0.34
	Italy	INRAN_SCAI_2005_06	9	0.37	0.29	[(c)]	[(c)]	9	0.12	0.11	[(c)]	[(c)]
	United Kingdom	DNSIYC_2011	699	0.76	0.74	0.28	1.28	699	0.22	0.22	0.11	0.33
1 to < 3 years	Finland	DIPP_2001_2009	245	0.95	0.95	0.56	1.39	245	0.26	0.26	0.19	0.37
	Germany	VELS	174	0.98	0.88	0.50	1.82	174	0.21	0.19	0.12	0.36
	Italy	INRAN_SCAI_2005_06	20	1.06	1.00	[(c)]	[(c)]	20	0.22	0.21	[(c)]	[(c)]
	United Kingdom	NDNS–Rolling Programme Years1–3	107	1.27	1.24	0.76	1.83	107	0.26	0.26	0.17	0.37
	United Kingdom	DNSIYC_2011	663	1.09	1.06	0.58	1.66	663	0.26	0.26	0.17	0.38
3 to < 10 years	Finland	DIPP_2001_2009	381	1.55	1.48	1.05	2.26	381	0.26	0.26	0.20	0.34
	France	INCA2	239	1.45	1.37	0.78	2.40	239	0.23	0.22	0.15	0.35
	Germany	EsKiMo	426	1.54	1.40	0.84	2.66	426	0.20	0.18	0.12	0.35
	Germany	VELS	146	1.10	1.01	0.60	2.04	146	0.20	0.18	0.12	0.37
	Italy	INRAN_SCAI_2005_06	94	1.52	1.44	0.91	2.24	94	0.21	0.20	0.13	0.31
	Netherlands	DNFCS 2007–2010	231	1.44	1.36	0.66	2.46	231	0.17	0.16	0.09	0.27
	United Kingdom	NDNS–Rolling Programme Years1–3	326	1.61	1.59	0.92	2.38	326	0.26	0.25	0.16	0.36
10 to < 18 years	Finland	NWSSP07_08	136	2.31	2.17	1.36	3.60	136	0.28	0.26	0.19	0.47
	France	INCA2	449	1.78	1.68	1.01	2.82	449	0.23	0.22	0.15	0.34
	Germany	EsKiMo	197	1.70	1.48	0.83	3.16	197	0.21	0.19	0.11	0.40
	Italy	INRAN_SCAI_2005_06	108	1.95	1.86	1.07	3.10	108	0.20	0.19	0.14	0.31
	Netherlands	DNFCS 2007–2010	566	2.05	1.80	0.88	4.05	566	0.19	0.18	0.10	0.33
	United Kingdom	NDNS–Rolling Programme Years1–3	340	2.05	1.97	1.10	3.42	340	0.25	0.24	0.15	0.38
18 to < 65 years	Finland	FINDIET2012	585	2.12	1.94	1.04	3.56	585	0.23	0.21	0.14	0.35
	France	INCA2	936	1.85	1.79	0.98	2.85	936	0.21	0.21	0.14	0.32
	Ireland	NANS_2012	634	3.09	2.92	1.52	4.98	634	0.31	0.30	0.20	0.47
	Italy	INRAN_SCAI_2005_06	1,068	1.77	1.70	1.04	2.78	1,068	0.20	0.19	0.13	0.29

