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Use of Vitamins in Foods

Toxicological and nutritional-physiological aspects

Part I

Imprint

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1 Preface

BfR (previously BgVV) has been involved since 2000 in the risk assessment of vitamins and minerals, including trace elements, in foods. External experts were also invited to participate in the drawing up of this two-volume report now available in the series "BfR-Wissenschaft", The aim was to obtain as solid a consensus as possible on scientific risk assessment. Technical experts from the German Nutrition Society (DGE), the Senate Commission on Food Safety (SKLM), the German Research Foundation (DFG), the Federal Institute for Medicinal Products and Medical Devices (BfArM), the Federal Research Centre for Nutrition and Food (BFEL), the Robert Koch Institute (RKI), the German Institute of Human Nutrition (DIfE) and the Research Institute of Child Nutrition (FKE) in Dortmund as well as individual experts took part. Part I of the report examines the "Use of Vitamins in Foods – Toxicological and Nutritional-Physiological Aspects". Part II is also published in the series "BfR-Wissenschaft" as edition 04/2004 and is entitled "Use of Minerals in Foods – Toxicological and Nutritional-Physiological Aspects".

The report is intended as a basis for discussion and a decision-making for risk managers in Germany and the European Union when setting maximum levels for nutrients in food supplements and fortified foods. The setting of maximum levels was announced in Articles 5 and 13 of "Directive 2002/46/EC of the European Parliament and the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements". The proposal for a "Regulation on the addition of vitamins and minerals and of certain other substances to foods" also envisages the setting of maximum levels for vitamins and minerals by the European Commission with the support of the Standing Committee on the Food Chain and Animal Health.

When setting maximum levels of this kind, consideration must be given both to the likely safe daily intakes of a vitamin and mineral (so-called tolerable upper intake levels) and to the uptake of these nutrients from common foods by the population. At the same time, the recommended daily intakes for a nutrient should be taken into account when setting a maximum level in a food. Whereas tolerable upper intake levels have been derived or defined by various scientific bodies, this report endeavours to identify ways of calculating maximum levels of vitamins and minerals in a single food from tolerable upper intake levels, daily intake and recommended daily intake.

Such "combined" maximum levels should allow supplementation levels sufficient for correcting nutrient deficits in population groups without exceeding the tolerable daily intake levels (to a major degree). Depending on the desired level of protection, various options for setting maximum levels are outlined in this two-volume report and the respective advantages and disadvantages are discussed. This does not constitute in any way a pre-emption of a political decision in favour of one of the identified options. This decision is to be taken on the Community level in Europe.

