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Dietary Reference Values for riboflavin

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Abstract

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) derives Dietary Reference Values (DRVs) for riboflavin. The Panel considers that the inflection point in the urinary riboflavin excretion curve in relation to riboflavin intake represents body saturation and can be used as a biomarker of adequate riboflavin status. The Panel also considers that erythrocyte glutathione reductase activation coefficient is a useful biomarker, but has limitations. For adults, the Panel considers that Average Requirements (ARs) and Population Reference Intakes (PRIs) can be determined from the weighted mean of riboflavin intake associated with the inflection point in the urinary riboflavin excretion curve reported in four intervention studies. PRIs are derived for adults and children assuming a coefficient of variation of 10%, in the absence of information on the variability in the requirement and to account for the potential effect of physical activity and the methylenetetrahydrofolate reductase 677TT genotype. For adults, the AR and PRI are set at 1.3 and 1.6 mg/day. For infants aged 7–11 months, an Adequate Intake of 0.4 mg/day is set by upward extrapolation from the riboflavin intake of exclusively breastfed infants aged 0–6 months. For children, ARs are derived by downward extrapolation from the adult AR, applying allometric scaling and growth factors and considering differences in reference body weight. For children of both sexes aged 1–17 years, ARs range between 0.5 and 1.4 mg/day, and PRIs between 0.6 and 1.6 mg/day. For pregnant or lactating women, additional requirements are considered, to account for fetal uptake and riboflavin accretion in the placenta during pregnancy or the losses through breast milk, and PRIs of 1.9 and 2.0 mg/day, respectively, are derived.

Keywords: riboflavin, biomarker, urinary excretion, glutathione reductase, average requirement, population reference intake, dietary reference value

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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 for the European population, including vitamin B2. The Panel considers in this Scientific Opinion that vitamin B2 is riboflavin.

Riboflavin or 7,8-dimethyl-10-ribityl-isoalloxazine, is a water-soluble compound naturally present in food of plant and animal origin as free riboflavin and, mainly, as the biologically active derivatives flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

Riboflavin is the integral part of the coenzymes FAD and FMN that act as the cofactors of a variety of flavoprotein enzymes such as glutathione reductase or pyridoxamine phosphate oxidase (PPO). FAD and FMN act as proton carriers in redox reactions involved in energy metabolism, metabolic pathways and formation of some vitamins and coenzymes. In particular, riboflavin is involved in the metabolism of niacin and vitamin B6 and FAD is also required by the methylenetetrahydrofolate reductase (MTHFR) in the folate cycle and thereby is involved in homocysteine metabolism. Signs of riboflavin deficiency are unspecific and include sore throat, hyperaemia and edema of the pharyngeal and oral mucous membranes, cheilosis, glossitis (magenta tongue), and normochromic normocytic anaemia characterised by erythroid hypoplasia and reticulocytopenia. No Tolerable Upper Intake Level has been set for riboflavin.

Dietary riboflavin associated with food protein is hydrolysed to free riboflavin and its absorption mainly takes place in the proximal small intestine through carrier-mediated, saturable transport process. The Panel considers an absorption efficiency of dietary riboflavin of 95%. Free riboflavin transported into enterocytes is subjected to phosphorylation to form FMN, subsequently converted to FAD. From the small intestine, riboflavin enters the plasma, where FAD is reported to be the major form. The uptake of riboflavin into the cells of organs such as the liver is facilitated and may require specific carriers. Absorbed riboflavin appears partly in the plasma, and partly is sequestered by the liver on the first pass through the portal vein from the gut. There is a positive transfer of riboflavin from the pregnant woman to the fetus. Most of the riboflavin in tissues including erythrocytes exists predominantly as FAD and FMN, covalently bound to enzymes. Unbound FAD and FMN are rapidly hydrolysed to free riboflavin that diffuses from cells and is excreted. When riboflavin is absorbed in excess, it is catabolised to numerous metabolites and little is stored in the body tissues. Urine is the main route for elimination of riboflavin.

The Panel reviewed possible biomarkers of riboflavin status and intake, i.e. urinary excretion of riboflavin, erythrocyte glutathione reductase activation coefficient (EGRAC), plasma and erythrocyte riboflavin, FAD and FMN, as well as PPO activity and activation coefficient. The Panel considers that the inflection point in the mean urinary riboflavin excretion curve in relation to riboflavin intake reflects body saturation and can be used to indicate adequate riboflavin status. The Panel also considers that EGRAC is a useful biomarker of riboflavin status and that EGRAC of 1.3 or less indicates adequate riboflavin status in all population groups. However, the Panel considers that the data on the relationship between riboflavin intake and EGRAC cannot be used alone to set DRVs for riboflavin, but can be used in support of data on the inflection in the urinary excretion curve in view of setting DRVs for riboflavin.

The Panel also notes that riboflavin status is modified by physical activity as urinary excretion of riboflavin is (generally) decreased and EGRAC increased when physical activity is increased, suggesting higher utilisation of riboflavin with increased energy expenditure. However, there is a lack of experimental data showing a clear quantitative relationship between riboflavin status biomarkers (urinary excretion of riboflavin and EGRAC) and energy expenditure (or physical activity). In addition, the Panel considers that relationship between riboflavin intake and biomarkers of riboflavin status is also influenced by MTHFR 677C>T polymorphism, as homozygosity for the T allele can increase the individual requirement for riboflavin, although the extent of this increase cannot be

defined. After having reviewed the existing evidence, the Panel concludes that available data on intake of riboflavin and health outcomes cannot be used to derive DRVs for riboflavin.

The Panel notes that new scientific data have become available for adults since the publication of the SCF report in 1993, and considers that updated average requirements (ARs) and population reference intake (PRIs) can be set for adults, children, pregnant and lactating women.

For adults, the Panel considers that an AR of 1.3 mg/day (after rounding) can be determined from the weighted mean of riboflavin intake associated with the inflection point in the mean urinary riboflavin excretion curve in relation to riboflavin intake as reported in four intervention studies in different non-EU countries. The Panel considers that the potential effect of physical activity and of MTHFR 677TT genotype on riboflavin requirement is covered by the data presented from the studies considered, thus is accounted for in the assumed CV applied to set the PRI for riboflavin. A CV of 10% was used to calculate PRIs from the ARs for adults, i.e. 1.6 mg/day after rounding, and the same CV was used for all other population groups. The Panel considers that there is no indication of different riboflavin requirement according to sex or between younger and older adults, and sets the same DRV for men and women (without correction per difference in body weight) of all ages.

For all infants aged 7–11 months, in the absence of sufficient data to set an AR, the Panel sets an AI of 0.4 mg/day based on the estimated intake of riboflavin of exclusively breastfed infants from birth to six months, and upward extrapolation by allometric scaling (on the assumption that riboflavin requirement is related to metabolically active body mass), taking into account the difference in reference body weight.

For children aged 1–17 years, the Panel sets ARs by downward extrapolation from the AR of adults, by allometric scaling (on the assumption that riboflavin requirement is related to metabolically active body mass), applying growth factors and taking into account the differences in reference body weight. The Panel considers unnecessary to set sex-specific ARs and PRIs for boys and girls of all ages. The Panel sets ARs ranging from 0.5 (children aged 1–3 years) to 1.4 mg/day (children aged 15–17 years) and PRIs ranging from 0.6 (children aged 1–3 years) to 1.6 mg/day (children aged 15–17 years).

For pregnant women, the Panel considers that data are insufficient to estimate the additional needs for dietary riboflavin during pregnancy based on fetal uptake and riboflavin accretion in the placenta during pregnancy. The Panel sets an AR of 1.5 mg/day, calculated by allometric scaling from the AR for non-pregnant women, considering the mean gestational increase in body weight of 12 kg, and also sets a PRI of 1.9 mg/day

For lactating women, an additional riboflavin requirement of 0.31 mg/day is calculated considering the secretion of riboflavin into milk during lactation (0.291 mg/day), the mean milk transfer during the first six months of lactation in exclusively breastfeeding women (0.8 L/day), and an absorption efficiency of 95%. An AR of 1.7 mg/day is calculated by the Panel, considering the additional requirement above the AR of non-lactating women, and a PRI of 2 mg/day is set for lactating women.

Based on data from 13 surveys in nine countries of the European Union, average riboflavin intake ranged across countries from 0.6 to 1.2 mg/day in infants, from 0.9 to 1.4 mg/day in children aged 1 to < 3 years, from 1 to 1.8 mg/day in children aged 3 to < 10 years, and from 1.2 to 2.2 mg/day in children aged 10 to < 18 years. Average riboflavin intake ranged between 1.4 and 2.2 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 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 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 the 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, 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 the 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;

[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] Energy intake was about 1,900 kcal/day for non-pregnant, about 2,300–2,400 kcal/day for pregnant and lactating women,

- Dietary fibre.

Following on from the first part of the task, the 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, the 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).

ASSESSMENT

1. Introduction

In 1993, the Scientific Committee on Food (SCF) adopted an opinion on the nutrient and energy intakes for the European Community (SCF, 1993). For riboflavin, the SCF set Average Requirements (ARs) and Population Reference Intakes (PRIs) for men and women. PRIs were also set for infants and children as well as for pregnant or lactating women.

The purpose of this Opinion is to review Dietary Reference Values (DRVs) for vitamin B2. In this Opinion, the Panel considers that vitamin B2 is the name of the compound riboflavin.

2. Definition/category

2.1. Chemistry

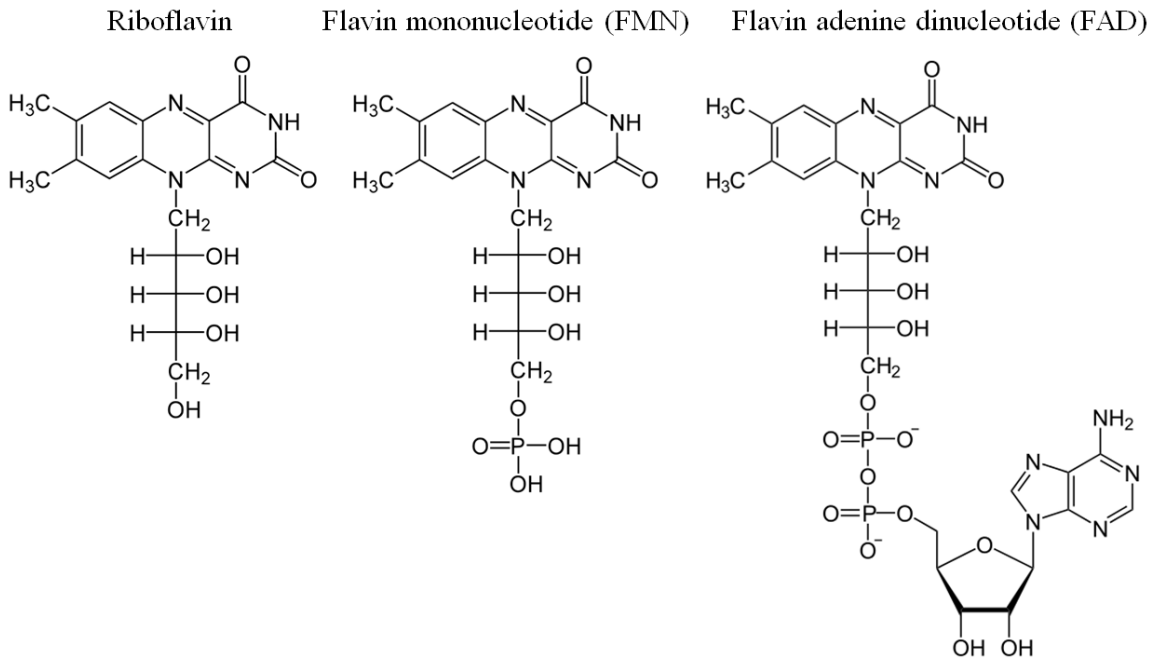
Flavins (from Latin flavin, 'yellow') is the name of a group of water-soluble yellow pigments to which riboflavin, flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD) belong.

Riboflavin, or 7,8-dimethyl-10-ribityl-isoalloxazine, is the tricyclic ring isoalloxazine bound to a ribityl side chain (IUPAC name: 7,8-Dimethyl-10-[(2*S*,3*S*,4*R*)-2,3,4,5-tetrahydroxypentyl]benzo[g]pteridine-2,4-dione) (Figure 1).

Riboflavin is water-soluble. In the diet, it is naturally present as free riboflavin and, mainly, as the biologically active derivatives FMN and FAD (Figure 1) (Powers, 2003; Said and Ross, 2012). FMN is also called riboflavin-5-phosphate (Merrill et al., 1981).

All three compounds are present in foods of plant or animal origin (Section 3.1.). Riboflavin-binding proteins have been found in egg white and yolk (Zanette et al., 1984; White and Merrill, 1988), as well as in cow milk (Kanno et al., 1991). Although relatively heat-stable, riboflavin is readily degraded by light in solutions (Section 2.2.2.1.). Riboflavin (E101(i)) and riboflavin 5'-phosphate sodium (E101(ii)) are also used as food colours (EFSA ANS Panel, 2013).

In this Opinion, the Panel used the terms 'total riboflavin' to refer explicitly to the sum of the three components (riboflavin, FMN and FAD) and 'free riboflavin' whenever it is necessary to make a distinction from FMN or FAD.



312

313 **Figure 1:** Chemical structures of riboflavin, FMN and FAD
314 Molecular masses: riboflavin: 376.4 g/mol; FMN: 456.3 g/mol, FAD: 785.6 g/mol

315 ## 2.2. Function of the nutrient

316 ### 2.2.1. Biochemical functions

317 Riboflavin is the integral part of the coenzymes FAD and FMN that act as the cofactors of
318 flavoprotein enzymes involved in a variety of reactions. FAD and FMN act as proton carriers in redox
319 reactions involved in energy metabolism (Section 2.5.), metabolic pathways and the formation of some
320 vitamins and coenzymes (McCormick, 2000; SCF, 2000; Said and Ross, 2012). In particular,
321 riboflavin is involved in the metabolism of niacin and vitamin B6 (McCormick, 1989, 2000; EFSA
322 NDA Panel, 2014a, 2016). FAD is also required as a cofactor for the methylenetetrahydrofolate
323 reductase (MTHFR; EC 1.7.99.5) that is a key enzyme in the folate cycle (EFSA NDA Panel, 2015b)
324 and it is required for the formation of 5-methyltetrahydrofolate which, in turn, is involved in the
325 remethylation of homocysteine to methionine (McKinley et al., 2001).

326 The enzyme glutathione reductase (EC 1.8.1.7) is using FAD as a cofactor to catalyse the reduction of
327 the oxidised form glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH), a critical
328 step in maintaining the reducing environment of the cell. In people with glucose-6-phosphate
329 dehydrogenase (G6PD) deficiency, the most common enzyme disorder caused by an enzyme defect,
330 with an estimated frequency of 0.4% of all births in the European Union (EU) (WHO Working Group,
331 1989; Cappellini and Fiorelli, 2008), glutathione reductase has an increased avidity for FAD, leading
332 to high *in-vitro* activity. Another enzyme, pyridoxamine phosphate oxidase (PPO, EC 1.4.3.5.) is
333 FMN-dependent, is involved in the conversion of pyridoxine and pyridoxamine to the coenzyme
334 pyridoxal phosphate and is present in various tissues including erythrocytes (Mushtaq et al., 2009).
335 The activity of glutathione reductase in erythrocyte (EGR) and that of PPO are discussed in Sections
336 2.4.2 and 2.4.4.

337 ### 2.2.2. Health consequences of deficiency and excess

338 2.2.2.1. Deficiency

339

Riboflavin deficiency (ariboflavinosis) is most often accompanied by other nutrient deficiencies, and was reported in populations from both developed and developing countries (Venkataswamy, 1967; Komindr and Nichoalds, 1980; Nichoalds, 1981). Clinical signs of riboflavin deficiency reported in humans (IOM, 1998) are unspecific and include e.g. sore throat, hyperaemia and edema of the pharyngeal and oral mucous membranes, cheilosis, glossitis (magenta tongue), seborrhoeic dermatitis, skin lesions including angular stomatitis (as reported in (Horwitt et al., 1950)) and normochromic normocytic anaemia characterised by erythroid hypoplasia and reticulocytopenia (Lane and Alfrey, 1965). The correction of riboflavin deficiency improved haematologic status in Gambian adults (Fairweather-Tait et al., 1992); the relationship between riboflavin status and haematologic status is further described in Sections 2.3.7 and 2.4.2.

Due to the photosensitivity of riboflavin, phototherapy used to treat hyperbilirubinemia in newborns was also associated with low riboflavin status as apparent by increases of erythrocyte glutathione reductase activation coefficient (EGRAC) values with the duration of phototherapy (see Section 2.4.2. on EGRAC) (Gromisch et al., 1977; Tan et al., 1978; Hovi et al., 1979; Parsons and Dias, 1991). The maximum absorption spectrum of riboflavin is at a wavelength similar to that at which the degradation of bilirubin occurs (Gromisch et al., 1977).

A woman with riboflavin deficiency (indicated by an EGRAC of 2.81), although no clinical symptoms of deficiency were reported, gave birth to a child with malformations of the urinary tract and with the clinical and biochemical signs of multiple acyl-coenzyme A (CoA) dehydrogenase deficiency (MADD) due to a heterozygous deletion of the solute carrier *SLC52A1* gene in the mother that codes for the human riboflavin transporter 1 (hRFT1) (Chiong et al., 2007; Ho et al., 2011).

2.2.2.2. Excess

A Tolerable Upper Intake Level (UL) for riboflavin could not be derived by the SCF because there was not sufficient clinical evidence for adverse effects of high riboflavin intakes (SCF, 2000). No adverse effects from 'high' riboflavin intakes from food or supplements have been reported (Rivlin, 2010). The Panel notes that revising the UL for riboflavin is not within the scope of the present Opinion.

2.3. Physiology and metabolism

2.3.1. Intestinal absorption

Dietary FMN and FAD associated with food protein are hydrolysed to free riboflavin (Merrill et al., 1981; Nichoalds, 1981). Acidification in the stomach releases the non-covalently bound coenzymes FAD and FMN, which are also hydrolysed to free riboflavin by non-specific phosphatases of the brush border and baso-lateral membranes of enterocytes in the upper small intestine (Merrill et al., 1981; Said and Ross, 2012).

Absorption of free riboflavin mainly takes place in the proximal small intestine through a carrier-mediated, saturable transport process (Jusko and Levy, 1967; Rivier, 1973; Meinen et al., 1977; Merrill et al., 1981; Daniel et al., 1983; Said and Ma, 1994; IOM, 1998; Said and Ross, 2012). A carrier-mediated absorption of riboflavin is also present in the colon (Sorrell et al., 1971; Yuasa et al., 2000; Said and Ross, 2012). A small amount of riboflavin circulates via the enterohepatic system (Said and Ross, 2012).

The absorbed quantity of oral doses of riboflavin (assessed by the urinary recovery of riboflavin) linearly increases according to intake up to about 25–30 mg riboflavin (Levy and Jusko, 1966; Jusko and Levy, 1967) (also reported in reviews (Jusko WJ and Levy G., 1975; Merrill et al., 1981)). This was confirmed by the pharmacokinetics study by Zempleni et al. (1996) using oral riboflavin doses, which calculated the maximum amount of riboflavin that can be absorbed as about 27 mg. IOM (1998) based its discussion on bioavailability of riboflavin on Zempleni et al. (1996), which showed that

absorption from the gut lumen was 95% complete within 4.4h. In a study in twenty healthy women using ^{13}C-labelled riboflavin in semi-skimmed milk or ^{15}N-labelled free riboflavin and FMN in spinach soup and urinary monitoring, there was no significant difference in true absorption between the spinach meal and the milk meal (Dainty et al., 2007).

Prevalence of riboflavin deficiency is high in chronic alcoholics (Said and Ross, 2012), and the proposed mechanism investigated in animals and *in vitro* is that ethanol consumption inhibits the release of riboflavin from dietary FMN and FAD and its absorption (Pinto et al., 1987). A significant negative association between dietary phytate forms and apparent absorption of dietary riboflavin (-0.86, $p < 0.05$) was observed in ileostomy patients (Agte et al., 2005).

The Panel notes that the absorbed quantity of riboflavin linearly increases up to an intake of 25–30 mg, and that absorption efficiency of dietary riboflavin is 95%.

2.3.2. Transport

Free riboflavin transported into enterocytes is subjected to adenosine triphosphate (ATP)-dependent phosphorylation by the cytosolic flavokinase (EC 2.7.1.26) to form FMN subsequently converted to FAD by the FAD-dependent FAD synthetase (EC 2.7.7.2).

Free riboflavin, FMN and FAD are transported in plasma bound to albumin and to immunoglobulins (Ig) (IgA, IgG and IgM), as shown in healthy subjects (Innis et al., 1985) and in patients (Innis et al., 1986). Hustad et al. (2002) (Section 2.4.3.1.) reported FAD as the major form in plasma in healthy individuals compared to free riboflavin or FMN (median concentrations were 74, 10.5 and 6.6 nmol/L, respectively).

FAD concentration in erythrocyte was reported to be higher than that of FMN (medians of 469 and 44 nmol/L respectively, with only traces of free riboflavin) (Hustad et al., 2002).