Age class	Country	Survey	Intakes expressed in mg/day					Intakes expressed in mg/MJ				
			n[(a)]	Average	Median	P5	P95	n[(a)]	Average	Median	P5	P95
	Netherlands	DNFCS 2007–2010	1,023	2.25	2.04	1.01	4.09	1,023	0.20	0.19	0.11	0.31
	Sweden	Riksmaten 2010	623	2.54	2.43	1.23	4.25	623	0.26	0.25	0.16	0.39
	United Kingdom	NDNS–Rolling Programme Years1–3	560	2.50	2.40	1.27	3.93	560	0.29	0.28	0.18	0.42
65 to < 75 years	Finland	FINDIET2012	210	1.75	1.62	0.91	2.98	210	0.22	0.21	0.13	0.34
	France	INCA2	111	1.90	1.83	0.99	3.28	111	0.22	0.21	0.15	0.34
	Ireland	NANS_2012	72	2.64	2.65	1.26	4.04	72	0.31	0.31	0.17	0.48
	Italy	INRAN_SCAI_2005_06	133	1.79	1.67	1.03	2.70	133	0.21	0.20	0.14	0.30
	Netherlands	DNFCS 2007–2010	91	1.75	1.73	0.98	2.66	91	0.19	0.19	0.12	0.28
	Sweden	Riksmaten 2010	127	2.41	2.27	1.39	3.90	127	0.28	0.27	0.20	0.40
	United Kingdom	NDNS–Rolling Programme Years1–3	75	2.54	2.44	0.92	3.67	75	0.31	0.30	0.18	0.44
≥ 75 years	France	INCA2	40	1.72	1.64	(c)	(c)	40	0.23	0.23	(c)	(c)
	Ireland	NANS_2012	34	2.26	2.21	(c)	(c)	34	0.30	0.29	(c)	(c)
	Italy	INRAN_SCAI_2005_06	69	1.66	1.66	1.05	2.48	69	0.19	0.19	0.14	0.28
	Sweden	Riksmaten 2010	42	2.31	2.32	(c)	(c)	42	0.28	0.27	(c)	(c)
	United Kingdom	NDNS–Rolling Programme Years1–3	56	2.01	1.92	(c)	(c)	56	0.28	0.28	(c)	(c)

DIPP: type 1 Diabetes Prediction and Prevention survey; DNFCS: Dutch National Food Consumption Survey; DNSIYC: Diet and Nutrition Survey of Infants and Young Children; EsKiMo: Ernährungsstudie als KIGGS-Modul; FC_PREGNANTWOMEN: food consumption of pregnant women in Latvia; FINDIET: the national dietary survey of Finland; INCA: étude Individuelle Nationale des Consommations Alimentaires; INRAN-SCAI: Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione - Studio sui Consumi Alimentari in Italia; NANS: National Adult Nutrition Survey; NDNS: National Diet and Nutrition Survey; NWSSP: Nutrition and Wellbeing of Secondary School Pupils; VELS: Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): n, number of subjects.

(b): Infants between 1 and 11 months. The proportions of breastfed infants were 58% in the Finnish survey, 40% in the German survey, 44% in the Italian survey, and 21% in the UK survey. Most infants were partially breastfed. For the Italian and German surveys, breast milk intake estimates were derived from the number of breastfeeding events recorded per day multiplied by standard breast milk amounts consumed on an eating occasion at different age. For the UK survey, the amount of breast milk consumed was either directly quantified by the mother (expressed breast milk) or extrapolated from the duration of each breastfeeding event. As no information on the breastfeeding events were reported in the Finnish survey, breast milk intake was not taken into consideration in the intake estimates of Finnish infants.

(c): 5th or 95th percentile intakes calculated from fewer than 60 subjects require cautious interpretation as the results may not be statistically robust (EFSA, 2011b) and, therefore, for these dietary surveys/age classes, the 5th and 95th percentile estimates are not presented in the intake results.

Appendix D – Vitamin B6 intakes in females in different surveys, estimated by EFSA according to age class and country