Professor Dr. Dr. Andreas Hensel
President of BfR

2 Glossary and Abbreviations

ACE beverages	Beverages fortified with provitamin A and the vitamins C and E
ADI	Acceptable Daily Intake
AFSSA	French Agency for Food Safety
ATBC Study	Alpha-Tocopherol, β-Carotene Cancer Prevention Trial
BfArM	Federal Institute for Medicinal Products and Medical Devices
BfR	Federal Institute for Risk Assessment
BGA	Federal Health Office
BgVV	Federal Institute for Health Protection of Consumers and Veterinary Medicine
CARET Study	β-Carotene Cancer and Retinol Efficiency Trial
CAS Number	Chemical Abstracts Service. System which allocates a number to a chemical substance (= CAS Number)
D-A-CH	Deutsche, Österreichische und Schweizerische Gesellschaft für Ernährung: Deutsche Gesellschaft für Ernährung e.V. (DGE), Österreichische Gesellschaft für Ernährung (ÖGE), Schweizerische Gesellschaft für Ernährungsforschung (SGE) and Schweizerische Vereinigung für Ernährung (SVE) (German, Austrian and Swiss nutrition societies)
DGE	Deutsche Gesellschaft für Ernährung e.V. (German Nutrition Society)
DiätVO - Verordnung über diätetische Lebensmittel	Ordinance on Foods for Special Dietary Purposes
DINF	Dietary Intake by Normal Food (upper percentile)
DONALD Study	Dortmund Nutritional and Anthropometric Longitudinally Designed Study
EAR	Estimated average requirement
EFSA	European Food Safety Authority
EPIC Study	European Prospective Investigation into Cancer and Nutrition-Study
Estimated values	Values for nutrients for which human requirements cannot yet be determined with the desired accuracy. The estimated values do, however, provide good pointers for adequate and safe intake (D-A-CH, 2000). ➢ **Reference values**
EU	European Union
EVM	Expert Group on Vitamins and Minerals (UK)
FDA	Food and Drug Administration (USA)
FF	Fortified foods
FNB	Food and Nutrition Board of the Institute of Medicine (IOM)
FS	Food supplements
FSA	Food Standards Agency (UK)
Guidance Level	Levels that would not be expected to be associated with adverse effects but acknowledging that such levels may not be applicable to all life stages or for life-long intake and where it was not possible to establish Safe Upper Levels. Guidance levels should not be used as Safe Upper Levels (Food Standards Agency. Safe Upper Levels for Vitamins and Minerals. Report of the Expert Group on Vitamins and Minerals. London, 2003)
Guidance values	Orientation aid for nutrient levels if there is a need, on health grounds, for intake to be controlled within specific ranges but not within strict limit values (D-A-CH, 2000) ➢ **Reference values**
IOM	Institute of Medicine of the National Academy of Science (NAS)
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LMBG	Food and Other Commodities Act
LOAEL	Lowest observed adverse effect level; lowest intake (or experimental dose) of a nutrient at which an adverse effect has been identified (FNB: Dietary Reference Intakes. Applications in Dietary Assessment. Food and Nutrition Board, Institute of Medicine. National Academic Press, Washington D.C., 2000)
MEF	Multi-Exposure Factor = estimated number of food supplements and fortified foods with the respective nutrient
MRDR Test	Modified Relative Dose Response Test
NAS	National Academy of Science (USA)
NHS	Nurses' Health Study

NOAEL	No observed adverse effect level; the highest intake (or experimental dose) of a nutrient at which no adverse effects have been observed in the individuals studied (FNB: Dietary Reference Intakes. Applications in Dietary Assessment. Food and Nutrition Board, Institute of Medicine. National Academic Press, Washington D.C., 2000)
NFCS	National Food Consumption Study
OTC products	Over-the-counter products
Percentile	A specific value in a set of ordered data below which a specific percentage of the data fall. For instance, the 10 percentile is the value which 10% of the data fall below and 90% of the data fall above.
PHS	Physician's Health Study
PRI	Population Reference Intakes of SCF
RDA	Recommended dietary allowances
Recommendations	Amounts of nutrients derived from levels of average requirements and increased by the two-fold standard deviation. The intake of these amounts covers requirements in almost 98% of all individuals in a population and protects against damage to health (D-A-CH, 2000) ➢ **Reference values**
Reference values	Levels for nutrients which are assumed to protect almost all persons in the respective population group from food-related damage to health and which ensure they can function fully. Furthermore, they are intended to build up a certain body reserve which is immediately available for sudden increases in requirements without any impairment to health (D-A-CH, 2000). A distinction is made between: ➢ **Recommendations** ➢ **Estimated values** ➢ **Guidance values**
Safe Upper Level (SUL)	Represents an intake that can be consumed daily over lifetime without significant risk to health. (Food Standards Agency. Safe Upper Levels for Vitamins and Minerals. Report of the Expert Group on Vitamins and Minerals. London, 2003)
SCF	Scientific Committee on Food
TDI	Tolerable daily intake
TL	Tolerable level in a single dietary supplement or in an individual fortified food product; Maximum level for individual food supplements or individual fortified foods
Tolerable Upper Intake Level (UL)	➢ The maximum level of total chronic daily intake of a nutrient (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans. (Scientific Committee on Food: Guidelines of the Scientific Committee on Food for the development of tolerable upper intake levels for vitamins and minerals (adopted on 19 October 2000). SCF/CS/NUT/UPPLEV/11 Final. 28 November 2000) ➢ The highest average daily nutrient intake level likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects increases. (FNB: Dietary Reference Intakes. Applications in Dietary assessment. Food and Nutrition Board, Institute of Medicine. National Academic Press, Washington D.C., 2000)
UF	Uncertainty factor
Upper Intake Level (UL)	The highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. (Nordic Council: Addition of vitamins and minerals. A discussion paper on health risks related to food and food supplements. Copenhagen 2001, TemaNord 2001: 519)
VERA	Nutrition survey and risk factor analysis
VitaminV - Verordnung über vitaminisierte Lebensmittel	Ordinance on Vitaminised Foods