Pregnancy increases the blood concentration of a carrier protein available for riboflavin and identified in umbilical cord serum and sera of pregnant women (Visweswariah and Adiga, 1987; Natraj et al., 1988), that is essential to normal fetal development (Foraker et al., 2003). In *an vitro* perfusion system (Dancis et al., 1985), radioactive riboflavin analysed via high performance liquid chromatography (HPLC) was transferred across placentas of term mothers without being converted to FMN or FAD, with a transfer rate towards the fetus that suggested a transport mediated by a carrier system, as later confirmed by Dancis et al. (1986). The transfer of riboflavin to the fetus is efficient (Dancis et al., 1988). Calculation of the difference in plasma concentration between the umbilical vein and the umbilical artery multiplied by umbilical plasma flow in term infants, showed that fetal free riboflavin uptake was 0.1 mg/kg per day (Zempleni et al., 1995). Riboflavin content of the placenta has been investigated (Baker et al., 1981; Zempleni et al., 1995). Content of FAD+FMN measured by HPLC in the placenta of full-term infants was higher than that of free riboflavin (Zempleni et al., 1995), as already suggested in a study of placentas of 54 mothers of full term infants showing high riboflavin status (Section 2.4.1.) in relation with its high FAD content (Ramsay et al., 1983).

The Panel notes that riboflavin is transported in the plasma (bound to albumin and immunoglobulins), or mainly in erythrocytes, and that there is a positive transfer of riboflavin from the pregnant woman to the fetus.

2.3.3. Distribution to tissues

At physiological concentrations, the uptake of riboflavin into the cells of organs is facilitated and may require specific carriers (Bowman et al., 1989; McCormick, 1989; IOM, 1998). Carrier-mediated processes have been identified for riboflavin transport in the liver and in human retinal pigment epithelium (Said and Ross, 2012). Dainty et al. (2007) (Section 2.3.1.) suggested that the absorbed

riboflavin partly appears in the plasma, and partly is sequestered by the liver on the first pass through the portal vein from the gut.

2.3.4. Storage

Most of the riboflavin in tissues, including erythrocytes (Section 2.3.2.), exists predominantly as FAD and as FMN, covalently bound to enzymes (Singer and Kenney, 1974; Hustad et al., 2002). Unbound FAD and FMN are hydrolysed to free riboflavin that diffuses from cells and is excreted, thus the intracellular phosphorylation of riboflavin to FMN and FAD is a form of metabolic trapping important for riboflavin homeostasis (Gastaldi et al., 2000; Powers, 2003). In men on a restricted riboflavin intake (0.5 mg/day for 9 months) compared to controls, the riboflavin content of erythrocytes became significantly lower in the restricted group within 45 days of the restriction and slowly decreased further during the ensuing months (Bessey et al., 1956). When riboflavin is absorbed in excess, little is stored in the body tissues and the excess is excreted, mainly in the urine (Sauberlich, 1999) (Section 2.3.6.2).

2.3.5. Metabolism

Riboflavin is converted to its coenzymes derivatives FAD and FMN in the cellular cytoplasm of most tissues, e.g. in the small intestine, liver, heart, and kidney (Darby W.J., 1981; Brown M.L., 1990; IOM, 1998). The first step of this metabolism is the ATP-dependent phosphorylation of riboflavin to FMN, catalysed by the enzyme flavokinase under hormonal control. In a second step, FMN is complexed with specific apoenzymes to form different flavoproteins, or is mainly converted to FAD by the FAD synthetase. The conversion to FAD is controlled by the FAD content of the tissues and an excess of FAD inhibits this conversion as shown in rats (Yamada et al., 1990). Riboflavin is metabolised only in small amounts (Sauberlich et al., 1974; Roughead and McCormick, 1991).When riboflavin is in excess in tissues, it is catabolised to numerous metabolites such as 7-hydroxymethylriboflavin and lumiflavin (Powers, 2003).

2.3.6. Elimination

2.3.6.1. Faeces

No significant faecal excretion of riboflavin has been reported.

2.3.6.2. Urine

Intakes of riboflavin in excess of tissue capacities are excreted in the urine (Section 2.3.4.). Riboflavin generally accounts for about 60–70% of all urinary flavins (McCormick, 1989; Sauberlich, 1999; Said and Ross, 2012), while riboflavin metabolites, including 7a-hydroxyriboflavin, 8a-sulfonylriboflavin, lumiflavin, 8a-hydroxyriboflavin, and l0-hydroxyethylflavin, could amount to 28–39% of total urinary flavins (Chastain and McCormick, 1987). Some urinary metabolites also reflect bacterial catabolism of riboflavin in the gastrointestinal tract (Chastain and McCormick, 1987; Powers, 2003)

Well-nourished subjects aged 3–62 years were given one capsule containing 1.7 mg riboflavin in addition to the dietary intake (mean total riboflavin intake was 2.13 mg/day, range 0.26–5.17 mg/day), and urinary excretion of riboflavin and its metabolites was investigated (Roughead and McCormick, 1991). The correlation between intake of riboflavin and the urinary excretion of all riboflavin metabolites was weak but positive (correlation coefficients of 0.04–0.25). There was a strong positive correlation ($r > 0.7$) between the urinary excretion of riboflavin expressed as a function of creatinine and that of almost all urinary metabolites of riboflavin.

Urinary excretion over 24 hours (expressed as total riboflavin excreted or in relation to urinary creatinine) can be measured directly by fluorometric methods (Chastain and McCormick, 1987; Roughead and McCormick, 1991; Gibson, 2005). A more sensitive and specific HPLC method includes a fixed-wave-length spectrofluorometer and allows the separation of urinary riboflavin from other molecules such as riboflavin-5-phosphate, non-riboflavin fluorescing molecules and photo-

degraded riboflavin, thus results for riboflavin excretion via the HPLC method with fluorometry tend to be lower than those with the fluorometric method alone (Smith, 1980; Gibson, 2005).

Urinary riboflavin has been shown to increase under conditions causing negative nitrogen balance and with the administration of antibiotics (IOM, 1998; Gibson, 2005).

2.3.6.3. Breast milk

Riboflavin is secreted into breast milk in concentrations that are sensitive to maternal riboflavin intake and can be increased, although slightly, by riboflavin supplementation (Deodhar et al., 1964; Nail et al., 1980; Bates C. J. et al., 1982).

The main flavins in breast milk are riboflavin and FAD, but FMN and some riboflavin metabolites (10-hydroxyethylflavin, 10-formylmethylflavin, 7α-hydroxyriboflavin, 8α -hydroriboflavin) are also present (Roughead and McCormick (1990)).

The Panel used a comprehensive search of the literature published from 1990 onwards as preparatory work to the present opinion in order to identify data on which DRVs for riboflavin may potentially be based (Buijssen et al., 2014), including data on breast milk concentration of 'total flavin' or total or free riboflavin (Ortega et al., 1999; Sakurai et al., 2005; Kodentsova and Vrzhesinskaya, 2006). The Panel also considered additional individual studies reviewed by SCF (2003) or by Bates and Prentice (1994) and Picciano (1995) on 'total flavin' or total or free riboflavin concentration in breast milk (Nail et al., 1980; Thomas et al., 1980; Ford et al., 1983; Dostálová et al., 1988; Roughead and McCormick, 1990).

Appendix A reports the mean concentration of 'total flavin' or total/free riboflavin in human milk from healthy lactating women in eight studies. One of them was conducted in Japan but was kept for completeness (Sakurai et al., 2005).

In seven studies conducted in Europe (Ford et al., 1983; Dostálová et al., 1988; Ortega et al., 1999) or in USA and Russia (Nail et al., 1980; Thomas et al., 1980; Roughead and McCormick, 1990; Kodentsova and Vrzhesinskaya, 2006), only two European studies (Ford et al., 1983; Dostálová et al., 1988) clearly stated that infants were full term, and five studies in the EU or in the USA measured content in mature milk (Nail et al., 1980; Thomas et al., 1980; Ford et al., 1983; Dostálová et al., 1988; Ortega et al., 1999). Riboflavin was measured by different methods (fluorometric, spectrophotometric methods, or microbiological methods). In the seven studies in Western countries, the mean concentration of total flavin or total or free riboflavin in milk of mothers (across all stages of lactation) ranged between 180 and 799 µg/L. Specifically in unsupplemented mothers, this range was 216–485 µg/L.

Among the studies considered, maternal riboflavin status was reported in plasma (EGRAC, see Section 2.4.2.) (Ortega et al., 1999), or in urine (Nail et al., 1980; Thomas et al., 1980) by different analytical methods. Mean maternal riboflavin intake was reported in four studies (Nail et al., 1980; Thomas et al., 1980; Roughead and McCormick, 1990; Ortega et al., 1999), but in one study it was not clear if the intake was from the diet, or supplements or both (Roughead and McCormick, 1990). Focussing on three studies carried out with unsupplemented mothers in Spain (Ortega et al., 1999), and in the USA (Nail et al., 1980; Thomas et al., 1980), for which the maternal riboflavin intake and status were reported, the mean riboflavin concentration in mature milk ranged between 243 and 485 µg/L (mid-point: 364 µg/L).

Considering a mean milk transfer of 0.8 L/day during the first six months of lactation in exclusively breastfeeding women (Butte and King, 2002; FAO/WHO/UNU, 2004; EFSA NDA Panel, 2009), and a concentration of riboflavin in mature human milk of 364 µg/L, the secretion of riboflavin into milk during lactation is estimated to be 291 µg/day, i.e. about 290 µg/day.

2.3.6.4. Conclusion on elimination

The Panel notes that urine is the main route for elimination of riboflavin. The Panel considers that the concentration of riboflavin in breast milk is increased by maternal oral supplementation, and that the average concentration of riboflavin in mature breast milk of unsupplemented women is about 360 µg/L.

2.3.7. Interaction with other nutrients

Regarding other B-vitamins, riboflavin is involved in the metabolism of niacin and vitamin B6 (EFSA NDA Panel, 2014a, 2016) and FAD is also required by the MTHFR in the folate cycle (EFSA NDA Panel, 2015b) (Section 2.2.1.).

In Gambian men with mean EGRAC of about 2.1., the correction of riboflavin deficiency by riboflavin supplementation (10 mg on 6 days per week for 4 weeks) improved haemoglobin concentration, but not plasma ferritin, packed cell volume or iron absorption assessed with labelled iron (Fairweather-Tait et al., 1992). In Nigerian adults, some of them anaemic and whose riboflavin intake and status were unknown, supplementation with riboflavin (5 mg/day for 8 weeks) significantly increased haemoglobin concentration, haematocrit concentration and erythrocyte count (Ajayi et al., 1990). The mechanism by which riboflavin deficiency results in disturbance of the production of erythrocytes is thought to be through impaired mobilisation of iron from ferritin (via reduced flavins) (EFSA NDA Panel, 2015a).

2.4. Biomarkers

2.4.1. Inflection of the urinary excretion of riboflavin

Urinary excretion of riboflavin reflects dietary intake when tissues are saturated (Section 2.3.6.2.). Within a few days, urinary excretion reacts to the lowering of riboflavin intake (Horwitt et al., 1950). Urinary riboflavin is subject to large variations, and it is most useful in studies in which riboflavin dietary intake is strictly controlled (e.g. (Boisvert et al., 1993)). It can be expressed as 24h urinary riboflavin excretion (Sauberlich et al., 1974) or as fasting (spot) urinary riboflavin (Guo et al., 2016), with or without correction by creatinine concentration to control for the completeness of collection. (Gershoff et al., 1956; Plough and Consolazio, 1959). Cut-off values for deficiency and adequacy/sufficiency have been proposed based on a number of controlled depletion/repletion studies reviewed by (Sauberlich et al., 1974): values of total 24h urinary excretion of riboflavin below 40 µg/day (or 27 µg/g creatinine) indicated deficiency, values between 40 and 120 µg/day (or 80 µg/g creatinine) indicated insufficiency, and values exceeding 120 µg/day indicated sufficiency in adults. Corresponding intakes were not available. The cut-off value of 120 µg/day was recently retained by (Said and Ross, 2012).

Two supplementation studies (Horwitt et al., 1948; Horwitt MK et al., 1949), one of them designed to assess thiamin requirement, were described by **Horwitt et al. (1950)** and later used by SCF (1993) (Section 4.1.). They were undertaken in the USA in men living in a 'mental institution' (hospital), who consumed different amounts of riboflavin over many months (up to two years in one group) and whose energy intake was not always reported. This study showed that urinary excretion of riboflavin increases as riboflavin intake increases. Across the two projects, 24h urinary riboflavin excretion was measured with microbiological and fluorometric methods in a total of 66 subjects. Across the two projects, riboflavin intake was 0.55 mg/day (basal diet alone), or 0.75, 0.85, 1.1, about 1.6, 2.05, 2.15, 2.55 and 3.55 mg/day; all intake values from basal or supplemented diets were obtained by chemical analyses (except the 1.6 mg/day provided by a hospital diet consumed *ad libitum)*. Differing numbers (unclear reporting: 11 up to either 39 or 42) of these subjects were investigated under several of these different riboflavin regimen. The highest increase in mean urinary excretion between two doses, i.e. mean urinary excretion from 97 to 434 µg/day, corresponded to total riboflavin intakes between 1.1 and 1.6 mg/day. The Panel notes that the inflection in the urinary excretion curve occurred at riboflavin intakes between 1.1 and 1.6 mg/day.

The results from the study by Horwitt et al. (1950) were taken into account in the review by Bro-Rasmussen (1958) on 14 studies in adults, pregnant women and children in Western countries published between 1941 and 1950. This review showed that, in adults, the inflection point at which the tissues are saturated with riboflavin and its excretion into the urine starts to increase corresponds to a dietary intake of riboflavin between 1.0 and 1.6 mg/day.

In a recent study on Chinese adult men **(Guo et al., 2016)**, 78 (73 completers) young healthy men (aged 18–22 years) in the army, were randomly assigned either to one of six groups that received, for six weeks, daily riboflavin supplements of 0, 0.2, 0.4, 0.6, 0.8, or 1.0 mg, respectively. They had no clinical signs of riboflavin deficiency, were physically active, and mean body weights were 62.9–68.8 kg according to groups, and mean energy intake was 13.9 MJ/day. The mean riboflavin intake from food was between 1.0 and 1.1 mg/day according to groups (mean baseline intake obtained from chemical analysis), therefore the total riboflavin intake (food + supplements) was 1, 1.3, 1.5, 1.6, 1.9, 2.0 mg/day for the six groups, respectively. In the group with a riboflavin intake of 1.5 mg/day, mean 'fasting' urinary riboflavin excretion (assessed fluorophotometrically in the morning urine) was 543 µg/g creatinine. With riboflavin intakes above 1.4 mg/day (calculated by the authors), riboflavin excretion showed a strong positive linear correlation with riboflavin intake (R^2 = 0.9667, $p < 0.01$). The Panel notes the inflection point of the curve of mean urinary excretion according to intake, calculated by the author as the intercept of two regression lines developed among different riboflavin intake groups, is 1.4 mg/day, which is a result similar to that of Horwitt et al. (1950) although the excretion values were not similar.

Older subjects in Guatemala (4 men and 10 women, mean age was 70.9 years), with light physical activity, participated in a 16-week intervention study **(Boisvert et al., 1993)** (Section 2.4.2.). Fourteen subjects were fed for 2–5 weeks a basal diet with a low content of riboflavin (weekly mean was 0.65–0.7 mg/day assessed by a microbiological assay), and with an average weekly energy content of 10.2 MJ/day. In the following periods (duration 2–5 weeks each), the diet was supplemented with increases of 0.2 mg riboflavin per period, amounting to a total riboflavin intake of 0.9, 1.1, 1.3 and 1.5 mg/day, respectively. There was a sharp increase in mean 24h urinary excretion of riboflavin (assessed by HPLC with fluorescence detection) for an intake between 1.1 and 1.3 mg/day. At these intakes, mean urinary excretion increased from about 12.4 to 79 µg/day but was higher than 141 µg/day only at intakes ≥ 1.5 mg/day. The Panel notes that the inflection point of the curve of mean urinary excretion according to intake, calculated by the authors as the intercept of two regression lines, was 1.13 mg/day.

In the USA **(Brewer et al., 1946)**, 14 young healthy women (aged 21–32 years, body weights in the range 45.5–68.2 kg) followed two six-day preliminary periods on a self-selected diet supplemented only in the second period with 3 mg/day of riboflavin. Then, the following phase was composed of several 12-day experimental periods. The subjects consumed a controlled experimental diet providing 0.79 mg/day riboflavin (analytically measured) for a first period of 12 days and, between each following 12-day experimental period, they followed a three-day intermediate period when they consumed again their self-selected diet supplemented with 3 mg/day riboflavin (to reach a high tissue content of riboflavin at the start of the following period). The controlled experimental diet provided 8.8–9.6 MJ/day throughout the study and the authors estimated the energy requirement for each subject on the basis of her activity, size and food habits. Three to nine subjects were studied at each of the following total riboflavin intakes: 0.79, 1.04, 1.26, 1.62, 2.23 and 2.72 mg/day (each value being the average over each 12-day experimental periods). Average 24h urinary excretion of riboflavin (measured by fluorometry or an adsorption procedure) was averaged for the last three days of each period, and values were: 0.07, 0.16, 0.13, 0.32, 1.18 and 1.31 mg/day. The Panel notes that the inflection point in the relationship between urinary excretion and intake occurred between the intakes of 1.26 and 1.62 mg/day (1.44 by interpolation). The Panel also notes that the authors plotted the averages for urinary excretion of riboflavin against riboflavin intake for (i) their study, (ii) five other studies in women published in 1941–1945 and (iii) all data combined. For each of these groups of data, one linear regression line was plotted for urinary riboflavin at intakes ranging from 0.5 to 2 mg/day, and another linear regression line was plotted for intakes ranging from 1.3 to 7 mg/day. The

621 points of intersection of these two lines were between intakes of 1.3 and 1.5 mg/day riboflavin, above
622 which a sharp increase in urinary excretion of riboflavin occurred. The Panel notes that this result is in
623 line with the other studies described above.

624 Intervention studies with riboflavin (alone or in combination) or depletion/repletion studies, conducted
625 in the EU (Van der Beek et al., 1988), USA (Keys et al., 1944; Davis et al., 1946; Roe et al., 1982;
626 Alexander et al., 1984; Roughead and McCormick, 1991), and India (Bamji, 1969), in healthy men
627 and women of a wide range of ages showed that the urinary excretion of riboflavin (or total flavin)
628 (collected as spot or 24h urine, either corrected by creatinine or not) increases with increased
629 intakes/supplements over a range of around 2 to 11 mg/day.

630 Data from the Verbundstudie Ernährungserhebung und Risikofaktoren Analytik (VERA Study), an
631 observational study made in a subsample of 2,006 adults (women n = 1,144) of the German National
632 Consumption Study I, showed that median 24h urinary excretion was 614 and 504 µg/day (Heseker et
633 al., 1992). The median riboflavin intake was 1.5 and 1.3 mg/day (2.5–97.5th percentiles: 0.8–3.4 and
634 0.5–2.9 mg/day) for men and women, respectively (Heseker et al., 1994).

635 The inclusion of HPLC measurements in the most recent analytical methods (e.g. (Roughead and
636 McCormick, 1991; Boisvert et al., 1993)) reduced the overestimation of riboflavin excretion compared
637 to older methods e.g. microbiological or fluorometric assays (Horwitt et al., 1950; Bro-Rasmussen,
638 1958), which could not separate the non-active flavin metabolites from the riboflavin fraction of the
639 vitamin in urine, thus improving its reliability as biomarker of nutritional status (Section 2.3.6.2.).

640 **The Panel considers** that 24h (preferably) or fasting urinary excretion of riboflavin is a suitable
641 biomarker of riboflavin short-term intake and of riboflavin status. The Panel also notes that urinary
642 excretion of riboflavin is not a sensitive marker of riboflavin intakes below 1.1 mg/day (Horwitt et al.,
643 1950). The Panel considers that the inflection of the mean urinary excretion curve in relation to
644 riboflavin intake reflects body saturation of riboflavin, and the saturation of all metabolic pathways of
645 riboflavin, thus indicating a level at which all riboflavin functions are fulfilled. Regressing urinary
646 excretion against intake can be useful to derive the requirement. The Panel notes that the
647 methodological limitations, especially in studies with older analytical methods (e.g. microbiological or
648 fluorometric assays), can influence the results for absolute values of urine riboflavin (Section 2.4.1.),
649 but assumes that the overall profile of the curve as a function of intake and the inflection point of this
650 curve are not affected.

651 **2.4.2. Erythrocyte glutathione reductase activation coefficient (EGRAC)**

652 The activity of EGR (Section 2.2.1.) expressed in terms of activation coefficient (AC) is the ratio of
653 the enzyme activity measured *in-vitro* with and without addition of the cofactor FAD. An EGRAC
654 of 1 indicates a complete saturation of EGR with intracellular FAD, while values higher than 1
655 indicate an incomplete saturation of the enzyme by intracellular FAD. EGRAC therefore provides
656 indirect information on the riboflavin status, to which it is inversely related, and is considered to
657 indicate the degree of tissue saturation with riboflavin (Sauberlich et al., 1974; Hoey et al., 2009).
658 EGRAC cannot be used in people with G6PD deficiency, as their glutathione reductase has an
659 increased avidity for FAD, leading to *in-vitro* activity that can be about 1.5 to 2 times higher than in
660 erythrocytes with normal G6PD activity (Thurnham, 1972; Nichoalds, 1981; Anderson et al., 1987;
661 Bates, 1987; Mushtaq et al., 2009), and that may prevent the identification of a low riboflavin status
662 (Sections 2.2.1. and 2.4.4.). EGRAC is sensitive to riboflavin intake, particularly below 1.0 mg/day
663 both in young and older adults (Bates et al., 1989; Boisvert et al., 1993), but is 'virtually unaffected
664 by daily variations in riboflavin intakes' (Boisvert et al., 1993). In an observational study on 927 free-
665 living adults aged 60 years or more, tobacco smokers had higher EGRAC than non-smokers
666 (Sadowski J.A., 1992).