Age class	Country	Survey	Intakes expressed in mg/day					Intakes expressed in mg/MJ				
			n[a]	Average	Median	P5	P95	n[a]	Average	Median	P5	P95
< 1 year[b]	Finland	DIPP_2001_2009	253	0.41	0.43	0.07	0.74	251	0.26	0.23	0.17	0.44
	Germany	VELS	75	0.62	0.59	0.30	1.00	75	0.21	0.21	0.10	0.31
	Italy	INRAN_SCAI_2005_06	7	0.55	0.65	[c]	[c]	7	0.19	0.18	[c]	[c]
	United Kingdom	DNSIYC_2011	670	0.68	0.65	0.27	1.22	670	0.22	0.22	0.11	0.34
1 to < 3 years	Finland	DIPP_2001_2009	255	0.89	0.86	0.51	1.34	255	0.26	0.25	0.20	0.37
	Germany	VELS	174	0.88	0.78	0.45	1.94	174	0.21	0.18	0.12	0.42
	Italy	INRAN_SCAI_2005_06	16	0.92	0.85	[c]	[c]	16	0.19	0.20	[c]	[c]
	United Kingdom	NDNS–Rolling Programme Years1–3	78	1.17	1.17	0.70	1.62	78	0.26	0.25	0.17	0.37
	United Kingdom	DNSIYC_2011	651	1.02	0.99	0.54	1.59	651	0.26	0.25	0.16	0.38
3 to < 10 years	Finland	DIPP_2001_2009	369	1.36	1.34	0.88	1.94	369	0.26	0.25	0.20	0.33
	France	INCA2	243	1.28	1.23	0.76	1.97	243	0.23	0.23	0.16	0.32
	Germany	EsKiMo	409	1.41	1.27	0.78	2.47	409	0.21	0.19	0.13	0.35
	Germany	VELS	147	0.99	0.90	0.50	1.85	147	0.19	0.17	0.11	0.33
	Italy	INRAN_SCAI_2005_06	99	1.47	1.43	0.77	2.20	99	0.20	0.19	0.13	0.30
	Netherlands	DNFCS 2007–2010	216	1.37	1.26	0.68	2.36	216	0.17	0.16	0.09	0.26
	United Kingdom	NDNS–Rolling Programme Years1–3	325	1.51	1.49	0.86	2.18	325	0.25	0.25	0.17	0.34
10 to < 18 years	Finland	NWSSP07_08	170	1.73	1.68	1.03	2.52	170	0.26	0.26	0.19	0.35
	France	INCA2	524	1.46	1.39	0.77	2.33	524	0.23	0.22	0.14	0.35
	Germany	EsKiMo	196	1.57	1.40	0.78	2.60	196	0.21	0.19	0.12	0.42
	Italy	INRAN_SCAI_2005_06	139	1.65	1.52	0.95	2.70	139	0.21	0.20	0.13	0.32
	Latvia[d]	FC_PREGNANTWOMEN_2011	12	2.19	2.22	[c]	[c]	12	0.22	0.23	[c]	[c]
	Netherlands	DNFCS 2007–2010	576	1.60	1.47	0.71	2.83	576	0.18	0.17	0.09	0.30
	United Kingdom	NDNS–Rolling Programme Years1–3	326	1.73	1.64	0.91	2.72	326	0.26	0.25	0.16	0.41
18 to < 65 years	Finland	FINDIET2012	710	1.58	1.51	0.87	2.46	710	0.22	0.21	0.14	0.35
	France	INCA2	1,340	1.47	1.42	0.81	2.32	1,340	0.23	0.22	0.15	0.35
	Ireland	NANS_2012	640	2.11	2.03	1.14	3.42	640	0.29	0.28	0.18	0.44
	Italy	INRAN_SCAI_2005_06	1,245	1.53	1.49	0.82	2.33	1,245	0.21	0.20	0.13	0.31
	Latvia[d]	FC_PREGNANTWOMEN_2011	990	2.02	1.93	1.15	3.06	990	0.24	0.23	0.14	0.37
	Netherlands	DNFCS 2007–2010	1,034	1.65	1.54	0.78	2.89	1,034	0.20	0.19	0.11	0.31

Age class	Country	Survey	Intakes expressed in mg/day					Intakes expressed in mg/MJ				
			n[(a)]	Average	Median	P5	P95	n[(a)]	Average	Median	P5	P95
	Sweden	Riksmaten 2010	807	1.97	1.89	0.99	3.16	807	0.29	0.25	0.16	0.39
	United Kingdom	NDNS–Rolling Programme Years1–3	706	1.90	1.90	0.99	2.88	706	0.29	0.28	0.17	0.44
65 to < 75 years	Finland	FINDIET2012	203	1.39	1.32	0.75	2.19	203	0.23	0.22	0.14	0.35
	France	INCA2	153	1.47	1.43	0.88	2.28	153	0.24	0.23	0.17	0.33
	Ireland	NANS_2012	77	2.16	2.14	1.29	3.17	77	0.32	0.31	0.22	0.47
	Italy	INRAN_SCAI_2005_06	157	1.53	1.48	0.81	2.29	157	0.23	0.21	0.14	0.33
	Netherlands	DNFCS 2007–2010	82	1.39	1.42	0.61	2.18	82	0.20	0.19	0.11	0.29
	Sweden	Riksmaten 2010	168	2.01	1.89	1.10	3.49	168	0.29	0.27	0.19	0.47
	United Kingdom	NDNS–Rolling Programme Years1–3	91	1.88	1.91	1.18	2.57	91	0.32	0.31	0.21	0.44
≥ 75 years	France	INCA2	44	1.36	1.32	(c)	(c)	44	0.23	0.22	(c)	(c)
	Ireland	NANS_2012	43	2.08	1.92	1.05	3.79	43	0.33	0.30	0.22	0.59
	Italy	INRAN_SCAI_2005_06	159	1.42	1.41	0.81	2.12	159	0.21	0.20	0.13	0.33
	Sweden	Riksmaten 2010	30	2.05	1.88	(c)	(c)	30	0.31	0.28	(c)	(c)
	United Kingdom	NDNS–Rolling Programme Years1–3	83	1.87	1.84	1.21	2.54	83	0.32	0.31	0.19	0.45