ZVerkV – Verordnung über Anforderungen an Zusatzstoffe und das Inverkehrbringen von Zusatzstoffen für technologische Zwecke (Zusatzstoff-Verkehrsverordnung)	Ordinance on Requirements to be met by Food Additives and Marketing of Food Additives authorised for Technological Purposes (Additives Marketing Ordinance)

3 Introduction

3.1 Description of the problem

The market of food supplements and fortified foods (conventional foods with added vitamins and/or minerals) is diverse and growing. It is, therefore, important to lay down uniform provisions and set maximum levels for these products to protect consumers from possible adverse health effects but also from misleading advertising. Directive 2002/46/EC adopted by the European Parliament in June 2002 is a first step towards the uniform regulation of food supplements in Europe. Firstly, the Directive contains a positive list of all substances that can be added to food supplements in the Member States of the European Union. The next step, which is planned, is to set maximum levels – referred to the daily dose recommended by the manufacturer.

The "Proposal for a Regulation of the European Parliament and of the Council on the addition of vitamins, minerals and certain other substances to food", which was submitted in November 2003 by the European Commission, aims to bring about the standardisation of provisions concerning the addition of vitamins and minerals to conventional foods (CEC, 2003). This proposal also envisages setting maximum levels for vitamins and minerals.

Both documents, Directive 2002/46/EU and the Proposal for a Regulation, call for other sources of vitamin and mineral intakes to be taken into account when setting maximum levels.

In this context, BfR has prepared this report on the toxicological and nutritional aspects of the use of vitamins and minerals in foods. In contrast to the work done by the European Scientific Committee on Food (SCF), the American Food and Nutrition Board (FNB) and the British Expert Committee on Vitamins and Minerals (EVM), this was not to constitute another attempt to derive tolerable upper intake levels for vitamins and minerals. Instead, the goal was to derive proposals for maximum levels of vitamins and trace elements in individual food supplements and for the nutrient fortification of individual conventional foods bearing in mind the assessments undertaken by scientific bodies, other relevant study findings and the data available for Germany on nutrient intake and supply situation.

This report only addresses the addition of vitamins and minerals to conventional foods including food supplements whereas foods for special dietary purposes (e.g. complete/incomplete foods for special medical purposes) and medicinal products are explicitly excluded. Furthermore, the proposed maximum levels mainly refer to adults, perhaps additionally to children and adolescents but, not to young children or infants as they are already taken into account in the Ordinance on foods for special dietary uses.

In January 2002 a report was already published which looked at the addition of minerals ("Toxicological and Nutritional-Physiological Aspects of the Use of Minerals and Vitamins in Foods. Part I: Minerals and Trace Elements)" (BgVV, 2002). In the meantime tolerable upper intake levels for several substances (calcium, zinc, copper, chromium, iodine) have been derived and published by the EU Scientific Committee on Food (SCF). This report updates the publication from 2002. Given its size, this report was broken down into two documents. Both are published in the series BfR-Wissenschaft. Part I is called "Use of Vitamins in Foods – Toxicological and Nutritional-Physiological Aspects" (BfR-Wissenschaft 03/2004). Part II bears the number 04/2004 in the BfR-Wissenschaft series and is entitled "Use of Minerals in Foods – Toxicological and Nutritional-Physiological Aspects". Both publications are available subject to a charge from the BfR Press and Public Relations Office.