827 In the 16-weeks supplementation study by **Boisvert et al. (1993)** (Section 2.4.1.) in 14 Guatemalan
828 older subjects with a mean baseline EGRAC of 1.64, EGRAC decreased significantly with increasing

riboflavin intake. In 10 out of 14 subjects, at a mean riboflavin intake of 1.3 mg/day, EGRAC was below the 'limit of normality' of 1.34 chosen by the authors. Based on the inflection point of the curve of mean urinary excretion of riboflavin according to mean EGRAC (that reflects body saturation), urinary excretion started to increase at EGRAC below 1.3–1.4. The Panel considers that from the results of this study, an EGRAC of 1.3 or less can be used to define adequacy in relation to changes in urinary excretion.

In an intervention study in Filipino women, either non-pregnant (n = 6), pregnant (n = 12, 2nd or 3rd trimester) or lactating (n = 11, mean of 7 weeks of lactation), and Filipino children aged 4–6 years (n = 20) and 10–12 years (n = 14), all with mean EGRAC at baseline ranging between 1.3 and 2, EGRAC was measured but not urinary excretion of riboflavin, and the content of the basal diet was analysed chemically **(Kuizon et al., 1998)**. The habitual riboflavin intake was low (0.25–0.34 mg/day in children, 0.45 mg/day in non-pregnant women and 0.30–0.53 mg/day in pregnant and lactating women), and the energy intake approximately met the WHO/FAO 1976 recommended dietary allowances (RDA)[2]. The adult participants went through four sequential feeding periods with duration of 8–10 days each: with a diet containing riboflavin at the usual level of their intake (period 1) or at increasing percentages of the 1976 Filipino RDA (0.5 mg/day) i.e. 80% (period 2), 100% (period 3) then 120% (period 4). Thus, mean riboflavin intake increased up to 1.09 mg/day in non-pregnant women, up to 1.56 mg/day in pregnant women and up to 1.6 mg/day in lactating women in the last period. The children went through two feeding periods at their usual level of intake, then two periods with increasing intake up to a mean of 1.21 mg/day. By regression analysis, the authors showed that the mean intake needed to reach an EGRAC below 1.3 were 0.72 mg/day (0.38 mg/1,000 kcal) in non-pregnant women, 1.36 mg/day (0.58 mg/1,000 kcal) in pregnant women, 1.31 mg/day (0.60 mg/1,000 kcal) in lactating women, 0.58 mg/day (0.43 mg/1,000 kcal) in children aged 4–6 years and 0.70 mg/day (0.38 mg/1,000 kcal) in children aged 10–12 years.

In an intervention study in Gambia (non-randomised), 278 infants followed between 0 and 2 years and their mothers, with mean EGRAC of 1.52 (cord blood) and 1.95 (at parturition), were studied to investigate the effect of supplementation on riboflavin status **(Bates C. J et al., 1982)** (Section 4.2.). Some infants (n = 175) were breastfed and received a weaning food supplemented with riboflavin (1.4 µg/g fresh weight) between 3 and 12 months of age in addition to the local weaning food. Their mothers were supplemented to increase the content of riboflavin in breastmilk. Another group of infants were breastfed and, received, at 3–4 months of age, a local weaning food which was a poor source of vitamin (content not given), and their mothers were not supplemented. The mean EGRAC corresponding to unsupplemented intakes (breast milk and weaning food) ranging between 0.13 and 0.21 mg/day in infants aged 0–12 months remained always above or only slightly below 1.3. However, mean EGRAC remained below 1.3 until 12 months (although it increased between 9 to 12 months in 20% of the infants) in infants receiving the supplemented weaning food and breastmilk from supplemented mothers (total intake 0.3–0.4 mg/day).

Usual intakes of 0.5 mg/day induced mean EGRAC in Gambian pregnant and lactating women of 1.75 and 1.82, respectively, with associated clinical signs of deficiency, especially in mothers close to parturition (Bates, 1981). In a study in India on pregnant and non-pregnant women, pregnant women with clinical signs of deficiency had a mean EGRAC significantly higher than that of non-pregnant women with clinical signs of deficiency or that of pregnant women without clinical signs of deficiency (2.64 vs 2.05 with $p < 0.001$, and 2.11 with $p < 0.05$, respectively) (Bamji and Prema, 1981) (Section 5.2.3.1). As regards association between EGRAC and some other health parameters, in a randomised controlled trial (RCT) in 123 women with EGRAC > 1.4, supplementation with 2 or 4 mg/day riboflavin for 8 weeks, compared with placebo, in addition to a mean intake of 1.1–1.3 mg/day according to groups, did not lead to any significant change in any of the haematological parameters investigated (Powers et al., 2011). However, in this study, a significant positive relationship ($p < 0.02$)

[2] Energy intake was about 1,900 kcal/day for non-pregnant, about 2,300–2,400 kcal/day for pregnant and lactating women, and about 1,350 and 1,800 kcal/day for children aged 4–6 years and 10–12 years, respectively.

was observed between baseline EGRAC and the change in haemoglobin concentration in the 4 mg/day group or both supplemented groups combined (but not in the 2 mg/day group). There was also a significant association between baseline tertile of EGRAC and the change in erythrocyte number ($p = 0.002$).

In a study on apparently healthy children (12–14 years) in Croatia, 20% of the 124 subjects had baseline EGRAC > 1.20 (Suboticanec et al., 1990). Then, 38 subjects were assigned to a 'riboflavin group' supplemented with 2 mg/day riboflavin for two months and 40 received a placebo (mean baseline EGRAC: 1.15 and 1.13 respectively, no randomisation, no information on energy or riboflavin intake from the diet). Mean EGRAC did not change significantly in the placebo group (1.12 compared to 1.13 at baseline), while it decreased significantly in the riboflavin group (1.00 compared to 1.15 at baseline, $p = 0.001$) in which there were no subjects with EGRAC > 1.20 anymore. This result in children is in line with results from intervention studies in the EU and the USA, which showed that EGRAC decreases with increasing riboflavin intake in healthy young and older adults. EGRAC exceeded 1.3 (i.e. 1.37–1.46) with low riboflavin intakes (e.g. 0.53 mg/day) (Van der Beek et al., 1988). It was above 1.2 at intakes of 0.6 mg/1,000 kcal (energy intake not given), but declined following increased riboflavin intakes of 0.8 and 1.0 mg/1,000 kcal (Roe et al., 1982). In older adults and adolescent rural Gambians with initial EGRAC ranging from 1.6 to 2.06, and median dietary daily riboflavin intake of 0.7 mg/day, EGRAC decreased with supplementation (doses ranging from 0.25 to 2.5 mg/day), reaching values of 1.3–1.4 with total intakes between 1.7 and 2.5 mg/day (Bates et al., 1989).

It was previously considered that an adequate riboflavin status was defined as an EGRAC of 1.2 or less (Glatzle et al., 1970; Sauberlich et al., 1974; Sadowski J.A., 1992; Benton et al., 1997; Sauberlich, 1999), insufficiency as EGRAC between 1.2 and 1.4, and deficiency as EGRAC greater than 1.4 (Sauberlich et al., 1974; Sadowski J.A., 1992; Sauberlich, 1999). A revised cut-off of 1.3 to define adequacy was used (Bates et al., 2016). From the comparison of the performance of the analytical methods used in the National Diet and Nutrition Survey (NDNS) in 1990 and 2003, Hill et al. (2009) concluded that the analytical method used in 1990 NDNS significantly underestimated the EGRAC compared to that used in 2003 NDNS ($p < 0.0001$), due to methodological differences. The authors concluded that the EGRAC analytical method should be standardised for measuring EGRAC in nutrition surveys. In a systematic review including 18 supplementation studies (Hoey et al., 2009), the authors explained that a cut-off of 1.3 had been proposed elsewhere as 'generally indicative of suboptimal status' or 'upper limit of a normal range' or 'upper limit of normality' (Bates C. J. et al., 1982; Powers et al., 1987; McNulty et al., 2006) and was a result of a 'change of assay methodology' compared to earlier studies.

Data on riboflavin intake and EGRAC are available from two large European observational studies. In the NDNS (years 5–6 i.e. 2012/13–2013/14), a survey representative of the UK population (Bates et al., 2016), for adults 19–64 years, intake (n = 965 men and women) and EGRAC (n = 526 men and women) were measured. Mean EGRAC was 1.36, with 55% of the adults with an EGRAC above 1.3, and the mean intake was 1.61 mg/day (2.5–97.5th percentiles: 0.54–3.14 mg/day). In earlier NDNS (1990 and 2003), EGRAC were not correlated with intake (Hill et al., 2009). Data from the VERA Study made in 2,006 adults in Germany (Section 2.4.1.) showed a median EGRAC of 1.33 and 1.37 (Heseker et al., 1992), with a median riboflavin intake of 1.5 and 1.3 (2.5–97.5th percentiles: 0.8–3.4 and 0.5–2.9 mg/day) for men and women, respectively (Heseker et al., 1994).

The Panel considers that EGRAC is a useful biomarker of riboflavin status. It is high in case of clinical symptoms of riboflavin deficiency, and decreases with increasing riboflavin intakes. The Panel notes that the analytical methods to assess EGRAC are not standardised. From a study in older adults, the Panel also considers that an EGRAC of 1.3 or less indicates adequate riboflavin status based on the inflection point observed in the relationship between mean EGRAC and mean urinary excretion. The Panel considers that this cut-off value may be used in younger adults, children, infants, pregnant women, lactating women.

2.4.3. Plasma and erythrocyte riboflavin, FAD, FMN

2.4.3.1 Plasma riboflavin, FAD and FMN concentration

Plasma/serum concentrations of riboflavin, FMN and FAD have been proposed to evaluate riboflavin status. However, no cut-off value for these biomarkers to assess riboflavin deficiency and/or adequacy has been proposed. Plasma/serum riboflavin reflects recent dietary intake and therefore is variable as reviewed by Sauberlich et al. (1974) and it is significantly lowered by tobacco smoking, as investigated by Ulvik et al. (2010).

In the RCT on Chinese adult men (Guo et al., 2016) (Section 2.4.1.), compared to the group with an intake of 1.0 mg/day, fasting plasma free riboflavin concentration was significantly higher at a mean total riboflavin intake of 1.5 mg/day ($p < 0.05$) and continued to increase for the higher intake levels investigated.

In a randomised, placebo-controlled study in Northern Ireland, 46 older subjects with EGRAC ≥ 1.2 (selected from a population of 124 individuals with mean age of 69 years) received for 12 weeks either a placebo (n = 23) or a daily riboflavin dose of 1.6 mg (n = 23) after an overnight fast (Hustad et al., 2002). Mean baseline dietary intake (1.6 mg/day) did not differ significantly between groups. Mean plasma free riboflavin and plasma FMN increased significantly after supplementation compared to baseline (13.2 to 19.5 nmol/L or by about 83%, $p = 0.001$, and 6.5 to 7.9 nmol/L or by about 27% ($p = 0.04$ respectively), while plasma FAD (i.e. the major form present in plasma, Section 2.3.2.) did not. Plasma FMN was strongly associated with the plasma concentration of its precursor riboflavin (Spearman correlation coefficient 0.58, $p < 0.01$), while the correlation coefficient of plasma FAD with its precursor FMN was lower (0.30, $p < 0.01$), and plasma riboflavin and FAD concentrations were not correlated. None of these plasma concentrations were correlated with EGRAC.

This result of Hustad et al. (2002) on a relationship of plasma riboflavin and FMN (but not FAD) with riboflavin intake is in line with a previous study in the USA, in which 10 men received a basal diet containing 0.55 mg/day riboflavin whilst 6 other men received the basal diet for 16 months and were supplemented with 2.55 mg/day riboflavin for 14 months, and 3.55 mg/day riboflavin for the last two months (Bessey et al., 1956) (Section 2.4.3.2.). Supplemented subjects were reported to have 'significantly' higher mean **plasma free riboflavin plus FMN** compared to the restricted group (about 19.2 vs 7.6 nmol/L) and higher mean **plasma total riboflavin** (about 83.2 vs 63.7 nmol/L), but a similar mean plasma FAD (about 62.4 vs 58.6 nmol/L) (statistics not reported).

However, the results of Hustad et al. (2002) are in contrast with an observational study (Vasilaki et al., 2010) that reported, in 119 healthy subjects, a positive correlation between plasma FMN and FAD, and between plasma free riboflavin and plasma FAD or FMN ($r = 0.5$, 0.49 and 0.55, respectively; $p < 0.001$).

The Panel notes that plasma free or total riboflavin reflects recent intakes, and that plasma free riboflavin and FMN increase with riboflavin supplementation, while plasma FAD is not a sensitive biomarker of riboflavin intake. The Panel notes that no plateauing of the riboflavin concentration in plasma was observed in the range of intake investigated (1–2 mg/day) and that no conclusion can be drawn regarding the interpretation of the results on plasma concentration for this range of intake. The Panel notes that plasma riboflavin, FMN or FAD are not correlated with EGRAC and that no normal range or cut-off value to assess riboflavin deficiency and/or adequacy has been proposed for these biomarkers.

2.3.4.2. Erythrocyte riboflavin, FAD, FMN concentration

The erythrocyte concentration of FAD and FMN (i.e. the main forms present in erythrocytes, Section 2.3.2.) has been considered for the evaluation of riboflavin status (Burch et al., 1948). A cut-off of 270 nmol/L for erythrocyte riboflavin has been proposed to define riboflavin deficiency (IOM,

1998). According to Sauberlich et al. (1974), who used the available evidence (ICNND, 1963) to set guidelines for the interpretation of erythrocyte concentrations of riboflavin in adults, concentrations of less than 270 nmol/L indicated deficiency, between 270 and 400 nmol/L in cells indicated insufficiency and more than 400 nmol/L in cells indicated sufficiency.

In a first intervention study in India in 16 healthy subjects and 11 subjects with clinical signs of riboflavin deficiency (Bamji, 1969), at baseline, the mean erythrocyte total riboflavin content in deficient subjects was significantly lower compared to healthy subjects (616.1 vs 854.0 nmol/L, $p < 0.01$) and increased to 920.7 nmol/L (significance not reported) after 7 days of supplementation with 10 mg/day riboflavin. In a second intervention study of 15–18 days described in the same paper, 4 healthy women received a basal diet containing 0.6 mg/day riboflavin, subsequently supplemented with riboflavin at weekly intervals to reach a total intake of 0.8, 1 and 1.2 mg/day respectively. The erythrocyte total riboflavin concentrations did not change except in one subject (statistics not given), which may indicate saturation.

In the study by Bessey et al. (1956) (Section 2.4.3.1), in which 10 men received a basal diet containing 0.55 mg/day riboflavin and 6 men received the basal diet for 16 months then were supplemented (2.55 mg/day for 14 months, then 3.55 mg/day for two months), the mean erythrocyte riboflavin concentration in the restricted group was lower than in the supplemented group (315.9 vs 602.1 nmol/L, statistics not provided). In a second study described in the same paper, 4 groups of 7-8 men each were fed, for 9 months (280 days), a basal diet (0.4 mg/day riboflavin) supplemented with different amounts, reaching a total intake of riboflavin ranging across groups between 0.5 (restricted group) and 2.4 mg/day. The restricted group was later supplemented with riboflavin (1.3 mg/day for 71 more days and 2.4 mg/day for the last two weeks). A fifth group was fed with a regular hospital diet (1.6 mg/day riboflavin). At baseline, all groups had similar erythrocyte riboflavin concentration (about 540 nmol/L). During the first 9 months, the restricted group had significantly lower erythrocyte riboflavin (range 321.3–569.7 nmol/L) ($p = 0.05$) compared to the three other groups that were not different from each other (range between 488.7 and 648 nmol/L) or from the 5th group on the hospital diet and did not change with time. This difference with the restricted group was significant when data were analysed between day 80 and day 280 when the erythrocyte concentration in the restricted group was the lowest i.e. about 405 nmol/L (but not anymore at the end of the study when the restricted group was additionally supplemented to 2.4 mg/day). The authors concluded that an erythrocyte riboflavin content of 540 nmol/L or more indicates an adequate intake, i.e. a concentration that occurred in most individuals at intakes of about 1.5 to 2.5 mg/day. At higher intakes (10 mg/day), the authors reported that, after a temporary increase of few hours, erythrocyte riboflavin content returned to values of 540–675 nmol/L, which may indicate saturation. The authors considered an erythrocyte riboflavin concentration of 405 nmol/L, on the contrary, as an indication that the intake of riboflavin (i.e. about 0.5 mg/day) should be increased.

In an observational study by Graham et al. (2005), the authors compared 84 pregnant women in Nepal (mean EGRAC = 1.7, mean erythrocyte riboflavin 141 nmol/L and unknown riboflavin intake) with unpublished data from healthy Californian adults, in which the 5th percentile of erythrocyte riboflavin concentration was 170 nmol/L. An erythrocyte concentration of riboflavin + FAD below 170 nmol/L detected 92% of subjects with EGRAC ≥ 1.4, whilst 73% of those with EGRAC < 1.4 had erythrocyte riboflavin+FAD concentrations above 170 nmol/L.

In the RCT by Hustad et al. (2002) in which 46 older adults with EGRAC ≥ 1.2 received either a placebo or a daily riboflavin dose of 1.6 mg for 12 weeks in addition to a baseline intake of 1.6 mg/day (Section 2.4.3.1.), after supplementation, the erythrocyte concentration of free riboflavin remained below the limit of quantification (< 1 nmol/L), while mean erythrocyte FMN increased compared to baseline (32 to 54 nmol/L or by 87%, $p < 0.001$), as well as mean erythrocyte FAD (463 to 525 nmol/L or by 14%, $p = 0.01$). Erythrocyte FMN was correlated with erythrocyte FAD (0.57, $p < 0.01$). Both erythrocyte FMN and FAD were correlated with EGRAC ($r = 0.45$, $p < 0.01$ and 0.30, $p < 0.05$, respectively). Neither erythrocyte FMN or FAD was correlated with plasma FMN or FAD.

In the observational study by Vasilaki et al. (2010), on 119 healthy subjects, erythrocyte FMN was also positively correlated with erythrocyte FAD ($r = 0.44$, $p < 0.001$). Erythrocyte FMN (but not FAD) was positively correlated with erythrocyte free riboflavin ($p < 0.001$). However, contrary to Hustad et al. (2002), erythrocyte FAD was weakly but significantly correlated with plasma FAD ($r = 0.21$, $p < 0.05$).

The Panel notes that the concentration of FAD and FMN in erythrocyte could be considered a marker of long-term riboflavin intake. Erythrocyte concentration of riboflavin (mainly FMN and FAD) is decreased with riboflavin deficiency. However, the Panel notes that limited data are available on a dose-response relationship with riboflavin intake: erythrocyte total riboflavin was not a sensitive biomarker of riboflavin intakes in the range 0.8–1.2 mg/day in one study, while in another study, erythrocyte FMN and to a lower extent erythrocyte FAD reacted to riboflavin supplementation of 1.6 mg/day in addition to the baseline intake of 1.6 mg/day. The Panel notes that erythrocyte FMN and FAD are correlated with EGRAC, while correlation with plasma values is unclear. A variety of cut-off values for erythrocyte concentration of riboflavin (or riboflavin + FAD) have been proposed to assess riboflavin deficiency or adequacy. The Panel thus considers that additional data are required before a conclusion on the suitability of the erythrocyte riboflavin content as a biomarker of riboflavin status can be made.

2.4.4. Pyridoxamine phosphate oxidase (PPO) activity and activation coefficient

As for EGR, an activation coefficient for PPO (Section 2.2.1.), i.e. PPOAC, can be defined: PPO is expressed as nmol pyridoxal phosphate formed per hour and g haemoglobin, and PPOAC as the ratio of the enzyme activity measured with and without the cofactor FMN *in-vitro* (Bates and Powers, 1985; Mushtaq et al., 2009).

PPO activity or PPOAC were proposed for measuring riboflavin status, especially in population with high prevalence of G6PD deficiency (Section 2.4.3.). In an intervention study (Bates and Powers, 1985), 72 pregnant and lactating Gambian women, the vast majority with clinical signs of riboflavin deficiency, were randomly assigned to receive either a placebo ($n = 38$) or a riboflavin supplement (5 mg/day, intake from food not reported, $n = 34$). In the non-supplemented group, at the end of the study compared to baseline, mean PPO activity in haemolysates decreased (3.60 vs 4.34, $p < 0.05$) and mean EGRAC increased (mean 2.88 vs 2.20, $p < 0.001$) significantly. In the supplemented group, three women were G6PD deficient. Before supplementation, PPO was 'similar' in G6PD-deficient and non-deficient subjects (mean of 4.44 and 5.43, respectively), while EGRAC was lower in the deficient subjects (mean of 1.41 vs 2.24) (significance not tested). After supplementation, in subjects who were not G6PD deficient, mean EGRAC significantly decreased compared to baseline (1.37 vs 2.24) and mean PPO significantly increased (16.41 vs 5.43) ($p < 0.001$). In the G6PD-deficient subjects, the authors reported a 'substantial stimulation' of PPO compared to baseline (mean value of 11.85 vs 4.44, significance not tested), while the EGRAC decrease after supplementation was 'small' (mean EGRAC 1.16 vs 1.41 at baseline).