DIPP: type 1 Diabetes Prediction and Prevention survey; DNFCS: Dutch National Food Consumption Survey; DNSIYC: Diet and Nutrition Survey of Infants and Young Children; EsKiMo: Ernährungsstudie als KIGGS-Modul; FC_PREGNANTWOMEN: food consumption of pregnant women in Latvia; FINDIET: the national dietary survey of Finland; INCA: étude Individuelle Nationale des Consommations Alimentaires; INRAN-SCAI: Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione - Studio sui Consumi Alimentari in Italia; NANS: National Adult Nutrition Survey; NDNS: National Diet and Nutrition Survey; NWSSP: Nutrition and Wellbeing of Secondary School Pupils; VELS: Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln.

(a): n, number of subjects.

(b): Infants between 1 and 11 months. The proportions of breastfed infants were 58% in the Finnish survey, 40% in the German survey, 44% in the Italian survey, and 21% in the UK survey. Most infants were partially breastfed. For the Italian and German surveys, breast milk intake estimates were derived from the number of breastfeeding events recorded per day multiplied by standard breast milk amounts consumed on an eating occasion at different age. For the UK survey, the amount of breast milk consumed was either directly quantified by the mother (expressed breast milk) or extrapolated from the duration of each breastfeeding event. As no information on the breastfeeding events were reported in the Finnish survey, breast milk intake was not taken into consideration in the intake estimates of Finnish infants.

(c): 5th or 95th percentile intakes calculated from fewer than 60 subjects require cautious interpretation as the results may not be statistically robust (EFSA, 2011b) and, therefore, for these dietary surveys/age classes, the 5th and 95th percentile estimates are not presented in the intake results.

(d): Pregnant women only.

Appendix E – Minimum and maximum percentage contribution of different food groups (FoodEx2 level 1) to vitamin B6 intake estimates in males

Food groups	Age						
	< 1 year	1 to < 3 years	3 to < 10 years	10 to < 18 years	18 to < 65 years	65 to < 75 years	≥ 75 years
Additives, flavours, baking and processing aids	< 1	< 1	0	0–1	0	0	0
Alcoholic beverages	0	< 1	< 1	< 1–2	2–9	2–7	2–5
Animal and vegetable fats and oils	0–1	< 1–4	< 1–5	< 1–4	< 1–4	< 1–6	< 1–5
Coffee, cocoa, tea and infusions	0	< 1–1	< 1–2	< 1–2	< 1–2	< 1–2	< 1–3
Composite dishes	< 1–4	< 1–6	< 1–6	< 1–7	< 1–11	< 1–9	< 1–8
Eggs and egg products	< 1	< 1–1	< 1–1	< 1–1	< 1–2	< 1–2	< 1–2
Fish, seafood, amphibians, reptiles and invertebrates	< 1–1	1–5	1–6	1–6	1–7	3–11	4–12
Food products for young population	30–53	2–11	< 1–1	< 1	< 1	_(a)	_(a)
Fruit and fruit products	9–19	12–20	5–11	3–8	3–9	5–12	5–12
Fruit and vegetable juices and nectars	< 1–8	1–21	3–25	3–25	1–8	1–5	< 1–6
Grains and grain-based products	4–12	11–21	14–26	12–27	11–22	12–21	13–20
Human milk	< 1–11(b)	< 1	_(a)	_(a)	_(a)	_(a)	_(a)
Legumes, nuts, oilseeds and spices	< 1–2	< 1–2	1–3	1–2	1–2	1–2	1–3
Meat and meat products	1–12	8–19	11–27	12–30	15–30	15–27	14–26
Milk and dairy products	5–12	13–21	10–22	7–16	5–12	5–12	7–8
Products for non-standard diets, food imitates and food supplements or fortifying agents	< 1	0	< 1	< 1	< 1–1	< 1	< 1–2
Seasoning, sauces and condiments	< 1–1	< 1–1	< 1–1	< 1–1	< 1–1	< 1–1	< 1