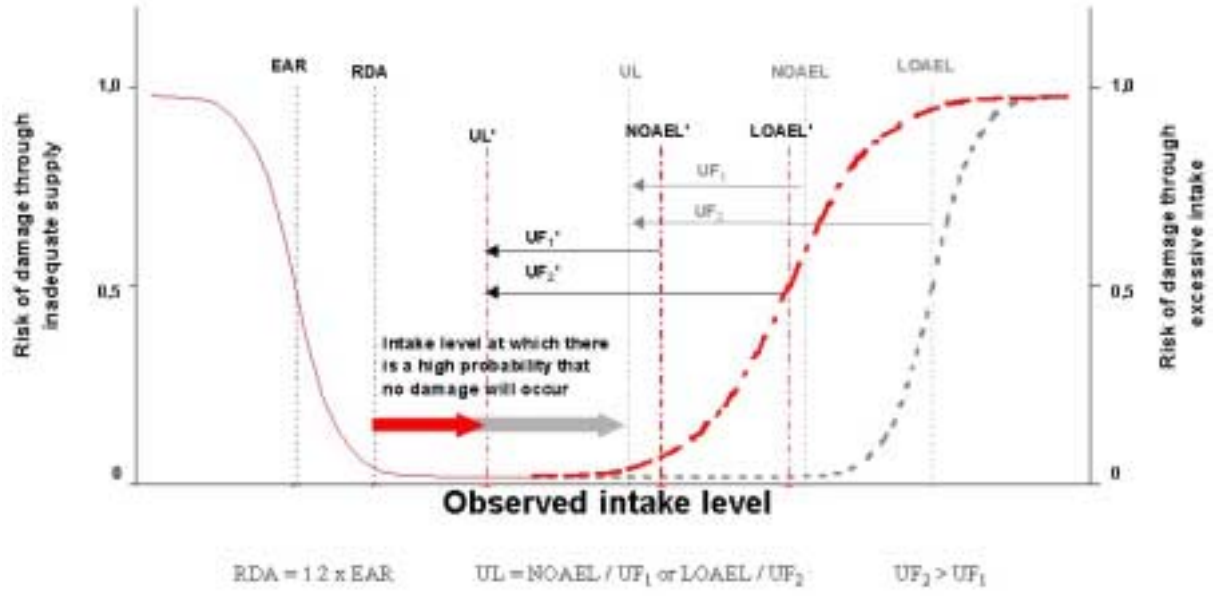
3.2 Principles for the risk assessment of vitamins and minerals including trace elements

The setting of tolerable upper levels for the daily intake of vitamins and minerals calls for comprehensive risk assessment based on generally recognised scientific data taking into account nutritional-physiological requirements.

The risk assessment of vitamins and minerals varies greatly from that of chemical residues or contaminants in foods. The special feature of essential nutrients, like minerals and vitamins, is that besides the risks related to high intakes there are also risks of inadequate supply or deficiency. In this context, the different sensitivities of individual consumer groups have to be taken into account as well (Dybing *et al.*, 2002; Grossklaus, 2002).

In the case of the classical toxicological procedure to set safe intakes or upper levels, the adverse effects identified are first placed in relationship to the dose (hazard characterisation). Based on the toxicological parameters like, for instance, NOAEL (No Observed Adverse Effect Level) and LOAEL (Lowest Observed Adverse Effect Level), tolerable upper intake levels (UL) are derived by the EU Scientific Committee on Food (SCF) or the European Food Safety Authority (EFSA) or other bodies using uncertainty factors (UF). SCF defines the UL as the maximum level of total chronic daily intake of a nutrient (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans (SCF, 2000).

Figure 1: Relationships between the recommended daily allowance (RDA) and LOAEL, NOAEL and the derivation of the UL for nutrients



using the example of a nutrient with a large margin between LOAEL/NOAEL and RDA (...) or a small margin between LOAEL/NOAEL and RDA (-·-) (modified in accordance with FNB, 2001)

Moreover, when determining the tolerable upper intake level/upper safe intake level of vitamins and minerals, it should be borne in mind that the area between the risk of insufficient dietary intake or deficiency and the risk of overdose or occurrence of toxic side effects may vary considerably for different nutrients. This is shown in Figure 1. The graph depicts the relative risk of the occurrence of deficiency or the occurrence of adverse side effects depending on the intake level of a nutrient.