In a study in the UK, haemolysate samples were selected from a previous intervention study in 145 young healthy non G6PD deficient women selected for EGRAC ≥ 1.40 (Mushtaq et al., 2009). A total of 68 samples were randomly selected from subjects who had received for eight weeks either a placebo ($n = 23$), or riboflavin (2 or 4 mg/day, $n = 23$ or 22 respectively). After supplementation, compared to baseline, mean EGRAC decreased significantly in both supplemented groups (1.34 and 1.25 for the 2 and the 4 mg/day group respectively compared to 1.59, $p = 0.002$ and $p < 0.001$), and the decrease was significantly larger than in the placebo group ($p < 0.001$). Both PPO and PPOAC responded to supplementation. There was a dose-response relationship with supplementation only for PPO activity as its increase in the 4 mg/day group was significantly higher than in the 2 mg/day ($p < 0.001$), while the decrease in PPOAC was not significantly different between the supplemented groups. There was a strong inverse correlation between PPO activity and PPOAC ($r=-0.65$, $p < 0.001$) and both correlated significantly with EGRAC (baseline or post-intervention, r between 0.41 and

0.57). A significant relationship was shown between PPO activity or PPOAC and riboflavin intake measured at baseline (r = 0.35 and 0.42 respectively, $p < 0.003$ or 0.002 respectively)

The Panel notes that PPO activity and PPOAC are promising biomarkers, as they respond to riboflavin intake from foods or supplements and could be used in populations with a high prevalence of G6PD deficiency. However, the Panel also notes that no criteria have been developed for these biomarkers to assess riboflavin adequacy.

2.4.5. Conclusion on biomarkers

The Panel considers that 24h (preferably) or fasting urinary excretion of riboflavin is a suitable marker of riboflavin short-term intake and of riboflavin status. The Panel considers that the inflection of the urinary excretion curve in relation to riboflavin intake reflects body saturation of riboflavin and can be used to indicate adequate riboflavin status. The Panel notes that analytical methods can influence the results for absolute values of urinary riboflavin, but assumes that the overall profile of the curve as a function of intake and the inflection point of this curve are not affected.

The Panel considers that EGRAC is a useful biomarker of riboflavin status and that EGRAC of 1.3 or less indicates adequate riboflavin status. The Panel also notes that EGRAC determination requires a single blood sample, and thus is more easily performed than urine collection over 24h. The Panel notes that there is a lack of standardisation of EGRAC measurement (thus comparison of results from different experimental/observational studies are difficult) and that EGR saturation with the coenzyme cannot be considered as representative for all riboflavin functions (described in Section 2.2.1.).

The Panel considers that plasma riboflavin, either free or total, responds to riboflavin intake but this biomarker has several limitations including its sensitivity to recent intakes. However, riboflavin status can be derived from fasting concentration of free riboflavin (or free riboflavin + FMN) in plasma determined in controlled conditions. Plasma FMN, but not FAD, responds to riboflavin supplementation. A normal range or cut-off for deficiency or adequacy for plasma riboflavin, FMN or FAD is not available. The Panel considers that that additional data are required before a conclusion on the suitability of the erythrocyte riboflavin concentration as a biomarker of riboflavin status can be made. The Panel also considers that PPO activity or PPOAC are promising biomarkers but notes that no criteria have been developed for them to assess riboflavin adequacy.

Overall, the Panel considers that the inflection point of the urinary excretion curve in relation to riboflavin intake is the most suitable biomarker to assess adequacy of riboflavin status. EGRAC can be used as a supportive biomarker of the urinary excretion in order to assess riboflavin status.

2.5. Effect of energy intake or expenditure or exercise

Twelve US young healthy normal-weight women at study entry consumed a diet with a mean riboflavin intake of 1.45 mg/day **(Belko et al., 1983)**. After a 2-week basal period, in which 'caloric intakes were adjusted to achieve weight management' and subjects (mean EGRAC of 1.27) were fed a basal diet providing 2,000 kcal/day and 1.2 mg/day riboflavin (0.14 mg/MJ or 0.6 mg riboflavin/1,000 kcal), the subjects went through a four-week sedentary period. Mean EGRAC first increased to 1.41 when subjects were fed only the basal diet and had to continue their normal daily activities while limiting 'any recreational exercise'. Then, mean EGRAC decreased to 1.24 when the riboflavin content of the diet was increased by 0.2 or 0.4 mg/1,000 kcal increments (up to 1.6 or 2 mg/day, i.e. 0.19 or 0.24 mg/MJ or 0.8 or 1.0 mg/1,000 kcal, depending on the subject). During the following period of exercise, mean EGRAC was 1.27 (first 3 weeks) when the diet did not change except for the increased energy intake by additional 240 kcal/day (total of 2,240 kcal, 9.37 MJ/day). Then, EGRAC decreased significantly compared to the first 3 weeks, only when riboflavin intake was increased by 0.4 mg/day (0.047 mg/MJ or 0.2 mg/1,000 kcal), in the second 3 weeks. Throughout the study, urinary excretion of riboflavin did not change during most of the exercise period, and remained significantly (but weakly) negatively correlated with EGRAC ($r = -0.23$, $p \leq 0.01$).

In a US study, 12 'overweight and obese' women were randomly divided in two groups of six **(Belko et al., 1984)**. Both groups had an initial baseline period of non-exercise, with a diet containing 1,200 kcal and 0.96 mg/day of riboflavin (0.19 mg/MJ or 0.8 mg/1,000 kcal), and two 5-week metabolic periods of either exercise or non-exercise (cross-over design). During the study, the riboflavin/calorie ratio was kept constant. EGRAC increased from a baseline mean of 1.28 to 1.40 during non-exercise and to 1.49 during exercise. Mean 24h urinary excretion of riboflavin (collection over 3 days) fell from about 48% of intake during baseline to about 30% of intake during non-exercise and to about 19% of intake during exercise (statistically significant effect of exercise: $p = 0.01$).

In another study in the USA **(Belko et al., 1985)** that also examined the effect of exercise on riboflavin status of 'moderately overweight' women (defined as in Belko et al. (1984)), 12 women consumed a diet providing 1,250 kcal/day. They were randomly assigned to receive either only the basal diet with a riboflavin content of 1.2 mg/day (0.23 mg/MJ, 0.94 mg/1,000 kcal) ($n = 6$, 'moderate riboflavin' group) or a diet containing 1.4 mg/day (0.28 mg/MJ or 1.16 mg riboflavin/1,000 kcal) ($n = 6$, 'high riboflavin' group). Within each group, they were then randomly assigned to sequences of exercise and non-exercise with a cross-over design. In both groups, mean EGRAC significantly increased during exercise, compared to non-exercise, from 1.16 to 1.20 ('high riboflavin' group) and from 1.31 to 1.36 ('moderate riboflavin' group) ($p < 0.05$). Mean urinary riboflavin excretion was significantly lower with exercise compared to non-exercise in the 'high riboflavin' group (0.176 versus 0.326 mg/day, $p < 0.05$) but not in the 'moderate riboflavin' group (0.072 versus 0.127 mg/day).

Fourteen healthy women participated in a 10-week exercise study in the USA **(Winters et al., 1992)**. After a 2-week basal period, the subjects were randomly allocated to either a diet ('low riboflavin, LRibo') providing 1.2 mg/day riboflavin (0.15 mg/MJ or 0.6 mg/1,000 kcal, mean energy intake of 1,801 kcal/day i.e. 7.54 MJ/day) or to the basal diet supplemented with riboflavin as FMN ('high riboflavin, HRibo') providing 1.8 mg/day riboflavin (0.22 mg/MJ or 0.9 mg/1,000 kcal, mean energy intake: 1,933 kcal/day i.e. 8.09 MJ/day). All subjects were then randomly allocated to two four-week metabolic periods of either exercise or non-exercise, when mean energy intake was 1,875 kcal/day (7.84 MJ/day) and about 1,976 kcal/day (8.27 MJ/day), respectively, for both LRibo and Hribo groups. The HRibo group had significantly lower EGRAC than the LRibo group during both non-exercise ($p < 0.0005$) and exercise (p not reported) periods (mean: Hribo: 1.07 (non-ex), 1.109 (ex), LRibo: 1.22 (non-ex), 1.283 (ex)). However, both the LRibo and HRibo groups showed significant increases in EGRAC during exercise periods ($p < 0.0001$ for both groups) compared to the non-exercise period. Mean urinary excretion of riboflavin in the HRibo group was approximately three times the value of that for the LRibo group during both no exercise (0.66 and 0.17 mg/day, respectively) and exercise (0.46 and 0.14 mg/day, respectively) and both groups showed a significant decline in urinary riboflavin excretion with exercise ($p < 0.01$). These results showed that EGRAC was lower and urinary excretion is higher in the group with the higher riboflavin intake, and that EGRAC was increased and urinary excretion decreased by exercise.

Six healthy sedentary to moderately active men with high baseline mean EGRAC of 1.53, and with a body mass index (BMI) 17.2–30.2 kg/m^2, were enrolled in a physical intervention metabolic study[3] in India **(Soares et al., 1993)**. The study was divided in two periods of maintenance (M1 and M2 of 16 and 12 days respectively), with an exercise period of 18 days (EXER) of daily exercise in between. The mean total energy expenditure (TEE) across metabolic periods (10.33, 11.01 and 10.64 MJ/day in M1, M2 and EXER, respectively) was not substantially different (p value not reported). In both maintenance periods, energy intake was 10.34 MJ/day and riboflavin intake was 1.04 mg/day (i.e. 0.10 mg/MJ or 0.42 mg/1,000 kcal). In the exercise period, additional energy was provided to compensate for the increased energy cost of exercise (energy intake in EXER 11.63 MJ/day), and the riboflavin intake was higher, 1.28 mg/day (or 0.11 mg/MJ or 0.46 mg/1,000 kcal). Mean EGRAC was statistically significantly different over the three metabolic periods ($p < 0.05$): 1.36 (M1), 1.57 (EXER), and 1.54 (M2). Mean urinary excretion expressed as % of intake was significantly lower in

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

the exercise period compared to period M1 ($p < 0.05$, 18.1% (EXER, i.e. mean of 232 µg/day) versus 26.2% (M1) or 22.3% (M2)).

Two experiments in a US study aimed at investigating the effect of exercise in healthy subjects **(Tucker et al., 1960)**. In the first experiment, seven 'normal' men on uncontrolled diets collected urine samples at rest (2h), then after they exercised, and after another period of rest after the exercise (1h). Hourly urinary riboflavin excretion during three periods of rest was significantly higher than during exercise (158, 138 and 150% of the exercise value, respectively, $p < 0.05$). In a second experiment, nine healthy men maintained a constant riboflavin intake of 2 mg/day throughout the study. During a control period of daily exercise, the energy composition of the diet needed for maintenance of their body weight was 3,300 kcal/day (i.e. 13.81 MJ/day), the riboflavin intake was 1.2 mg/day (0.15 mg/MJ or about 0.6 mg/1,000 kcal), and the mean urinary excretion of riboflavin was 285 µg/day. Then, the training session intensity was increased and also the diet composition was changed up to 5,500–6,000 kcal/day for maintenance of body weight (i.e. 23.03–25.12 MJ/day, with a riboflavin intake of 0.69–0.77 mg/day, 0.09–0.08 mg/MJ or about 0.33–0.36 mg/1,000 kcal). This physical activity reduced the mean urinary riboflavin excretion to 137 µg/day by the third day ($p < 0.01$).

The Panel notes that some results indicate that riboflavin status is modified by physical activity. This is supported by the influence of exercise on EGRAC (that increased) (Belko et al., 1984; Belko et al., 1985; Winters et al., 1992; Soares et al., 1993) and urinary excretion of riboflavin that generally decreased (Tucker et al., 1960; Belko et al., 1984; Belko et al., 1985; Winters et al., 1992; Soares et al., 1993) but not always (Belko et al., 1983). This suggests a higher utilisation of riboflavin with increased energy expenditure, thus these results support the idea that riboflavin requirement could be related to physical activity. However, the Panel notes the limitations of these studies. Only one study (Soares et al., 1993) reported total energy expenditure (TEE) in a small number of subjects, over a very wide range (8.3–19.6 MJ/day) although mean TEE did not differ during the different experimental periods in which riboflavin intake was changed. The Panel considers this a strong limitation. The Panel also notes the lack of information on the method of measurement of riboflavin intake in some of the studies, the particular aim of some of the studies (i.e. weight management studies in overweight or obese women), their short duration or small sample size, and the high variability in the characteristics of the subjects (e.g. large range of BMIs). The Panel considers that, from the studies available, there is a lack of experimental data showing a clear quantitative relationship between riboflavin status biomarkers (urinary excretion of riboflavin and EGRAC) and energy expenditure (or physical activity).

2.6. Effects of genotype

FAD is required as a cofactor for the enzyme MTHFR (Section 2.2.1.). A common polymorphism of the gene encoding this enzyme, MTHFR C677T polymorphism, is reported to have unfavourable metabolic and health consequences related to riboflavin. Homozygosity for the T allele is associated with up to 70% reduced enzyme activity, which is caused by an increased propensity of the enzyme to dissociate from its FAD cofactor (Guenther et al., 1999; Yamada et al., 2001) and it results in impaired folate metabolism and high plasma total homocysteine concentrations (Jacques et al., 1996; Hustad et al., 2007).

Studies found that impaired functioning of the MTHFR enzyme is dependent on riboflavin status measured by EGRAC, and that elevated plasma total homocysteine concentrations are evident only in individuals with 677TT genotype and poor riboflavin status (McNulty et al., 2002; Garcia-Minguillan et al., 2014). Plasma riboflavin also emerged as a factor influencing plasma total homocysteine in men and women from the Framingham Offspring Cohort (Jacques et al., 2002), as well as in a cohort of 423 healthy Norwegian blood donors (Hustad et al., 2000). An RCT with supplementation with 1.6 mg/day riboflavin (baseline intake not reported) showed increased riboflavin status (measured by EGRAC) to the same extent in all genotype groups (CC, CT and TT), but plasma total homocysteine lowering was found only in people with TT genotype without any effect in those with CC and CT

genotypes (McNulty et al., 2006). Thus, both observational studies and an RCT show consistent results. This genotype-specific effect of riboflavin on homocysteine concentrations is probably a result of stabilising the variant enzyme and restoring MTHFR activity.

Meta-analyses of observational studies showed that the MTHFR C677T polymorphism increases the risk of high blood pressure by 24–87% (Qian et al., 2007; Niu et al., 2012; Wu et al., 2014; Yang et al., 2014). A meta-analysis of genome-wide association studies, based on data from 200,000 Europeans, listed the MTHFR gene among 12 independent genetic variants associated with an increased risk of high blood pressure (Ehret et al., 2011), but mechanisms are reported to be only speculative (McNulty et al., 2017). There is also evidence that riboflavin supplementation can modify the effect of the MTHFR C677T polymorphism on blood pressure. Results from three RCTs conducted in patients with premature cardiovascular disease (Horigan et al., 2010; Wilson et al., 2012) and in hypertensive individuals without overt cardiovascular disease (Wilson et al., 2013) showed that the high blood pressure in individuals with TT genotype is highly responsive to riboflavin supplementation at a dose of 1.6 mg/day for 16 weeks in addition to the usual diet, with an average decrease by 6–13 mmHg. Importantly, the effect of riboflavin on blood pressure was independent of the effect of antihypertensive drugs taken by these patients.

Two observational studies suggested that bone mineral density (BMD) is positively associated with riboflavin intake in case of MTHFR 677TT homozygosity (a genotype that has been reported to be associated with reduced BMD and increased risk of fracture) (Macdonald et al., 2004; Abrahamsen et al., 2005).

Some genetic defects that result in a 'deficiency' of riboflavin relative to the increased need for riboflavin have been described and respond to riboflavin administration in amounts above the reference values. These gene defects lead to disturbed transport of riboflavin or of riboflavin coenzymes at the plasma membrane or between intracellular organelles or to insufficient synthesis of FAD or to dysfunctional flavoproteins and can result in a typical organic aciduria (Gregersen et al., 1986; Barile et al., 2016). The organic aciduria is the consequence of decreased activities of multiple acyl-CoA dehydrogenases that are involved in fatty acid, choline and amino acid metabolism (similar to multiple acyl-CoA-dehydrogenase deficiency (MADD, MIM #231680)). Increased excretion of dicarboxylic acids in these cases results from microsomal and peroxisomal handling of fatty acids that cannot undergo ß-oxidation (Hoppel et al., 1979; Goodman, 1981; Veitch et al., 1988).

Mutations of human riboflavin transporters RFT 2 and 3 (coded for by *SLC52A2* and *SLC* 52*A3*, respectively, and expressed in brain/salivary glands and intestine, prostate/testis/stomach/pancreas, respectively) have been identified in patients with Brown-Vialetto-van Laere syndrome (homo- or compound heterozygous mutation of *SLC52A2)* and Fazio-Londe syndrome (homo- or compound heterozygous mutation of *SLC52A3)*, autosomal recessive progressive neurologic disorders with early onset of sensorineural hearing loss, bulbar dysfunction and severe muscle weakness leading to respiratory insufficiency (Bosch et al., 2011; Haack et al., 2012; Subramanian et al., 2015).

Several mutations in the gene coding for the FAD synthetase (FLAD1) that occurs in a cytsolic and a mitochondrial isoform have been identified in patients with riboflavin-responsive MADD and combined respiratory chain deficiency. Riboflavin responsiveness was observed in cases with mutations expressing proteins with residual enzyme activity (Olsen et al., 2016).

Schiff et al. (2016) reported on a unique case of a 14 year old girl with recurrent exercise intolerance and biochemical features of the MADD syndrome, which both responded promptly to high doses of riboflavin. The authors identified a mutation of the FAD transporter (coded for by *SLC52A32*) that transports FAD from the cytosol to the mitochondrion where the flavoprotein dehydrogenases are located.

The Panel notes that the data indicate that MTHFR 677TT genotype, with a prevalence of 12 to 24% of European populations, can increase the individual requirement for riboflavin, although the extent of

this increase cannot be defined. The Panel considers that this polymorphism should be considered in determining the requirements for riboflavin.

3. Dietary sources and intake data

3.1. Dietary sources

The primary dietary sources of riboflavin include milk, milk products, eggs, and offal according to the European Nutrient Composition Database of the European Food Safety Authority (EFSA) (Section 3.2). Cow's milk contains mainly free riboflavin, and smaller amounts of FMN and FAD. Milk and dairy products make the greatest contribution to riboflavin intake in Western diets (Powers, 2003). Due to its photosensitivity, riboflavin can be lost from breast milk used in enteral nutrition of newborns (Bates et al., 1985).

Currently, riboflavin as well as riboflavin 5'-phosphate sodium (Section 2.1.) can be added to food[4] and food supplements[5]. The riboflavin content of infant and follow-on formulae and of processed cereal-based foods and baby foods for infants and children is regulated[6].

3.2. Dietary intake

EFSA estimated dietary intake of riboflavin from food consumption data from the EFSA Comprehensive 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) from nine countries (Finland, France, Germany, Ireland, Italy, Latvia, the Netherlands, Sweden and the UK). Individual data from these nationally representative surveys (except for the Finnish surveys in children) undertaken between 2000 and 2012 were available to EFSA, and classified according to the FoodEx2 food classification system (EFSA, 2011a). Riboflavin intake calculations were performed only on subjects with at least two reporting days. The data cover all age groups from infants to adults.

Composition data for riboflavin 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 were used to calculate riboflavin 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 riboflavin values in the seven composition databases used varied between 15% (Germany) and 85% (Sweden). For countries not having any food composition database, i.e. Ireland and Latvia, food composition data were used from the UK and Germany, respectively. EFSA estimates are based on consumption of foods that may be fortified or not (and without taking dietary supplements into account).

Data on infants (1–11 months old) were available from Finland, Germany, Italy and UK. The proportions of breastfed infants were between 21% and 58% according to the survey considered and most breastfed infants were partially breastfed (see table footnotes of Appendices C–D). 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 riboflavin intake estimates for infants.

[4] 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.

[5] 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.

[6] 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.

Average riboflavin intake ranged from 0.6 to 1.2 mg/day (0.2–0.4 mg/MJ) in infants (< 1 year), from 0.9 to 1.4 mg/day (0.2–0.4 mg/MJ) in children aged 1 to < 3 years, from 1 to 1.8 mg/day (0.2–0.3 mg/MJ) in children aged 3 to < 10 years and, from 1.2 to 2.2 mg/day (0.2–0.3 mg/MJ) in children aged 10 to <18 years. Average riboflavin intake ranged between 1.4 and 2.2 mg/day (0.2 mg/MJ) in adults (≥ 18 years).

The main food groups contributing to riboflavin intake among infants and children aged 1–< 3 years were 'food products for young population' and 'milk and milk products'. From the age of 3 years onwards, the main contributors of riboflavin were the food groups 'milk and milk products', 'grains and grain-based products' and "meat and meat products'. Within these three food groups, liquid milk types, fresh meat and breakfast cereals were the most contributing foods among adults. Differences in main contributors to riboflavin intakes between sexes were minor.