Food groups	Age						
	< 1 year	1 to < 3 years	3 to < 10 years	10 to < 18 years	18 to < 65 years	65 to < 75 years	≥ 75 years
Starchy roots or tubers and products thereof, sugar plants	1–17	5–16	7–26	9–27	7–22	8–20	11–23
Sugar, confectionery and water-based sweet desserts	0	< 1–1	< 1–1	< 1–1	< 1	< 1	< 1
Vegetables and vegetable products	2–9	5–7	4–9	4–10	4–14	4–14	5–14
Water and water-based beverages	0	0	< 1–2	< 1–9	< 1–6	< 1–1	< 1–1

(a): '–' Means that there was no consumption event of the food group for the age and sex group considered, while '0' means that there were some consumption events, but that the food group does not contribute to the intake of the nutrient considered, for the age and sex group considered.
(b): The lower bound of this range corresponds to the data from the Finnish survey, which did not assess the amount of breast milk consumed.

Appendix F – Minimum and maximum percentage contribution of different food groups (FoodEx2 level 1) to vitamin B6 intake estimates in females

Food groups	Age						
	< 1 year	1 to < 3 years	3 to < 10 years	10 to < 18 years	18 to < 65 years	65 to < 75 years	≥ 75 years
Additives, flavours, baking and processing aids	0	0	0	0–1	0	0	0
Alcoholic beverages	< 1	< 1	< 1	< 1	< 1–3	< 1–3	< 1–1
Animal and vegetable fats and oils	< 1–2	< 1–5	< 1–5	< 1–4	< 1–4	< 1–4	< 1–4
Coffee, cocoa, tea and infusions	0–4	< 1–1	< 1–2	< 1–2	< 1–4	< 1–4	1–3
Composite dishes	< 1–2	< 1–6	< 1–6	1–8	< 1–11	< 1–9	< 1–9
Eggs and egg products	< 1	< 1–1	< 1–2	< 1–2	< 1–2	1–2	1–2
Fish, seafood, amphibians, reptiles and invertebrates	0–1	1–8	1–6	1–7	2–8	3–12	4–12
Food products for young population	29–53	2–10	< 1	< 1	< 1	_[a]	< 1
Fruit and fruit products	11–15	12–15	5–12	4–16	5–14	7–16	7–13
Fruit and vegetable juices and nectars	< 1–9	1–22	3–24	4–24	1–8	1–9	1–7
Grains and grain-based products	3–12	12–22	16–23	13–26	13–27	12–23	11–22
Human milk	< 1–4[b]	< 1	_[a]	_[a]	_[a]	_[a]	_[a]
Legumes, nuts, oilseeds and spices	< 1–3	< 1–3	1–2	1–2	1–3	1–2	1–2
Meat and meat products	1–11	8–17	11–27	12–26	14–25	12–24	12–24
Milk and dairy products	2–17	12–24	10–23	6–16	6–14	7–13	8–13
Products for non-standard diets, food imitates and food supplements or fortifying agents	< 1	< 1	0	< 1	< 1–3	< 1–1	< 1–1
Seasoning, sauces and condiments	< 1	< 1–1	< 1–1	< 1–1	< 1–1	< 1–1	< 1

Food groups	Age						
	< 1 year	1 to < 3 years	3 to < 10 years	10 to < 18 years	18 to < 65 years	65 to < 75 years	≥ 75 years
Starchy roots or tubers and products thereof, sugar plants	4–17	6–14	8–28	9–27	6–19	7–19	9–17
Sugar, confectionery and water-based sweet desserts	0	< 1–1	< 1–1	< 1–1	< 1–1	< 1	< 1–1
Vegetables and vegetable products	4–9	5–8	4–9	5–12	6–15	6–15	7–14
Water and water-based beverages	0	0	< 1–1	0–7	< 1–4	< 1–1	< 1

(a): '–' Means that there was no consumption event of the food group for the age and sex group considered, while '0' means that there were some consumption events, but that the food group does not contribute to the intake of the nutrient considered, for the age and sex group considered.
(b): The lower bound of this range corresponds to the data from the Finnish survey, which did not assess the amount of breast milk consumed.