4. Overview of dietary reference values and recommendations

4.1. Adults

D-A-CH (2015) considered data on 24h riboflavin urinary excretion (with a target of at least 120 µg in 24h urine) and on EGRAC (with a target of EGRAC < 1.2) (Sauberlich et al., 1974). The reference values were derived in consideration of the reference values for energy intake, due to the functions of riboflavin in energy metabolism (German Nutrition Society, 2015). In long-term studies in adults consuming 9.4 MJ/day and increasing riboflavin doses (starting from an intake of 0.55 mg/day at which signs of deficiency were observed), a major change in 24h urinary riboflavin excretion occurred between intakes of 1.1 and 1.6 mg/day (0.12 and 0.17 mg/MJ), which was assumed to indicate tissue saturation (Horwitt et al., 1950) (Section 2.4.1.). Other data in children, men and women showed that, at a riboflavin intake of about 0.12 mg/MJ, adequate EGRAC and riboflavin urinary excretion are observed (Horwitt et al., 1950; Kuizon et al., 1998). An intake of 0.12 mg/MJ was considered as the Average Requirement (AR). For older adults (65 years and over), there was no indication of a requirement for riboflavin different from the one of younger adults (Boisvert et al., 1993) (Sections 2.4.1 and 2.4.2.). The PRIs for the age ranges 19–< 51, 51–< 65 and ≥ 65 years were calculated considering a CV of 10 % and the reference values for energy.

In NNR 2012, because of the lack of new studies, the Nordic Council of Ministers (2014) maintained their previous AR of 0.12 mg/MJ (NNR, 2004) based on data on urinary excretion and on EGRAC (Roe et al., 1982; Belko et al., 1983; National Research Council, 1989; Toh et al., 1994). A Recommended Intake (RI) was derived at 0.14 mg/MJ, which corresponded to about 1.5–1.6 mg/day for men and 1.2–1.3 mg/day for women with moderate physical activity. The Nordic Countries stressed that, when planning diets, the riboflavin content should not be lower than 1.2 mg/day even at an energy intake below 8 MJ/day (FAO/WHO, 1967; National Research Council, 1989). A Lower level of Intake of 0.8 mg/day was set based on depletion/repletion studies (Horwitt et al., 1950; FAO/WHO, 1967; IOM, 1998). Data on riboflavin intake/status and health outcomes could not be used to set DRVs (de Vogel et al., 2008; Kabat et al., 2008; Sharp et al., 2008; Maruti et al., 2009; Shrubsole et al., 2009; Bassett et al., 2012b; Key et al., 2012).

WHO/FAO (2004) set a Recommended Nutrient Intake for men and women respectively at 1.3 and 1.1 mg/day. The WHO/FAO reported on studies on riboflavin status measured by EGRAC (Belko et al., 1983; Belko et al., 1984; Bates et al., 1989; Kuizon, 1992), on a daily intake of 1.7 mg/day that was largely excreted in the urine (Roughead and McCormick, 1991), and noted that riboflavin tissue saturation occurred at intake above 1.1 mg/day. Two studies undertaken in older adults were cited (Alexander et al., 1984; Boisvert et al., 1993), but no specific value was set.

Afssa (2001) set PRIs based on data on urinary riboflavin excretion (Horwitt et al., 1948), adapted according to the energy requirements proposed by Afssa (2001), and discarded some studies where riboflavin status was measured as EGRAC or urinary riboflavin excretion (Roe et al., 1982; Kuizon, 1992; Boisvert et al., 1993) based on their small sample size. PRIs of 1.6 mg/day (for men) and 1.5 mg/day (for women) and 1.6 mg/day (for both sexes at 75 years of age or above) were proposed.

The Health Council of the Netherlands (2000) set an AI of 1.1 mg/day for men and of 0.8 mg/day for women based on studies on urinary excretion (Horwitt et al., 1950; Horwitt, 1966). Urinary excretion increases at riboflavin intakes of 1.1–1.6 mg/day. A ratio between riboflavin urine excretion and riboflavin intakes appeared to be constant for an intake of 1.1 mg/day, which meant that a saturation of tissues occurred. The Council set different PRIs for men and women on the basis of different energy intakes (Bates et al., 1989; Zempleni et al., 1996). For older adults (over 51 years old), it was considered that data on urinary excretion studies and EGRAC did not suggest the setting of different PRIs from those determined for younger adults (Bates et al., 1989; Lowik et al., 1990; Boisvert et al., 1993; Bates, 1997).

IOM (1998) considered mainly studies on subjects receiving riboflavin from food or food and supplements, and reporting occurrence of clinical signs of deficiency, and/or measuring EGRAC and/or erythrocyte concentration of riboflavin or changes in the riboflavin urinary excretion curve. These studies were undertaken in women (Sebrell et al., 1941; Williams et al., 1943; Brewer et al., 1946; Davis et al., 1946; Roe et al., 1982; Belko et al., 1983; Kuizon, 1992), in men (Keys et al., 1944; Horwitt M et al., 1949; Horwitt et al., 1950; Bessey et al., 1956) or both (Boisvert et al., 1993). Based on these references, the IOM considered that normal EGRAC values were associated mostly with intakes below 1.3 mg/day. Deficiency was observed for intakes of about 0.5–0.6 mg/day with measures of urinary riboflavin excretion by microbiological assays (Sebrell et al., 1941; Horwitt et al., 1950). IOM (1998) considered that the difference in riboflavin requirement between sexes was explained by size and energy expenditure. For men (19–70 years), an Estimated Average Requirement (EAR) of 1.1 mg/day and, using a coefficient of variation (CV) of 10 % (due to a lack of data on the variation in requirements), an RDA of 1.3 mg/day was determined. Regarding women, an EAR at 0.9 mg/day and a RDA at 1.1 mg/day were set. It was considered that the limited data available in older adults (Alexander et al., 1984; Boisvert et al., 1993) did not support a requirement for older adults (above 70 years old) different from that of younger adults.

SCF (1993) assessed the intake at which clinical signs of deficiency appear, from clinical data with controlled riboflavin intakes and from epidemiological studies that reported deficiency for an intake range of 0.5–0.8 mg/day. The SCF also took into account the inflection in urinary riboflavin excretion according to increasing riboflavin intakes considered as an indication of tissue content (Horwitt et al., 1950; Bro-Rasmussen, 1958). For all adults, a Lowest Threshold Intake was defined at 0.6 mg/day. For men, an AR was determined at 1.3 mg/day by interpolation between the intakes of 1.1 and 1.6 mg/day, between which a sharp increase in urinary riboflavin excretion occurred (Horwitt et al., 1950). A PRI was set at 1.6 mg/day for men also on the basis of urinary excretion studies. For women, the AR was derived from the AR for men taking into account body weight. Thus, for women, the AR and PRI were 1.1 and 1.3 mg/day, respectively. SCF acknowledged the conventional expression of riboflavin requirements on the basis of energy intake, but did not relate riboflavin requirement to energy expenditure, as flavoproteins are involved in a number of reactions not limited to energy metabolism and therefore riboflavin requirements are not related only to energy expenditure. The SCF considered that no evidence was available to set specific values for older adults.

The UK COMA (DH, 1991) took into account the appearance of clinical signs of riboflavin deficiency at intakes below 0.5–0.8 mg/day (Adamson et al., 1945; Burgess, 1946; Horwitt M et al., 1949; Nicol, 1949; Horwitt et al., 1950; Bro-Rasmussen, 1958; Bates, 1981). The UK COMA also took into account the sharp increase in urinary riboflavin excretion for intakes higher than 0.11 mg/MJ (0.44 mg/1,000 kcal) (FAO/WHO, 1967; DHSS, 1969, 1979) and the riboflavin intakes in British adults (Gregory et al., 1990), i.e. 1.3 mg/day (for men) and 1.1 mg/day (for women), associated with EGRAC values below 1.3 considered as representing saturation of tissues with riboflavin (Glatzle et al., 1970; Thurnham et al., 1970). The Reference Nutrient Intakes were determined at 1.3 mg/day (men) and 1.1 mg/day (women). The corresponding Lower Reference Nutrient Intakes (LRNI) was 0.8 mg/day for all adults and the EARs were 1.0 and 0.9 mg/day for men and women respectively. The limited data in older adults (Thurnham et al., 1970) and the decreased resting energy expenditure and riboflavin intakes with age were considered as insufficient evidence to set specific values for older adults.

1407 An overview of DRVs for riboflavin for adults is given in Table 1.

1408 **Table 1:** Overview of Dietary Reference Values for riboflavin for adults

	D-A-CH (2015) [(a)]	NCM (2014) [(a)]	WHO/FAO (2004)	Afssa (2001)	NL (2000)	IOM (1998)	SCF (1993)	DH (1991) [(a)]
Age (years)	≥ 19	18–30	≥ 19	20–74	≥ 19	≥ 19	≥ 18	≥ 19
PRI men (mg/day)	1.4	1.6	1.3	1.6	1.5	1.3	1.6	1.3
PRI women (mg/day)	1.1	1.3	1.1	1.5	1.1	1.1	1.3	1.1
Age (years)	≥ 51	31–60		≥ 75				
PRI men (mg/day)	1.3	1.5		1.6				
PRI women (mg/day)	1.0	1.2		1.6				
Age (years)	> 65	61–74						
PRI men (mg/day)	1.3	1.4						
PRI women (mg/day)	1.0	1.2						
Age (years)		≥ 75						
PRI men (mg/day)		1.3						
PRI women (mg/day)		1.2						

1409 NCM: Nordic Council of Ministers. NL: The Netherlands.
1410 (a): DRVs in mg/day obtained from the DRVs in mg/MJ and the respective energy requirement.

1411 ## 4.2. Infants and children

1412 D-A-CH (2015) indicated that there were no data on riboflavin requirement for infants aged
1413 4-12 months, whereas data in children 4–12 years old with riboflavin deficiency showed a return of
1414 EGRAC to normal values of about 1.2 with a riboflavin intake of 0.12 mg/MJ (Kuizon et al., 1998).
1415 D-A-CH (2015) set PRIs ranging from 0.4 mg/day for infants aged 4–12 months to 1.6 mg/day for
1416 boys aged 15–< 19 years, on the basis of an AR of 0.12 mg/MJ as for adults, a CV of 10 % and the
1417 reference values for energy.

1418 For children, the Nordic Council of Ministers (2014) used the same AR and RI expressed in mg/MJ as
1419 for adults, and converted them into mg/day.

1420 WHO/FAO (2004) set a Recommended Nutrient Intake at 0.3 mg/day for infants up to six months of
1421 age based on the daily average riboflavin content in breast milk (0.35 mg/L and an average breast milk
1422 consumption of 0.75 L/day) (IOM, 1998). For older children, RNIs increased gradually with age.

1423 Afssa (2001) set PRIs for children from the adult PRIs adjusted for the energy requirement of each age
1424 range.

1425 The Health Council of the Netherlands (2000) derived an Adequate Intake (AI) for infants up to five
1426 months at 0.4 mg/day on the basis of a daily human milk consumption of 0.8 L and a riboflavin
1427 concentration in milk of 0.53 mg/L (Fomon and McCormick, 1993). For children and adolescents, data
1428 to set ARs were considered too limited (Oldham, 1944; Snyderman et al., 1949; Lo, 1985). Thus, an
1429 interpolation between the reference values of infants (under 5 months) and of adults was used to set
1430 AIs of children and adolescents, following the IOM (1998) approach. It was considered that the AI for
1431 children aged 14–18 years should be in line with the requirement of adults due to the linear increase of
1432 the requirement. Thus, for this age range, an AI of 1.5 mg/day and of 1.1 mg/day, respectively, for
1433 boys and girls was set.

1434 Considering a riboflavin concentration of 0.35 mg/L in human milk (WHO, 1965) and a milk
1435 consumption of 0.78 L/day, and after rounding, IOM (1998) set an AI at 0.3 mg/day for infants up to
1436 six months of age. The IOM set the AI at 0.4 mg/day for infants aged 7 to 12 months based on upward
1437 extrapolation from the value for younger infants and rounding, which was confirmed by downward

extrapolation from the adult EAR. The approach of adding riboflavin intakes from breast milk consumption and from solid foods was discarded as providing a value considered as too high. For children aged from 1 to 18 years, due to the limited data (Oldham, 1944; Sauberlich et al., 1973) to base an EAR, IOM (1998) decided to extrapolate the EARs from adults to children using allometric scaling (power 0.75) with growth factors, and rounding. A CV of 10% was also used to set RDAs.

SCF (1993) derived a PRI of 0.4 mg/day for infants 6–11 months based on data on changes in EGRAC in Gambian infants according to the level of supplementation (Bates C. J et al., 1982), and PRIs for children from the PRIs of adults on the basis of energy requirement due to lack of data to assess children's requirement.

The UK COMA (DH, 1991) set reference nutrient intakes for children by interpolation between the adult and infant values, and corresponding LRNIs and EARs were set. The reference nutrient intakes for infants were set at 0.4 mg/day, according to the average concentration of riboflavin in breast milk in the UK (0.31 mg/L) (DHSS, 1977), and a supplement intake of 0.4 mg/day which led to a satisfactory EGRAC value (1.15) in Gambian infants (Bates C. J et al., 1982).

An overview of DRVs for riboflavin for children is given in Table 2.

Table 2: Overview of Dietary Reference Values for riboflavin for children

	D-A-CH (2015) [(a)]	NCM (2014) [(a)]	WHO/FAO (2004)	Afssa (2001)	NL (2000)[(b)]	IOM (1998)	SCF (1993)	DH (1991)
Age (months)	4–12	6–11	0–6	0–12	6–11	0–6	6–11	7–9
PRI (mg/day)	0.4	0.5	0.3	0.4	0.4	0.3	0.4	0.4
Age (years)	1–4	1–< 2	7–12	1–3	1–3	7–12	1–3	1–3
PRI (mg/day)	0.7	0.6	0.4	0.8	0.5	0.4[(a)]	0.8	0.6
Age (years)	4–7	2-5	1–3	4–6	4–8	1–3	4–6	4–6
PRI (mg/day)	0.8	0.7	0.5	1	0.7	0.5	1.0	0.8
Age (years)	7–10	6–9	4–6	7–9	9–13	4–8	7–10	7–10
PRI Boys (mg/day)	1.0	1.1	0.6	1.3	1.0	0.6	1.2	1.0
PRI Girls (mg/day)	0.9	1.1	0.6	1.3	1.0	0.6	1.2	1.0
Age (years)	10–13	10–13	7–9	10–12	14–18		11–14	11–14
PRI Boys (mg/day)	1.1	1.3	0.9	1.4	1.5		1.3	1.2
PRI Girls (mg/day)	1.0	1.2	0.9	1.3	1.1		1.3	1.1
Age (years)	13–15	14–17	10–18	13–15		9–13	15–17	15–18
PRI Boys (mg/day)	1.4	1.7	1.3	1.6		0.9	1.5	1.3
PRI Girls (mg/day)	1.1	1.4	1.0	1.4		0.9		1.1
Age (years)	15–19			16-19		14–18		
PRI Boys (mg/day)	1.6			1.6		1.3		
PRI Girls (mg/day)	1.2			1.5		1.0		

(a) DRVs in mg/day obtained from the DRVs in mg/MJ and the respective energy requirement.
(b) AI
NCM: Nordic Council of Ministers. NL: The Netherlands.

4.3. Pregnancy and lactation

For pregnancy and lactation, D-A-CH (2015) considered the average requirement of 0.12 mg/MJ set for other women, a CV of 10% and the respective reference values for energy, i.e. an additional energy requirement of 1.04 and 2.09 MJ/day in the second and third trimester of pregnancy, respectively, and a reference value of 10 MJ/day for exclusively breastfeeding women during the first four to six months of lactation.

The Nordic Council of Ministers (2014) recommended an increase in intake of 0.3 mg/day for pregnancy and of 0.4 mg/day for lactation, to be added to the RI for non-pregnant non-lactating women.

WHO/FAO (2004) acknowledged that EGRAC increases during pregnancy and that riboflavin intakes and fetal growth are associated (Bates, 1981; Vir et al., 1981; Kuizon, 1992; Badart-Smook et al., 1997). An increase in intake of 0.3 mg/day for pregnant women (added to the reference value for non-pregnant women) was considered necessary for the growth of both maternal and fetal compartments. A transfer of 0.3 mg/day riboflavin to breast milk (IOM, 1998) and an efficiency of milk production of 70% (WHO, 1965) were considered to set the additional riboflavin intake for lactating women at 0.4 mg/day (rounded value, to be added to the RNI for non-lactating women).

Afssa (2001) set a PRI of 1.6 mg/day for pregnancy to cover fetus growth, and an additional intake of 0.3 mg/day for lactation to cover riboflavin losses through breast milk.

The Health Council of the Netherlands (2000) decided to increase the value during pregnancy and set an AR of 1.0 mg/day and a PRI of 1.4 mg/day. Indeed, it was acknowledged that a higher intake of riboflavin is required to lower EGRAC, which rises during pregnancy (Kuizon, 1992) and that urinary excretion decreases during the 3rd trimester (Bro-Rasmussen, 1958; FAO/WHO, 1967). For lactating women, it was considered that an amount of 0.4 mg/day of riboflavin is excreted in human milk. Thus, an AR of 1.2 mg/day and a PRI of 1.7 mg/day were set.

During pregnancy, IOM (1998) added 0.3 mg/day to the EAR for non-pregnant women, to allow for the growth in maternal compartments and of the foetus. IOM (1998) noted that urinary excretion of riboflavin is lower during pregnancy, that clinical signs of deficiency could appear more frequently for low intakes (less than 0.8 mg/day) (Brzezinski et al., 1952; Jansen and Jansen, 1954) and that EGRAC tends to increase during pregnancy (Heller et al., 1974; Bates, 1981; Vir et al., 1981). As 0.3 mg/day of riboflavin is considered to be transferred to breast milk during the first six months of lactation, and considering a milk production efficiency of 70% (WHO, 1965), IOM (1998) set an extra intake of 0.4 mg/day during lactation to be added to the EAR of non-lactating women. The same CV of 10% was used to set RDAs for pregnancy and lactation, i.e. 1.4 and 1.6 mg/day respectively.

SCF (1993) stated that EGRAC data could not be used to set riboflavin requirement during pregnancy. An increase in intake of 0.3 mg/day was recommended, added to the PRI of non-pregnant women, to take into account the increased tissue synthesis by the fetus and the mother. In order to meet the increased metabolic burden and the losses in breast milk, the SCF proposed an increase in riboflavin intake of 0.4 mg/day during lactation, to be added to the PRI for non-lactating women.

The UK COMA (DH, 1991) considered an intake of 0.3 mg/day for pregnant women to be added to the reference value of non-pregnant women, to meet the need of the fetus (DHSS, 1979) and considered that EGRAC data in pregnancy could not be interpreted. For lactation (either before or after four months), an extra-intake of 0.5 mg/day was recommended, to be added to the RNI for non-lactating women, based on the riboflavin concentration in breast milk and its metabolic cost (DHSS, 1979).

An overview of DRVs for riboflavin for pregnant or lactating women is given in Table 3.

Table 3: Overview of Dietary Reference Values for riboflavin for pregnant or lactating women

	D-A-CH (2015) [(a)]	NCM (2014)	WHO/FAO (2004)	Afssa (2001)	NL (2000)	IOM (1998)	SCF (1993)	DH (1991)
PRI Pregnancy (mg/day)	1.3 (2nd trimester) 1.4 (3rd trimester)	1.6	1.4	1.6	1.4	1.4	1.6	1.4
PRI Lactation (mg/day)	1.4	1.7	1.6	1.8	1.7	1.6	1.7	1.6

(a) DRVs in mg/day obtained from the DRVs in mg/MJ and the respective energy requirement.
NCM: Nordic Council of Ministers. NL: The Netherlands.

5. Criteria (endpoints) on which to base dietary reference values

5.1. Clinical signs of deficiency

Riboflavin intakes of less than 0.5 to 0.6 mg/day riboflavin for several months led to clinical signs of deficiency, but intakes of about 0.8 mg/day were sufficient to avoid them in men or women (Sebrell et al., 1941; Williams et al., 1943; Keys et al., 1944; Horwitt et al., 1950), and non-pregnant women with a riboflavin intake of 0.45 mg/day for 8–10 days showed no clinical signs of deficiency (Kuizon et al., 1998) (Section 2.4.2.). Regarding biomarkers, skin lesions were reported in three men (at an intake of 0.55 mg/day riboflavin and 2,200 kcal/day) with a urinary excretion of riboflavin below 40 µg/day (Horwitt et al., 1950) (Section 2.4.1). However, older subjects with urinary riboflavin excretion < 10 µg/g creatinine (and EGRAC > 2) did not show clinical signs of riboflavin deficiency either before or during their participation to the study by Boisvert et al. (1993) (Sections 2.4.1 and 2.4.2.).

The Panel notes that clinical signs of deficiency are unspecific (Section 2.2.2.1.), take several months to develop and are unreliable to assess adequacy or inadequacy of the riboflavin supply. The Panel considers that clinical signs of deficiency cannot be used as criteria to set DRVs.

5.2. Indicators of riboflavin requirements

The Panel considers that the inflection point of the urinary excretion curve according to intake is the most suitable biomarker to assess adequacy of riboflavin status. EGRAC can be used as a supportive biomarker of the urinary excretion in order to assess riboflavin status (Section 2.4.5.). The Panel also considers that riboflavin status is modified by physical activity, urinary excretion of riboflavin is (generally) decreased and EGRAC increased when physical activity is increased, suggesting higher utilisation of riboflavin with increased energy expenditure (Section 2.5.). However, there is a lack of experimental data showing a clear quantitative relationship between riboflavin status biomarkers (urinary excretion of riboflavin and EGRAC) and energy expenditure (or physical activity) (Section 2.5). In addition, the Panel considers that the relationship between riboflavin intake and biomarkers of riboflavin status is also influenced by MTHFR 677C>T polymorphism, as homozygosity for the T allele can increase the individual requirement for riboflavin, although the extent of this increase cannot be defined (Section 2.6.).

5.2.1. Adults

The Panel notes that new scientific data have become available for adults since the publication of the SCF report in 1993. These data are either on the inflection of the urinary excretion curve or on EGRAC, according to riboflavin intake.

5.2.1.1. Inflection of the urinary excretion of riboflavin

The Panel considers that the inflection of the urinary excretion curve in relation to riboflavin intake reflects body saturation of riboflavin and can be used to indicate adequate riboflavin status (Section 2.4.1). The Panel considers that the inflection in the urinary excretion curve reflects the overall saturation of all metabolic pathways of riboflavin (provided the collection of urinary samples are complete), thus indicating a level at which all riboflavin functions are fulfilled.

Four intervention studies investigated the inflection of the curve in 24h or fasting urinary excretion of riboflavin according to riboflavin intake in adults. These were: one study of the longest duration and in 66 US men from a ‘mental institution’ (Horwitt et al., 1950) that was used by SCF (1993) for setting DRVs, one study in 73 young physically active Chinese men (Guo et al., 2016), and two other studies of smaller size i.e. one study in 14 low-physically active older Guatemalan men and women (Boisvert et al., 1993) and one study in 14 young and healthy US women (Brewer et al., 1946) (Section 2.4.1.). The Panel acknowledges that the study by Brewer et al. (1946) was published before the SCF report in 1993 in which it was not considered. However, so far, it is the only available study that provides

information on the inflection point of the urinary excretion curve according to riboflavin intake in healthy women. The Panel notes that no study was available on subjects representative of the healthy European population. However, the Panel notes that the average body weights of the young men (Guo et al., 2016) and the young women (Brewer et al., 1946) investigated were close to the reference body weights for adults in the EU, i.e. 68.1 kg for men and 58.5 kg for women.

The inflection in the urinary excretion curve occurred at riboflavin intakes between 1.1 and 1.6 mg/day (Horwitt et al., 1950) (inflection point estimated at 1.3 mg/day by SCF by interpolation, Section 4.1.). The inflection occurred at intakes between 1.3 and 1.5 mg/day in adult men (Guo et al., 2016), between 1.1 and 1.3 mg/day in older men and women (Boisvert et al., 1993) and between 1.26 and 1.62 mg/day in adult women (Brewer et al., 1946). The inflection point, calculated as the intercept of two regression lines of mean urinary excretion versus intake, was calculated by the authors to be 1.4 mg/day (Guo et al., 2016), to be 1.13 mg/day (Boisvert et al., 1993), or to be between 1.3 and 1.5 mg/day (Brewer et al., 1946).

The Panel notes the consistency in these results, and considers that, from these different studies, no difference in riboflavin requirement could be shown between sex and between younger and older adults.

5.2.1.2. Erythrocyte glutathione reductase activation coefficient (EGRAC)

The Panel considers that EGRAC is a useful biomarker of riboflavin status in all population groups, reflecting the saturation of EGR with the coenzyme (Section 2.4.2.). As discussed in Section 2.4.2., the Panel considers that an EGRAC of 1.3 or less is indicative of adequate riboflavin status in all population groups.

Two intervention studies investigated the relationship between dietary riboflavin intake and EGRAC in adults: in 14 older low-physically active Guatemalan men and women (Boisvert et al., 1993), and in 6 non-pregnant women in the Philippines (Kuizon et al., 1998) (Section 2.4.2.). The mean intake at which the mean weekly EGRAC was below 1.3 was 1.37 mg/day in older adults (Boisvert et al., 1993). However, according to the regression analysis in the study by Kuizon et al. (1998), Filipino women reached EGRAC values of 1.3 with an average riboflavin intake of 0.72 mg/day. The Panel notes that the mean intake of 1.37 mg/day estimated by Boisvert et al. (1993) falls within the range of intake corresponding to the inflection point of the urinary excretion curve from four intervention studies discussed previously (Section 5.2.1.1.). The Panel however notes the discrepancy in the results from the only two intervention studies on riboflavin intake and EGRAC.

Two large observational studies in Europe (VERA in Germany and NDNS 2012–2014 in the UK) also provide data on riboflavin intake and EGRAC (Section 2.4.2.). The Panel notes that mean/median EGRAC values from NDNS and VERA are 1.3 or higher at mean/median intake higher than that calculated to reach an EGRAC of 1.3 from experimental data mentioned above (Boisvert et al., 1993; Kuizon et al., 1998).

The Panel concludes that the data on the relationship between riboflavin intake and EGRAC cannot be used alone to set DRVs for riboflavin for adults, but can be used in support of data on the inflection in the urinary excretion curve in view of setting DRVs for riboflavin.

5.2.1.3. Conclusions on riboflavin requirements in adults

The Panel concludes that, among the available biomarkers to estimate riboflavin requirements in adults, the inflection point in the urinary excretion curve represents the primary biomarker of riboflavin requirement. This inflection point was estimated to occur at an intake of riboflavin between 1.13 and 1.4 mg/day (Brewer et al., 1946; Horwitt et al., 1950; Boisvert et al., 1993; Guo et al., 2016).

The Panel concludes that, using this biomarker, there is no indication of different riboflavin requirement according to sex or between younger and older adults.

In relation to the fact that MTHFR genotype can influence the requirement for riboflavin (Section 2.6.), in the intervention studies used for setting DRVs for riboflavin for adults, no information is provided on this genotype. The Panel however considers that these studies were conducted in different countries (USA, Guatemala, Philippines, China) and population groups, and therefore assumes that their participants represent the diversity of this polymorphism. The Panel also notes that these studies included subjects that were either physically active (Guo et al., 2016) or with a low physical activity (Boisvert et al., 1993). The Panel considers that the potential effect of physical activity and of MTHFR 677TT genotype on riboflavin requirement is covered by the data presented from the studies considered.The Panel notes that the subjects in these studies were either physically active (Guo et al., 2016) or with a low physical activity (Boisvert et al., 1993). The Panel considers that the potential effect of physical activity and of MTHFR 677TT genotype on riboflavin requirement is covered by the data presented from the studies considered.

5.2.2. Infants and children

One intervention study in infants and children aged between 0 and 2 years in Gambia (Bates C. J et al., 1982) (Sections 2.4.2. and 4.2., used by SCF) showed that 0.13 to 0.21 mg/day riboflavin intake was not sufficient to support an EGRAC below 1.3 in infants up to 1 year. With a total intake of 0.3–0.4 mg/day (from food and supplements), most of the infants aged 3–9 months had an EGRAC below 1.3, but at 9–12 months, EGRAC increased above 1.3 in about 20% of the infants.

Based on a regression analysis, another intervention study in Filipino children (Kuizon et al., 1998) (Section 2.4.2.) showed that EGRAC reached values of 1.3 with an average riboflavin intake of 0.58 and 0.70 mg/day in children aged 4–6 years (n = 20) and 10–12 years (n = 14), respectively. One large observational study in Europe (NDNS 2012–2014 in the UK) (Section 2.4.2.) provides information on mean EGRAC and mean riboflavin intake in children. The Panel notes that mean EGRAC were at or above 1.3 for children aged 4–10 years and 11–18 years but below 1.3 in children aged 1.5–3 years, while mean intakes were higher than the average intake derived in the intervention study by Kuizon et al. (1998).

In view of the limitations in the use of EGRAC discussed previously (Sections 2.4. and 5.2.1.), **the Panel concludes** that these two intervention studies can provide only supportive evidence for setting DRVs for riboflavin for infants and children. The Panel concludes that data on riboflavin intake of breastfed infants during the first 6 months of lactation (Section 2.3.6.3.) can be used to derive a DRV for infants aged 7–11 months.

5.2.3. Pregnant and lactating women

5.2.3.1. Pregnant women

The Panel assessed whether data were available on the riboflavin transfer from the mother to the fetus and the riboflavin accretion in the fetus and placenta during pregnancy (Section 2.3.2.). Mean EGRAC measured in placenta of full-term infants was significantly lower than in cord blood or maternal plasma (0.92 versus 1.18 and 1.31 respectively) in relation with the high FAD placental content (Ramsay et al., 1983). **The Panel considers** that riboflavin demand is increased during pregnancy in relation with the riboflavin uptake by the fetus and concentration in the placenta (Baker et al., 1981; Dancis et al., 1985; Dancis et al., 1986; Zempleni et al., 1995), but that these data cannot be used to set DRVs for riboflavin for pregnant women.

A progressive fall in maternal riboflavin status assessed by urinary excretion or EGRAC during the third trimester was reported in studies conducted in countries with low riboflavin intake (0.5, or less than 1 mg/day, (Jansen and Jansen, 1954; Bates, 1981)) with clinical signs of deficiency towards the

end of pregnancy (Jansen and Jansen, 1954; Bamji and Prema, 1981; Bates, 1981) (Sections 2.4.2. and 4.3.)

Urinary riboflavin excretion decreases during the third trimester of pregnancy (Bro-Rasmussen, 1958; FAO/WHO, 1967) (Section 4.3.). EGRAC increases during pregnancy (Heller et al., 1974; Vir et al., 1981; Kuizon, 1992) (Section 4.3.).

In the intervention study in 12 Filipino pregnant women (2nd or 3rd trimester) (Kuizon et al., 1998) (Section 2.4.2.), based on a regression analysis, EGRAC reached values of 1.3 and below with an average riboflavin intake of 1.36 mg/day, which was higher than for non-pregnant women.

The Panel concludes that studies investigating riboflavin fetal uptake and riboflavin placental concentration provide evidence that pregnant women need more riboflavin than non-pregnant women. However, these data are not sufficient to estimate the additional need for dietary riboflavin during pregnancy. In view of the limitations in the use of EGRAC discussed previously (Sections 2.4. and 5.2.1.), the Panel concludes that the intervention study in Filipino pregnant women can provide only supportive evidence for setting DRVs for riboflavin for pregnant women.

5.2.3.2. Lactating women

From the available studies undertaken on healthy lactating mothers (Appendix A, Section 2.3.6.3.), the Panel estimated a riboflavin secretion of 0.291 mg/day in breast milk during the first six months of lactation, based on the three studies (Nail et al., 1980; Thomas et al., 1980; Ortega et al., 1999) undertaken in Spain and the USA providing the concentration of riboflavin in mature breast milk of healthy unsupplemented mothers as well as information on the maternal riboflavin intake and status.

In an intervention study in 10 Filipino lactating women (Kuizon et al., 1998) (Section 2.4.2.), based on a regression analysis, EGRAC reached values of 1.3 with an average riboflavin intake of 1.31 mg/day, which was higher than for non-lactating women.

The Panel concludes that an additional intake of riboflavin for lactating women is required to compensate for the amount of riboflavin secreted in breast milk during the first six months of exclusive breastfeeding. In view of the limitations in the use of EGRAC discussed previously (Sections 2.4. and 5.2.1.), the Panel concludes that the intervention study in Filipino lactating women can provide only supportive evidence for setting DRVs for riboflavin for lactating women.

5.3. Riboflavin intake and health consequences

Trials (randomised or not), prospective cohort, case-control and systematic reviews of observational studies are discussed in this Section. The relationship between riboflavin intake and chronic disease outcomes has been investigated in trials, and also in observational studies where associations between intake and disease outcomes may be confounded by uncertainties inherent to the methodology used for the assessment of riboflavin intake and by the effect of dietary, lifestyle, or other undefined factors on the disease outcomes investigated. A comprehensive search of the literature published between 1990 and 2014 was performed as preparatory work to this assessment in order to identify new data on relevant health outcomes upon which DRVs for riboflavin could be based (Buijssen et al., 2014). An additional literature search (in Pubmed) was performed to identify new data published until mid-2016 on riboflavin intake and health outcomes. The Panel only considered studies that include assessment of riboflavin intake, whereas studies on the relationship of levels of riboflavin biomarkers (Section 2.4.) and health outcomes with no quantitative data on riboflavin intake are not considered.

The Panel considers that evidence from only one observational study on a particular outcome is not sufficient to provide strong evidence of a relationship and thus cannot be used for setting DRVs for riboflavin. Thus, data on riboflavin intake and bone mineral density in postmenopausal women (Rejnmark et al., 2008), the risk of overactive bladder syndrome (Dallosso et al., 2004), the risk of premenstrual syndrome (Chocano-Bedoya et al., 2011), 'psychological distress' (Mishra et al., 2008),

cognition (McNeill et al., 2011), risk of total cardiovascular diseases (Zee et al., 2007), or cancer at some sites (gastric adenocarcinoma (Eussen et al., 2010); pancreatic cancer (Chuang et al., 2011); prostate cancer (Bassett et al., 2012a); oral carcinoma (Petridou et al., 2002); ovarian cancer (Kabat et al., 2008); oesophageal cancer (Siassi et al., 2000); cervical cancer (Liu et al., 1993); renal cell carcinoma (Gibson et al., 2010)) are not considered below. In addition, intervention studies investigating riboflavin supplementation, in addition to intake, at levels higher than the observed average intake of riboflavin in the EU (Appendices C and D) were also not considered by the Panel in this Section (e.g. 15 mg twice weekly or between 5 and 400 mg/day (Tremblay et al., 1984; Powers et al., 1987; Weight et al., 1988; Prasad PA et al., 1990; Schoenen et al., 1998; Condo et al., 2009; Di Lorenzo et al., 2009; Bruijn et al., 2010). Trials using combined supplementation with riboflavin and another nutrient (Blot et al., 1993), ecological studies or narrative reviews were also not considered. Since the reports by SCF (1993), more data have become available on risk of cancer and other health outcomes (risk of cataract, pregnancy-related outcomes, physical performance or all-cause mortality) which are described below.

5.3.1. Riboflavin intake and the risk of cancer

Regarding the risk of **lung cancer**, after adjustments for potential confounders, there was no association with riboflavin intake among women (Kabat et al., 2008), and a significant linear inverse association only in current smokers (Bassett et al., 2012b).

Regarding the risk of **colorectal cancer**, after adjustments for potential confounders, no association with riboflavin intake was found in most cohorts (de Vogel et al., 2008; Kabat et al., 2008; Shrubsole et al., 2009; Key et al., 2012; Yoon et al., 2016). In one cohort (Zschabitz et al., 2013) included in the systematic review below, no association was shown with riboflavin intake from food or from supplement, but a significantly increased risk was observed in the highest quartile of total riboflavin intake (food and supplements) compared to the lowest one. A significantly lower odds ratio (OR) in the highest tertile of riboflavin intake compared to the lowest one in K-ras mutation negative, but not K-ras mutation positive, colorectal adenomas was observed in one case-control study (Wark et al., 2006). In one systematic review (Liu et al., 2015a) of five cohort studies (de Vogel et al., 2008; Shrubsole et al., 2009; Bassett et al., 2013; Zschabitz et al., 2013), a significant inverse association with riboflavin intake was observed (relative risk (RR) 0.86, 95% confidence interval (CI) 0.76–0.97, (heterogeneity index (I^2) =0%). In another systematic review from the same author (Liu et al., 2015b) on 5 cohort studies and 4 case-control studies (La Vecchia et al., 1997; Jedrychowski et al., 2001; de Vogel et al., 2008; Ma et al., 2009; Shrubsole et al., 2009; van Lee et al., 2011; Bassett et al., 2013; Zschabitz et al., 2013) a significant inverse association with riboflavin intake was also observed (pooled OR for the highest versus the lowest categories of intake: 0.83, 95%CI 0.75–0.91, I^2=0%). The Panel considers that no quantitative value could be drawn from these two systematic reviews to support a DRV for riboflavin.

For **endometrial cancer**, after adjustments for potential confounders, in most prospective cohort or case-control studies, there was no association between riboflavin intake (from food and supplements or from food only) and OR or hazard ratio (HR) of endometrial cancer (Xu et al., 2007b; Xu et al., 2007a; Liu et al., 2013) or the RR of type-I endometrial cancer (Uccella et al., 2011). However, a significant positive (non-linear) association was found between supplemental intake of riboflavin or total intake from food and supplements (but not intake from food only) and the risk of type-II endometrial cancer (total intake: RR (95%CI) = 2.41 (1.13–5.13) for > 3.57 versus 0.23–1.61 mg/day; p trend = 0.026; supplements: RR (95%CI) = 1.94 (1.12–3.34) for > 1.70 versus 0 mg/day; p trend = 0.011) (Uccella et al., 2011).

For **breast cancer,** after adjustments for potential confounders, in three prospective cohort studies, there was no association between riboflavin intake and the risk of breast cancer (Kabat et al., 2008; Maruti et al., 2009; Bassett et al., 2013).

The Panel considers that the available studies on riboflavin intake and risk of various types of cancer are **inconsistent and cannot be used to derive DRVs for riboflavin.**

5.3.2. Riboflavin intake and other health outcomes

Two prospective cohort studies found inconsistent results on riboflavin intake and the risk of **cataracts.** There was a significant inverse non-linear association between total intake of riboflavin (food and supplements) and the odds for nuclear lens opacities (Jacques et al., 2001). However, there was no association between intake (total or from food only) and the risk of cataract extraction (Hankinson et al., 1992).

Regarding **all-cause mortality**, one prospective cohort study did not provide evidence for an association between mortality and the use of riboflavin supplements (users compared to non-users, either in smokers or non-smokers: HR (95%CI) 0.71 (0.45–1.11) and 1.60 (1.00–2.56) respectively) (Brzozowska et al., 2008). However, another prospective cohort study found a significant inverse non-linear association between riboflavin intake and the risk of mortality (RR (95%CI: 0.38 (0.16–0.90) for intake above 2.70 mg/day compared to intake below 1.92 mg/day, after adjustment for potential confounders (Fortes et al., 2000).

Pregnancy-related outcomes have been investigated in three studies (Badart-Smook et al., 1997; Smedts et al., 2008; Robitaille et al., 2009). After adjustments for potential confounders, there was a statistically significant positive linear association between riboflavin intake at 22nd week of gestation and birth length or weight (Badart-Smook et al., 1997). There was no association between riboflavin intake (assessed at 16 months after pregnancy as a proxy for usual intake in the preconception period) and the odds of congenital heart defects, after adjustments for potential confounders in particular for folate and nicotinamide intake (Smedts et al., 2008). Low riboflavin intake of women before conception was associated with the risk of transverse limb deficiencies in their infants with an adjusted OR (95% CI): 2.94 (1.04–8.32) for women not using folic acid supplements and with a riboflavin intake < 1.35 mg/day, compared with those also unsupplemented and with an intake > 2.57 mg riboflavin/day) (Robitaille et al., 2009).

Regarding **physical performance**, trials on increasing intake of riboflavin (0.15 versus 0.22 µg/kJ, in a cross-over study) (Winters et al., 1992) (Section 2.5.), riboflavin supplementation (2 mg/day versus placebo (Suboticanec et al., 1990), Section 2.4.2.) or riboflavin restriction (to about 55% of the Dutch RDA, in a double-blind complete factorial study) (van der Beek et al., 1994)) did no show any effect of these dietary changes on the parameters investigated (e.g. maximal oxygen capacity, onset of blood lactate accumulation, anaerobic threshold by gas exchange or peak power).

The Panel considers that the available studies on riboflavin intake and several health outcomes or all-cause mortality **cannot be used to derive DRV for riboflavin**.

5.3.3. Conclusions on riboflavin intake and health consequences

The Panel considers that studies on riboflavin intake and health outcomes or all-cause mortality cannot be used to set DRVs for riboflavin.

6. Data on which to base dietary reference values

The Panel considers that, since the release of the DRVs for riboflavin by SCF (1993), new data are available to update the AR and the PRI proposed by the SCF (1993).

6.1. Adults

The Panel concludes that, among the available biomarkers to estimate riboflavin requirements in adults, the inflection point in the urinary excretion curve, estimated to occur at an intake of riboflavin between 1.13 and 1.4 mg/day (Brewer et al., 1946; Horwitt et al., 1950; Boisvert et al., 1993; Guo et al., 2016) (Section 5.2.1.3.) represents the primary biomarker for assessing the riboflavin requirement. The Panel concludes that there is no indication in the available studies of different riboflavin requirement according to sex or between younger and older adults.

The Panel sets the same DRV for men and women and for older and younger adults, without correction for difference in body weight between sex and age group. The AR for riboflavin in adults is determined from the mean of the riboflavin intake, weighted for the number of subjects in each study, associated with the inflection point in the urinary excretion curve, i.e. of 1.3 (n = 66), 1.4 (n = 73), 1.13 (n = 14), 1.4 (n = 14) mg/day obtained in US men (Horwitt et al. (1950), Chinese young men (Guo et al., 2016), US young women (Brewer et al., 1946), and older Guatemalan men and women (Boisvert et al., 1993), respectively (Section 5.2.1.1.). Based on this calculation, an AR of 1.34 mg/day riboflavin is derived for men and women, rounded down to the nearest one decimal place to 1.3 mg/day.

The Panel concludes that the effect of physical activity and of MTHFR 677TT genotype on riboflavin requirement is covered by the data from the key studies considered (Section 5.2.1.3.), thus is accounted for in the assumed CV applied to set the PRI for riboflavin. Assuming a CV of 10% (in the absence of information on the variability in the requirement), the Panel sets a PRI of 1.61 mg/day for men and women, rounded down to the nearest one decimal place to 1.6 mg/day.

6.2. Infants aged 7–11 months

Considering that there is no evidence for an insufficient riboflavin intake of fully breastfed infants of healthy mothers during the first six months of life, the amount of riboflavin provided in human milk is considered to be adequate. For infant 7–11 months of age, the Panel concludes that no sufficient data are available to set an AR from the available study (Bates C. J et al., 1982) (Section 5.2.2), and set an AI by upwards extrapolation of riboflavin intake from breast milk in exclusively breastfed infants aged 0–6 months, by allometric scaling (on the assumption that riboflavin requirement is related to metabolically active body mass).

Considering a mean milk transfer of 0.8 L/day during the first six months of lactation in exclusively breastfeeding women and a concentration of riboflavin in mature breast milk of unsupplemented mothers of term infants of 364 µg/L (Section 2.3.6.3.), the Panel calculated the secretion of riboflavin into milk during lactation as 0.291 mg/day. For the calculation (Table 4), the Panel used calculated averages of the median weights of male and female infants, aged 3 months (6.1 kg) and 9 months (8.6 kg); the median weight-for-age data came from the WHO Multicentre Growth Reference Study Group (2006).

$$AI_{\text{infants 7-11 months}} = \text{riboflavin intake}_{\text{infants 0-6 months}} \times (\text{weight}_{\text{infants 9 mo}} / \text{weight}_{\text{infants 3 mo}})^{0.75}$$

Following this approach, the Panel calculates an AI for riboflavin for infants aged 7–11 months of 0.4 mg/day.

Table 4: Reference body weight and Adequate Intake (AI) of riboflavin for infants aged 7–11 months

Age	Reference body weight (kg)	AI (mg/day)
7–11 months	8.6 [(a)]	0.4

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

6.3. Children

The Panel notes that there are no sufficient data in children on which to base an AR for riboflavin (Section 5.2.2.). Therefore, the ARs were calculated by downward extrapolation from the AR of adult men and women (the unrounded value of 1.34 mg/day was used in the calculation, Section 6.1.). Allometric scaling was used on the assumption that riboflavin requirement is related to metabolically active body mass:

$$AR_{\text{children}} = AR_{\text{adults}} \times (\text{weight}_{\text{children}} / \text{weight}_{\text{adult}})^{0.75} \times (1 + \text{growth factor})$$

For the calculations (Table 5), median body weights of boys and girls (van Buuren et al., 2012) were used (for the age ranging from 4 to 17 years) as well as median body weights of 18- to 79-year-old men and women 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, 2014b). For the calculation of the PRI, as for adults (Section 6.1.), a CV of 10% was assumed. The calculated values were rounded to the nearest one decimal place.

As for adults, the Panel considered unnecessary to set sex-specific AR and PRIs for boys and girls of all ages.

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

Age	Reference body weight (kg)		Calculated ARs (mg/day)			Calculated PRIs (mg/day)			Proposed PRIs[f] (mg/day)
	Boys	Girls	Boys	Girls	Mean	Boys	Girls	Mean	Boys and girls
1–3 years	12.2 [a]	11.5 [a]	0.46	0.49	0.48	0.55	0.59	0.57	0.6
4–6 years	19.2 [b]	18.7 [b]	0.55	0.60	0.58	0.66	0.72	0.69	0.7
7–10 years	29.0 [c]	28.4 [c]	0.80	0.88	0.84	0.96	1.06	1.01	1.0
11–14 years	44.0 [d]	45.1 [d]	1.07	1.19	1.13	1.29	1.43	1.36	1.4
15–17 years	64.1 [e]	56.4 [e]	1.38	1.34	1.36	1.66	1.61	1.64	1.6

(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): Values for PRIs were calculated based on the unrounded ARs and rounded to the nearest one decimal place.

6.4. Pregnancy

The Panel concludes that data are not sufficient to estimate the additional need for dietary riboflavin during pregnancy based on fetal uptake and riboflavin accretion in the placenta during pregnancy, and that only one study shows a higher requirement in pregnant women compared to non-pregnant women (Kuizon et al., 1998) (Section 5.2.3.1).

Thus, the Panel calculates the additional riboflavin intake needed by pregnant women by allometric scaling (on the assumption that riboflavin requirement is related to metabolically active body mass). It was calculated from the AR of non-pregnant women (the unrounded value of 1.34 mg/day was used in the calculation, Section 6.1.), using the reference body weight for non-pregnant women and the mean gestational increase in body weight.

The reference body weight of 18–79-year-old women (58.5 kg) was previously calculated from the measured body heights of 19,969 women in 13 EU Member States and assuming a BMI of 22 kg/m^2

(see Appendix 11 in EFSA NDA Panel (2013)). A mean gestational increase in body weight of 12 kg, for women with a singleton pregnancy and a pre-pregnancy BMI in the range between 18.5 and 24.9 kg/m^2, was also previously considered (EFSA NDA Panel, 2013). Thus, the calculation is based on the equation below:

$$AR_{pregnant} = AR_{non\text{-}pregnant} \times (weight_{pregnant} / weight_{non\text{-}pregnant})^{0.75}$$

The calculated AR is 1.5 mg/day.

The Panel notes that the accretion in fetal tissues mostly occurs in the last months of pregnancy. In order to allow for the extra need related to the growth of maternal tissues (e.g. placenta), the Panel applies this additional requirement to the whole period of pregnancy.

As for non-pregnant adults (Section 6.1.), assuming a CV of 10%, and rounding to the nearest one decimal place, a PRI of 1.9 mg/day riboflavin is derived.

6.5. Lactation

The Panel concludes that concentration of riboflavin in breast milk rises with riboflavin intake of the mother (Sections 2.3.6.3.), and that an additional intake of riboflavin is required to balance the losses through breast milk (Section 5.2.3.2.). The Panel notes that only one study shows a higher requirement in lactating women compared to non-lactating women (Kuizon et al., 1998)

Thus, the Panel set an AR for lactating women by adding to the AR for non-lactating woman (the unrounded value of 1.34 mg/day was used in the calculation, Section 6.1.) an additional requirement to account for the losses through breast milk (Section 5.2.3.2.). This additional requirement can be calculated as the secretion of riboflavin into milk during lactation (0.291 mg/day (Section 2.3.6.3.)) corrected for absorption efficiency of 95% (Section 2.3.1.). The Panel calculated an AR of 1.65 mg/day. Considering, as for adults, a CV of 10% (Section 6.1.) and rounding to the nearest one decimal place, the Panel set a PRI of 2 mg/day for exclusively breastfeeding women during the first six months of lactation.

CONCLUSIONS

The Panel concludes that ARs and PRIs for riboflavin for adults can be derived from the weighted mean of riboflavin intake associated to the inflection point in the urinary riboflavin excretion curve reported in four intervention studies in non-EU countries. The Panel considers that the potential effect of physical activity and of MTHFR 677TT genotype on riboflavin requirement is covered by the data presented from the studies considered, thus is accounted for in the assumed CV applied to set the PRI for riboflavin. A CV of 10% was used to calculate PRIs from the ARs for adults and similarly for all other population groups. Based on the study on men in a 'mental institution' considered by SCF (1993), data on young women published before the release of the SCF report and newly available intervention studies in young and older men and women, the Panel sets an AR and a PRI for all adults, without correction for body weight of men and women. For infants aged 7–11 months, no sufficient data are available to set an AR, thus the Panel sets an AI, based on upward extrapolation by allometric scaling from the estimated intake of riboflavin of exclusively breastfed infants from birth to six months. For all children aged 1–17 years, the Panel sets ARs by downward extrapolation from the adult AR, by allometric scaling, applying growth factors and taking into account the differences in reference body weight. As for adults, the Panel considers unnecessary to set sex-specific AR and PRIs for boys and girls of all ages. For pregnant women, the Panel derives the AR by allometric scaling from the requirement for non-pregnant women, considering the mean gestational increase in body weight, to account for fetal uptake and riboflavin accretion in the placenta during pregnancy. For lactating women, the AR is increased compared to the AR for non-lactating women, to account for the secretion through breast milk, after correction for an absorption efficiency of 95% (Table 6)

1907 **Table 6:** Summary of Dietary Reference Values for riboflavin

Age	Average Requirement (mg/day)	Population Reference Intake (mg/day)[a]
7–11 months	-	0.4[b]
1–3 years	0.5	0.6
4–6 years	0.6	0.7
7–10 years	0.8	1.0
11–14 years	1.1	1.4
15–17 years	1.4	1.6
≥18years [g]	1.3	1.6
Pregnancy	1.5	1.9
Lactation	1.7	2.0

1908 (a): Values for ARs and PRIs are rounded to the closest one decimal place in this table. PRI are calculated with unrounded
1909 AR values (e.g. for adolescents and adults: 1.36 and 1.34).
1910 (b): Adequate Intake
1911

1912 ## RECOMMENDATION FOR RESEARCH

1913 The Panel suggests to undertake further research on:

1914 - biomarkers of riboflavin intake and status, and their dose–response relationship with
1915 riboflavin intake

1916 - the standardisation of EGRAC measurement, its relationship with urinary excretion of
1917 riboflavin and the cut-off value for EGRAC to assess adequacy for riboflavin

1918 - the requirement for riboflavin in some population groups e.g. infants, children, pregnant or
1919 lactating women and the potential influence of age and sex

1920 - the effect of physical activity and energy expenditure on riboflavin requirement

1921 - the quantification of the effect of genetic polymorphism on riboflavin requirement, in
1922 particular the polymorphism of MTHFR 677 C>T.

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APPENDICES

Appendix A. Concentrations of total flavin, or free or total riboflavin in breast milk of healthy mothers

Reference	Number of women (number of samples)	Country	Maternal dietary riboflavin intake (diet and/or supplements) (mg/day)	Maternal status (riboflavin concentration in urine or in plasma)	Stage of lactation	Riboflavin concentration in milk (µg/L)	Analytical method for breast milk content	Comments
Kodentsova and Vrzhesinskaya (2006)	n = 78 (preterm + normal delivery) including: normal delivery = 35	Russia	Intake recorded but not reported. Dietary intake recorded only for 25 breastfeeding women with normal delivery, including 15 not supplemented.	Urine: not reported/not measured. Plasma: spectrophotometry, by the method of titration with riboflavin-binding apoprotein. Measured but not reported.	3–10 days post-partum	Mean ± SD (range) Not supplemented 266 ± 40 (81–358) Supplemented 330 ± 41 (152–600)	Spectrophotometry, by the method of titration with riboflavin-binding apoprotein.	Volume of milk determined by weighing infants before and after breastfeeding. 24-hours milk samples (a single sample of breast milk from fasting women was taken).
Sakurai et al. (2005)	n = 691 samples Number of women not reported (only values from Group A reported (n = 114). Lower number of samples analysed than planned due to problems in the analytical procedure (e.g. insufficient sample volume)	Japan	Intake not reported – Unsupplemented women.	Status not reported	Various stages of lactation: 1–365 days 6–10 days 11–20 days 21–89 days 90–180 days 181–365 days	Total riboflavin Mean ± SD 377 ± 156 340 ± 97 380 ± 126 397 ± 126 385 ± 133	HPLC (absorbance was monitored at 530 nm using a fluorescence detector).	No explicit information on whether the infants were born at term or preterm. However, mean birth weight in group A was 3142 ± 425 g). Concentrations reported in this table for breast milk are the sum of riboflavin, FMN and FAD. The concentrations of each individual

Reference	Number of women (number of samples)	Country	Maternal dietary riboflavin intake (diet and/or supplements) (mg/day)	Maternal status (riboflavin concentration in urine or in plasma)	Stage of lactation	Riboflavin concentration in milk (µg/L)	Analytical method for breast milk content	Comments
								compound are also reported in the reference. Concentration of FAD is higher than FMN and riboflavin.
Ortega et al. (1999)[(a)]	n = 57	Spain	Five-day food record + food frequency intake questionnaire. Measured during their third trimester of gestation (between weeks 32 and 36). The authors said that maternal intake after giving birth did not change drastically (intake measured but not reported). Mean ± SD	Urine: not measured/not reported. Plasma: EGRAC Mean ± SD			Fluorometry	Prospective cohort study. Milk samples taken in the morning by manual expression of a 5 mL sample from each breast at the beginning and end of feeds. No explicit information on whether the infants were born at term or preterm. However, mean length of pregnancy: 39 wee ks, average weight of newborns: 3.3 kg.
	non-supplemented group = 25 (Group L: riboflavin intake < RI)[(a)]		1.37 ± 0.11	1.21 ± 0.35	Transitional milk: 13–14 days post-partum Mature milk: 40 days post-	216.39 ± 94.78 273.04 ± 95.72		

Reference	Number of women (number of samples)	Country	Maternal dietary riboflavin intake (diet and/or supplements) (mg/day)	Maternal status (riboflavin concentration in urine or in plasma)	Stage of lactation	Riboflavin concentration in milk (µg/L)	Analytical method for breast milk content	Comments
					partum.			
	supplemented group = 32 (Group H: riboflavin intake > RI) [(a)]		Total intake (supplements + diet): 2.52 ± 1.00 From supplements: 0.13 ± 0.50	1.06 ± 0.16[(b)]	Transitional milk: 13–14 days post-partum Mature milk: 40 days post-partum.	356.86 ±263.51 374.06 ± 164.34		
Roughead and McCormick (1990)	n = 5	USA	24 hours dietary record for the day previous to milk collection on four out of the five subjects. Total flavin (range): 1.13–2.91 No information on supplementation.	Status not reported	Not reported	Total flavin (mean ± SD of all measurements per subject): Min.: 180 ± 3 (containing 35.6 % riboflavin, 60.5% FAD) Max.: 799 ± 25 (containing 30.7 % riboflavin, 61.6% FAD)	Fluorescence spectrophotometry. n ≥ 4 measurements per subject. Analytical HPLC was used to analyse types and quantities of all flavins (riboflavin, FAD and other compounds).	No information on whether the infants were born at term or preterm, or on the stage of lactation. Total flavin concentrations reported in milk, with percentages of FAD, riboflavin, 10-hydroxyethylflavin, 10-formylmethylflavin, 7α-hydroxyriboflavin, 8α -hydroriboflavin
Dostálová et al. (1988)	n = 26 (number of samples)	Switzerland	Intake not reported. Not supplemented.	Urine: not reported EGRAC: measured but not reported.	Colostrum	Total riboflavin Mean ± SD (range)	Fluorometry	Mothers of infants born at term (37th-43rd week of gestation).

Reference	Number of women (number of samples)	Country	Maternal dietary riboflavin intake (diet and/or supplements) (mg/day)	Maternal status (riboflavin concentration in urine or in plasma)	Stage of lactation	Riboflavin concentration in milk (µg/L)	Analytical method for breast milk content	Comments
	(12)				*3–5 days post partum	*307 ± 150 (91-629)		Colostrum samples represent a 12-hr pool of milk portions before (foremilk) and after (hindmilk) each feed.
					Transitional milk			
	(4)				*6–10 days post partum	*240 ± 110 (82-333)		
					Mature milk			
	(4)				*Two weeks	*471 ± 121 (323-605)		
	(18)				*Four months	*485 ± 149 (foremilk: 291-492, hindmilk: 539-681)		Transitional and mature milk. obtained as a pool of fore- and hindmilk.
Dostálová et al. (1988)	Number not reported	Finland	Supplemented with a multivitamin supplement containing 2 mg riboflavin	Urine: not measured/reported Plasma: EGRAC measured but not reported.		Total riboflavin Mean ± SD (range)	Fluorometry	Mothers of infants born at term.
					Colostrum			Each of the mothers donated a complete milk sample on the 3rd and 4th day after delivery, and at 2, 4, 6 and 7.5 months of lactation. Colostrum and mature milk samples were collected during a 24-h period pooling fore-and hindmilk of each feed.
	(55)				*3 or 4 days post partum	*422 ± 85 (208–633)		
					Mature milk			
	(55)				*Eight weeks	*584 ± 144 (340–984)		
	(55)				*Four months	*573 ± 139 (274–984)		
	(55)				*Six months	*563 ± 176 (104–889)		
	(55)				*7.5 months	*601 ± 205 (142–1 111)		
Ford et al. (1983)[(c)]	n = 35	UK	Intake not reported. No information on supplementation.	Not reported		Mean (range)	Standard microbiological methods	Mothers of infants born at term (at the 39th week or later).

Reference	Number of women (number of samples)	Country	Maternal dietary riboflavin intake (diet and/or supplements) (mg/day)	Maternal status (riboflavin concentration in urine or in plasma)	Stage of lactation	Riboflavin concentration in milk (µg/L)	Analytical method for breast milk content	Comments
	6 (17) 10 (22) 24 (24)				Colostrum 1–5 days post partum. Transitional milk 6–15 days post partum Mature milk 16–244 days post partum	288 (120–500) 279 (130–733) 310 (200–440)		Composites of samples expressed manually over the day during the baby's feeding times. Mature milk samples were composite samples made up of milk taken in mid feed at four different times spread over the day on four successive days.
Thomas et al. (1980)	n = 12	USA	4-days diet record.	Plasma: not reported. Urine (mg/day, mean ± SD)	Six months post partum	Mean ± SD	Spectrophotometric determination	No information on whether the infants were born at term or preterm. Fore milk samples (25 mL) were collected four times per day at four-hours intervals (0, 4, 8, 12 hours in the non-supplemented group, and at 4, 8, 12 hours in the supplemented

Reference	Number of women (number of samples)	Country	Maternal dietary riboflavin intake (diet and/or supplements) (mg/day)	Maternal status (riboflavin concentration in urine or in plasma)	Stage of lactation	Riboflavin concentration in milk (µg/L)	Analytical method for breast milk content	Comments
	non-supplemented group = 6		1.87 ± 0.95 (mean ± SD)	0.442 ± 0.231 Spectro-photometric determination 24-hours urinary collection on the 3rd day of milk collection.		243 ± 35		group) for three days.
	supplemented group = 6		Food: 3.31 ± 1.25 (mean ± SD) Supplements: 2.0	0.450 ± 0.248		274 ± 46		

Reference	Number of women (number of samples)	Country	Maternal dietary riboflavin intake (diet and/or supplements) (mg/day)	Maternal status (riboflavin concentration in urine or in plasma)	Stage of lactation	Riboflavin concentration in milk (µg/L)	Analytical method for breast milk content	Comments
Nail et al. (1980)	n = 12	USA	Intake (mg/day): 24-h dietary recall before each milk collection period. 3-day diet record kept by the subjects during 3-day intervals of milk collection. Mean ± SD	Plasma: not reported. Urine excretion urinary collection at day 7 and 45. Modification of the Standards methods of the Infant formula Council and spectrophotometric determination mg/day (mean ± SD)		Mean ± SD	Modification of the Standards methods of the Infant formula Council and spectrophotometric determination	No information on whether the infants were born at term or preterm. Both supplemented and unsupplemented mothers had consumed the supplement during pregnancy. Milk samples (25 mL) were collected four times per day in the morning upon arising and at four-hours intervals (0, 4, 8, 12 hours after) for three days.
	non-supplemented group = 5		2.57 ± 1.34	0.73 ± 0.32	Colostrum 5–7 days post partum	367 ± 128		
			2.63 ± 0.91	0.74 ± 0.49	Mature milk 43–45 days post partum	485 ± 123		
	supplemented group = 7		4.44 ± 0.59 (total) (diet: 2.44 ± 0.59, supplements: 2.0)	2.16 ± 1.78	Colostrum 5–7 days post partum	880 ± 168		Milk was expressed after ingestion of the vitamin supplement.
			4.95 ± 1.28 (total) (diet: 2.90 ± 1.28, supplements: 2.0)	2.76 ± 1.13	Mature milk 43–45 days post partum.	710 ± 187		

(a) "Recommended intake (RI) for the Spanish population, for women in the second half of pregnancy: 0.6 mg/1000 kcal + 0.2 mg, with a minimum provision of 1.6 mg/day". Given that no subject showed high energy intakes, a RI was established as 1.6 mg/day (Instituto de Nutrición (CSIC), 1994)
(b): The difference between EGRAC of group H and L was not statistically significant.
(c): cited in Dostálová et al. (1988).

For the concentration of riboflavin in breast milk, the molecular mass (MM) of 376.4 g/mol was used to convert the values reported in nmol/L to µg/L.
Abbreviations: EGRAC: erythrocyte glutathione reductase activation coefficient, FAD: flavin adenine dinucleotide, FMN: flavin mononucleotide, HPLC: high-performance liquid chromatography, RI: Recommended Intake, SD: standard deviation

Appendix B. Dietary surveys in the EFSA Comprehensive European Food Consumption Database included in EFSA's nutrient intake calculation for riboflavin

Country	Dietary survey (Year)	Year	Method	Days	Age (years)	Number of subjects						
						Infants[a] < 1 year	Children 1–< 3 years	Children 3–< 10 years	Children 10–< 18 years	Adults 18–< 65 years	Adults 65–< 75 years	Adults ≥ 75 years
Finland/1	NWSSP	2007-2008	48-hour dietary recall [b]	2x2[b]	13–15				306			
Finland/2	FINDIET2012	2012	48-hour 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-hour dietary recall	2	15–45				12[d]	991[c]		
Netherlands	DNFCS 2007-2010	2007-2010	24-hour dietary recall	2	7–69			447	1 142	2,057	173	
Sweden	RIKSMATEN	2010-2011	Dietary records (Web)[e]	4	18–80					1,430	295	72
United Kingdom/1	DNSIYC-2011	2011	Dietary record	4	0.3–1.5	1,369	1,314					
United Kingdom/2	NDNS Rolling Programme (Years 1–3)	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-hour dietary recall comprising two consecutive days.

(c): Four subjects from the VELS study (one aged between 1 and < 3 years and 3 aged between 3 to < 10 years) and one subject from Latvian study (one adult) were not considered in the assessment due to the fact that only one 24-hour dietary recall day was available

(d): 5^{th} or 95^{th} 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 5^{th} and 95^{th} percentile estimates are not presented in the intake results.

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

2659 **Appendix C. Riboflavin 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[(c)]	Average	Median	P5	P95	n	Average	Median	P5	P95
< 1 year[(a)]	Germany	VELS	84	0.7	0.8	0.3	1.2	84	0.2	0.2	0.1	0.4
	Finland	DIPP_2001_2009	247	0.7	0.7	0.0	1.5	245	0.3	0.3	0.1	0.5
	United Kingdom	DNSIYC_2011	699	1.2	1.3	0.5	1.8	699	0.4	0.4	0.2	0.5
	Italy	INRAN_SCAI_2005_06	9	0.6	0.5	- [(b)]	- [(b)]	9	0.2	0.2	- [(b)]	- [(b)]
1 to < 3 years	Germany	VELS	174	1.0	0.9	0.4	1.5	174	0.2	0.2	0.1	0.3
	Finland	DIPP_2001_2009	245	1.3	1.3	0.5	2.1	245	0.4	0.4	0.2	0.5
	United Kingdom	NDNS Rolling Programme Years 1–3	107	1.4	1.4	0.6	2.4	107	0.3	0.3	0.2	0.4
	United Kingdom	DNSIYC_2011	663	1.4	1.4	0.7	2.1	663	0.3	0.3	0.2	0.5
	Italy	INRAN_SCAI_2005_06	20	1.2	1.1	0.7	2.1	20	0.2	0.2	0.2	0.4
3 to < 10 years	Germany	EsKiMo	426	1.4	1.3	0.8	2.4	426	0.2	0.2	0.1	0.3
	Germany	VELS	146	1.1	1.0	0.6	1.7	146	0.2	0.2	0.1	0.3
	Finland	DIPP_2001_2009	381	1.8	1.8	0.9	2.6	381	0.3	0.3	0.2	0.4
	France	INCA2	239	1.6	1.5	0.9	2.5	239	0.3	0.3	0.2	0.4
	United Kingdom	NDNS Rolling Programme Years 1–3	326	1.4	1.3	0.7	2.3	326	0.2	0.2	0.1	0.4
	Italy	INRAN_SCAI_2005_06	94	1.5	1.4	0.9	2.3	94	0.2	0.2	0.1	0.3
	Netherlands	DNFCS 2007-2010	231	1.3	1.3	0.6	2.3	231	0.2	0.2	0.1	0.2
10 to < 18 years	Germany	EsKiMo	197	1.5	1.4	0.8	2.3	197	0.2	0.2	0.1	0.3
	Finland	NWSSP07_08	136	2.2	2.2	1.1	3.7	136	0.3	0.3	0.2	0.4
	France	INCA2	449	1.7	1.7	0.9	2.8	449	0.2	0.2	0.1	0.3
	United Kingdom	NDNS Rolling Programme Years 1–3	340	1.5	1.4	0.7	2.7	340	0.2	0.2	0.1	0.3
	Italy	INRAN_SCAI_2005_06	108	1.7	1.7	1.0	2.6	108	0.2	0.2	0.1	0.2
	Netherlands	DNFCS 2007-2010	566	1.7	1.6	0.8	3.2	566	0.2	0.2	0.1	0.3
18 to < 65 years	Finland	FINDIET2012	585	2.0	1.9	0.9	3.8	585	0.2	0.2	0.1	0.3
	France	INCA2	936	1.9	1.8	0.9	2.9	936	0.2	0.2	0.1	0.3
	United Kingdom	NDNS Rolling Programme Years 1–3	560	1.8	1.7	0.8	3.2	560	0.2	0.2	0.1	0.3
	Ireland	NANS_2012	634	2.2	2.1	1.0	3.9	634	0.2	0.2	0.1	0.4

Age class	Country	Survey	Intakes expressed in mg/day					Intakes expressed in mg/MJ				
			n[(c)]	Average	Median	P5	P95	n	Average	Median	P5	P95
	Italy	INRAN_SCAI_2005_06	1,068	1.7	1.7	1.0	2.6	1068	0.2	0.2	0.1	0.3
	Netherlands	DNFCS 2007-2010	1,023	1.9	1.8	0.9	3.3	1023	0.2	0.2	0.1	0.3
	Sweden	Riksmaten 2010	623	1.8	1.7	0.9	2.9	623	0.2	0.2	0.1	0.3
65 to < 75 years	Finland	FINDIET2012	210	1.7	1.6	0.7	2.9	210	0.2	0.2	0.1	0.3
	France	INCA2	111	1.9	1.8	0.9	2.9	111	0.2	0.2	0.2	0.3
	United Kingdom	NDNS Rolling Programme Years 1–3	75	1.9	1.9	0.8	3.0	75	0.2	0.2	0.1	0.3
	Ireland	NANS_2012	72	1.9	1.8	0.9	2.9	72	0.2	0.2	0.1	0.3
	Italy	INRAN_SCAI_2005_06	133	1.7	1.7	1.0	2.5	133	0.2	0.2	0.1	0.3
	Netherlands	DNFCS 2007-2010	91	1.6	1.6	0.8	2.4	91	0.2	0.2	0.1	0.3
	Sweden	Riksmaten 2010	127	1.6	1.6	0.8	2.4	127	0.2	0.2	0.1	0.3
≥ 75 years	France	INCA2	40	1.6	1.6	- [(b)]	- [(b)]	40	0.2	0.2	- [(b)]	- [(b)]
	United Kingdom	NDNS Rolling Programme Years 1–3	56	1.8	1.6	- [(b)]	- [(b)]	56	0.3	0.2	- [(b)]	- [(b)]
	Ireland	NANS_2012	34	1.7	1.6	- [(b)]	- [(b)]	34	0.2	0.2	- [(b)]	- [(b)]
	Italy	INRAN_SCAI_2005_06	69	1.7	1.6	1.0	2.5	69	0.2	0.2	0.13	0.3
	Sweden	Riksmaten 2010	42	1.6	1.6	- [(b)]	- [(b)]	42	0.2	0.2	- [(b)]	- [(b)]

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 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. The consumption of breast milk was taken into account if the consumption was reported as human milk (Italian survey) or if the number of breast milk consumption events was reported (German and UK surveys). For the German 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 age groups: the amount of breast milk consumed on one eating occasion was set to 135 g/eating occasion for infants between 6-7 months of age and to 100 g/eating occasion for infants between 8–12 months of age (Kersting and Clausen, 2003). 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.

(b): 5^{th} or 95^{th} 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 5^{th} and 95^{th} percentile estimates are not presented in the intake results.

(c): n, number of subjects.

2677 **Appendix D. Riboflavin intakes in females in different surveys, estimated by EFSA according to age class and country**

Age class	Country	Survey	Intakes expressed in mg per day					Intakes expressed in mg per MJ				
			n[(c)]	Average	Median	P5	P95	n	Average	Median	P5	P95
< 1 year[(a)]	Germany	VELS	75	0.7	0.6	0.3	1.0	75	0.2	0.2	0.1	0.4
	Finland	DIPP_2001_2009	253	0.7	0.7	0.0	1.5	251	0.3	0.4	0.1	0.5
	United Kingdom	DNSIYC_2011	670	1.1	1.1	0.4	1.6	670	0.4	0.4	0.1	0.5
	Italy	INRAN_SCAI_2005_06	7	0.7	0.9	- [(b)]	- [(b)]	7	0.2	0.3	- [(b)]	- [(b)]
1 to < 3 years	Germany	VELS	174	0.9	0.9	0.5	1.4	174	0.2	0.2	0.1	0.3
	Finland	DIPP_2001_2009	255	1.3	1.3	0.4	2.1	255	0.4	0.4	0.2	0.6
	United Kingdom	NDNS Rolling Programme Years 1–3	78	1.2	1.2	0.5	1.9	78	0.3	0.3	0.2	0.4
	United Kingdom	DNSIYC_2011	651	1.3	1.3	0.6	2.0	651	0.3	0.3	0.2	0.5
	Italy	INRAN_SCAI_2005_06	16	1.1	1.0	- [(b)]	- [(b)]	16	0.2	0.2	- [(b)]	- [(b)]
3 to < 10 years	Germany	EsKiMo	409	1.2	1.2	0.7	2.0	409	0.2	0.2	0.1	0.3
	Germany	VELS	147	1.0	0.9	0.5	1.5	147	0.2	0.2	0.1	0.3
	Finland	DIPP_2001_2009	369	1.6	1.6	0.9	2.4	369	0.3	0.3	0.2	0.4
	France	INCA2	243	1.4	1.4	0.8	2.1	243	0.3	0.2	0.2	0.4
	United Kingdom	NDNS Rolling Programme Years 1–3	325	1.3	1.2	0.6	2.0	325	0.2	0.2	0.1	0.3
	Italy	INRAN_SCAI_2005_06	99	1.4	1.4	0.8	2.1	99	0.2	0.2	0.1	0.3
	Netherlands	DNFCS 2007-2010	216	1.3	1.3	0.6	2.4	216	0.2	0.2	0.1	0.3
10 to < 18 years	Germany	EsKiMo	196	1.3	1.3	0.7	2.2	196	0.2	0.2	0.1	0.3
	Finland	NWSSP07_08	170	1.7	1.7	0.8	2.9	170	0.3	0.3	0.1	0.4
	France	INCA2	524	1.4	1.4	0.7	2.3	524	0.2	0.2	0.1	0.3
	United Kingdom	NDNS Rolling Programme Years 1–3	326	1.2	1.1	0.6	2.2	326	0.2	0.2	0.1	0.3
	Italy	INRAN_SCAI_2005_06	139	1.4	1.4	0.9	2.1	139	0.2	0.2	0.1	0.3
	Latvia	FC_PREGNANTWOMEN_2011 [(c)]	12	1.9	1.5	- [(b)]	- [(b)]	12	0.2	0.2	- [(b)]	- [(b)]
	Netherlands	DNFCS 2007-2010	576	1.4	1.3	0.7	2.5	576	0.2	0.2	0.1	0.3
18 to < 65 years	Finland	FINDIET2012	710	1.6	1.5	0.8	2.8	710	0.2	0.2	0.1	0.4
	France	INCA2	1,340	1.5	1.5	0.8	2.4	1,340	0.2	0.2	0.1	0.4
	United Kingdom	NDNS Rolling Programme Years 1–3	706	1.4	1.3	0.7	2.3	706	0.2	0.2	0.1	0.4

Age class	Country	Survey	Intakes expressed in mg per day					Intakes expressed in mg per MJ				
			n[(c)]	Average	Median	P5	P95	n	Average	Median	P5	P95
	Ireland	NANS_2012	640	1.6	1.5	0.8	2.6	640	0.2	0.2	0.1	0.3
	Italy	INRAN_SCAI_2005_06	1,245	1.5	1.5	0.9	2.3	1,245	0.2	0.2	0.1	0.3
	Latvia	FC_PREGNANTWOMEN_2011 [(d)]	990	1.7	1.6	0.9	2.7	990	0.2	0.2	0.1	0.3
	Netherlands	DNFCS 2007-2010	1,034	1.5	1.4	0.7	2.6	1,034	0.2	0.2	0.1	0.3
	Sweden	Riksmaten 2010	807	1.4	1.4	0.8	2.3	807	0.2	0.2	0.1	0.3
65 to < 75 years	Finland	FINDIET2012	203	1.4	1.3	0.6	2.3	203	0.2	0.2	0.1	0.4
	France	INCA2	153	1.5	1.4	0.7	2.2	153	0.2	0.2	0.1	0.4
	United Kingdom	NDNS Rolling Programme Years 1–3	91	1.5	1.5	0.8	2.6	91	0.3	0.2	0.1	0.4
	Ireland	NANS_2012	77	1.6	1.6	0.8	3.1	77	0.2	0.2	0.1	0.4
	Italy	INRAN_SCAI_2005_06	157	1.5	1.5	0.8	2.3	157	0.2	0.2	0.1	0.3
	Netherlands	DNFCS 2007-2010	82	1.4	1.4	0.8	2.2	82	0.2	0.2	0.1	0.3
	Sweden	Riksmaten 2010	168	1.4	1.3	0.8	2.3	168	0.2	0.2	0.1	0.3
≥ 75 years	France	INCA2	44	1.5	1.4	- [(b)]	- [(b)]	44	0.3	0.2	- [(b)]	- [(b)]
	United Kingdom	NDNS Rolling Programme Years 1–3	83	1.6	1.5	0.9	2.7	83	0.3	0.3	0.2	0.4
	Ireland	NANS_2012	43	1.6	1.5	- [(b)]	- [(b)]	43	0.3	0.3	- [(b)]	- [(b)]
	Italy	INRAN_SCAI_2005_06	159	1.4	1.4	0.8	2.0	159	0.2	0.2	0.1	0.3
	Sweden	Riksmaten 2010	30	1.5	1.4	- [(b)]	- [(b)]	30	0.2	0.2	- [(b)]	- [(b)]

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 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. The consumption of breast milk was taken into account if the consumption was reported as human milk (Italian survey) or if the number of breast milk consumption events was reported (German and UK surveys). For the German 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 age groups: the amount of breast milk consumed on one eating occasion was set to 135 g/eating occasion for infants between 6–7 months of age and to 100 g/eating occasion for infants between 8–12 months of age (Kersting and Clausen, 2003). 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.

(b): 5^{th} or 95^{th} 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 5^{th} and 95^{th} percentile estimates are not presented in the intake results.

(c): n, number of subjects. (d): Pregnant women only.

Appendix E. Minimum and maximum percentage contribution of different food groups (FoodEx2 level 1) to riboflavin 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	0–2	0–3	0–1	0	0
Alcoholic beverages	< 1	< 1	< 1	< 1–1	2–8	1–5	2–4
Animal and vegetable fats and oils	0	< 1	< 1	< 1	< 1	< 1–1	< 1
Coffee, cocoa, tea and infusions	< 1	< 1–1	< 1–2	< 1–2	2–13	1–13	4–14
Composite dishes	< 1–2	< 1–5	< 1–7	< 1–11	< 1–12	1–11	< 1–12
Eggs and egg products	< 1–1	1–2	1–5	1–5	1–4	2–4	2–4
Fish, seafood, amphibians, reptiles and invertebrates	< 1	< 1–2	< 1–3	< 1–3	1–3	2–5	2–6
Food products for young population	46–68	4–24	< 1–2	< 1	< 1	–	–
Fruit and fruit products	1–4	2–4	2–3	1–3	1–4	2–5	2–5
Fruit and vegetable juices and nectars	< 1–1	< 1–4	1–5	1–5	1–3	< 1–1	< 1–1
Grains and grain-based products	1–5	3–14	4–22	5–22	10–19	9–20	11–19
Human milk	< 1 – 25 [(a)]	< 1–1	–	–	–	–	–
Legumes, nuts, oilseeds and spices	< 1	< 1–1	< 1–1	< 1–1	1	1	1
Meat and meat products	< 1–4	3–8	7–16	9–19	11–20	13–21	12–18
Milk and dairy products	16–27	53–63	38–69	32–63	24–48	22–47	29–34
Products for non-standard diets, food imitates and food supplements or fortifying agents	< 1	0–1	< 1–1	< 1–1	< 1–1	< 1	< 1–2
Seasoning, sauces and condiments	< 1–1	< 1–3	< 1–2	< 1–3	< 1–3	< 1–2	< 1–2
Starchy roots or tubers and products thereof, sugar plants	< 1–1	< 1–1	1–3	1–4	1–3	1–3	1–2
Sugar, confectionery and water-based sweet desserts	0	< 1–2	1–5	1–6	< 1–2	< 1–1	< 1–1
Vegetables and vegetable products	1–4	1–4	1–7	2–9	2–12	3–13	2–11
Water and water-based beverages	0	0	< 1–2	< 1–10	< 1–6	< 1–1	< 1–1

'-' 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.

(a): The 25% refers to the Italian INRAN_SCAI_2005_06 study with only n = 9.

Appendix F. Minimum and maximum percentage contribution of different food groups (FoodEx2 level 1) to riboflavin 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	0–2	0–3	0	< 1	0
Alcoholic beverages	< 1	< 1	< 1	< 1	< 1–2	< 1–2	< 1–1
Animal and vegetable fats and oils	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Coffee, cocoa, tea and infusions	< 1–3	< 1–6	< 1–1	1–3	2–14	2–13	6–14
Composite dishes	< 1–1	< 1–5	< 1–7	1–11	1–13	< 1–11	1–11
Eggs and egg products	< 1–1	1–3	1–5	1–5	2–4	2–4	2–4
Fish, seafood, amphibians, reptiles and invertebrates	< 1–1	< 1–2	< 1–2	< 1–4	1–3	1–5	2–4
Food products for young population	32–71	5–20	< 1–1	< 1	< 1	–	< 1
Fruit and fruit products	2–4	2–4	1–4	1–5	2–5	3–7	2–6
Fruit and vegetable juices and nectars	< 1–1	< 1–4	1–4	1–4	< 1–3	1–2	1–2
Grains and grain-based products	2–6	3–15	4–21	6–25	10–29	11–19	9–20
Human milk	< 1–10 [(a)]	< 1–1	–	–	–	–	–
Legumes, nuts, oilseeds and spices	< 1–1	< 1–1	1	1	1–2	1–2	1
Meat and meat products	1–3	3–7	7–16	8–17	10–18	9–18	10–16
Milk and dairy products	10–38	49–61	37–70	32–62	28–51	27–49	31–39
Products for non-standard diets, food imitates and food supplements or fortifying agents	< 1	< 1	0–2	< 1–1	< 1–3	< 1–2	< 1–1
Seasoning, sauces and condiments	< 1–1	< 1–2	< 1–3	< 1–4	< 1–3	< 1–1	< 1–1
Starchy roots or tubers and products thereof, sugar plants	< 1–1	1	1–3	1–4	1–3	1–2	1–2
Sugar, confectionery and water-based sweet desserts	0–1	< 1–2	1–5	< 1–6	< 1–2	< 1–1	< 1–2
Vegetables and vegetable products	1–4	1–4	2–7	2–10	3–13	3–14	3–13
Water and water-based beverages	0	0	< 1–1	0–8	< 1–4	< 1–1	< 1

'-' 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.

(a): The 10 % refers to the Italian INRAN_SCAI_2005_06 study with only n = 7.

ABBREVIATIONS

Afssa	Agence Française de Sécurité Sanitaire des Aliments
AI	adequate intake
AR	average requirement
ATP	adenosine triphosphate
BMD	bone mineral density
BMI	body mass index
CI	confidence interval
CoA	coenzyme A
COMA	Committee on Medical Aspects of Food Policy
CV	coefficient of variation
D-A-CH	Deutschland- Austria- Confoederatio Helvetica
DH	UK Department of Health
DIPP	Type 1 Diabetes Prediction and Prevention survey
DNFCS	Dutch National Food Consumption Survey
EsKiMo	Ernährungsstudie als KIGGS-Modul
DRV	dietary reference value
EAR	estimated average requirement
EGR	erythrocyte glutathione reductase
EGRAC	erythrocyte glutathione reductase activation coefficient
FAD	flavin adenine dinucleotide
FAO	Food and Agriculture Organization
FC_PREGNANTWOMEN	Food consumption of pregnant women in Latvia
FFQ	food frequency questionnaire
FLAD	flavin adenine dinucleotide synthetase
FMN	Flavin mononucleotide
FNB	US Food and Nutrition Board
G6PD	glucose-6-phosphate dehydrogenase

GSH	glutathione
GSSG	glutathione disulfide
G6PD	glucose-6-phosphate dehydrogenase
HR	hazard ratio
HPLC	high performance liquid chromatography
I^2	heterogeneity index
Ig	immunoglobulin
INCA	Étude 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	International Union of Pure and Applied Chemistry
LRNI	lower reference nutrient intake
LTI	lower threshold intake
MADD	multiple acyl-CoA dehydrogenase deficiency
MTHFR	methylenetetrahydrofolate reductase
NANS	National Adult Nutrition Survey
NDNS	National Diet and Nutrition Survey
NHANES	National Health And Nutrition Examination Survey
NNR	Nordic Nutrition Recommendations
OR	odds ratio
PPO	pyridoxamine phosphate oxidase
PPOAC	pyridoxamine phosphate oxidase activation coefficient
PRI	population reference intake
RCT	randomised controlled trial
RDA	recommended dietary allowances
RFT	riboflavin transporters
RI	recommended intake

RNI	recommended nutrient intake
RR	relative risk
SCF	Scientific Committee for Food
SD	standard deviation
SLC	solute carrier
TEE	total energy expenditure
UK	United Kingdom
UL	tolerable upper intake level
UNU	United Nations University
USA	United States of America
USDA	United States Department of Agriculture
VELS	Verzehrsstudie zur Ermittlung der Lebensmittelaufnahme von Säuglingen und Kleinkindern für die Abschätzung eines akuten Toxizitätsrisikos durch Rückstände von Pflanzenschutzmitteln
VERA	Verbundstudie Ernährungserhebung und Risikofaktoren Analytik
WHO	World Health Organization

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