The recommended dietary allowance for a nutrient (RDA or PRI, see Glossary) indicates the amount of a substance for which the probability of deficiency in a population group is not more than 2.5%. Higher nutrient intake than the RDA/PRI may, depending on the nutrient, lead quickly (see Fig. 1 LOAEL') or may lead with a larger margin of safety to adverse side effects (see Fig. 1, LOAEL). The UL is the intake level at which chronic daily intake, with a high degree of probability, will not lead to adverse side effects. The exceeding of this dose goes hand in hand with a higher probability of the occurrence of adverse side effects. In general, the margin between the RDA and UL is large (see Fig. 1 RDA ↔ UL). However, there are nutrients like vitamin A, for which the margin between the RDA and the defined UL is small (see Fig. 1, RDA ↔ UL'). The use of these nutrients in food supplements or fortified foods is, therefore, linked to a higher risk of adverse side effects than in the case, for instance, for nicotinamide where there is a large margin between the RDA and UL.

Regarding hazard characterisation, there are *considerable gaps in knowledge* about some nutrients. Although animal studies are available for the derivation of NOAEL and/or LOAEL, their transferability to man is unsure and very few studies in human beings are available. In some cases there are major differences between the individual bioavailability of nutrients and often a toxicological assessment is only possible of the amounts taken in via supplements and not of total daily intake (Hages *et al.*, 1999). Moreover, there may be interactions amongst various nutrients or with other food components that have to be taken into account. Furthermore, consideration must also be given to differences in gender and age as well as special physiological conditions and specificities in dietary habits (Dybing *et al.*, 2002).

Because of these differences in quantitative risk assessment, indications can only be given on a case by case basis whether and, if so, to what extent measures are required or whether they are necessary in line with the principle of hazard avoidance or the precautionary principle and whether they are imperative or not absolutely imperative.

3.3 Method to derive maximum levels for individual products

3.3.1 Structure of the report

The assessment of individual vitamins and minerals (Chapters 4-17 (Part I) and Chapters 4-18 (Part II)) was undertaken using the following structure (Figure 2). The individual chapters are based on principles of risk analysis and are tailored to deriving maximum levels in individual products (food supplements/fortified foods) (CAC, 2003).

Figure 2: Structure of risk assessment for the derivation of maximum levels in individual foods

1	Summary
2	Nutrient description
2.1	Characterisation and identification
2.2	Metabolism, function, requirements
2.3	Exposure (dietary and other sources, nutritional status)
3	Risk characterisation
3.1	Hazard characterisation (NOAEL, LOAEL)
3.2	Deficiency, possible risk groups
3.3	Excessive intake, possible risk groups
4	Tolerable upper intake level

continued from Figure 2: Structure of risk assessment for the derivation of maximum levels in individual foods

4.1	Derivation of maximum levels in food supplements
4.2	Derivation of maximum levels in fortified foods
5	Gaps in knowledge (optional)
6	References

Based on the supply status, nutrients can be divided into four categories following the example of AFSSA (Agence Française de Sécurité Sanitaire des Aliments) (Table 1) (AFSSA, 2002).

Table 1: Supply categories taking into account intake and/or supply status

Supply category	Criteria
1	Risk of a clinically manifest deficiency or a depletion of body stores in specific age groups with specific physiological conditions, specific eating habits, in specific regions
2	Uncertainty about the risk of a clinically manifest deficiency or a depletion of body stores because of the lack of or the questionable validity of a biomarker, inadequate food tables, lack of epidemiological studies
3	No indication of inadequate nutrient intake or there is nutrient intake in the range of recommended intake
4	Indication of nutrient intake above recommended intake

modified in accordance with AFSSA, 2002

With regard to the risk that nutrients can cause adverse effects, they can, following the classification of the Nordic Council (2001), be roughly divided into three categories depending on how large the margin is between recommended/observed intakes and the defined UL (Table 2). However, in individual cases (e.g. manganese, beta-carotene; see Table 3) the criteria used to define risk categories could not be applied.

Table 2: Various degrees of probability that a nutrient leads to adverse side effects

Risk category	Criterion
High risk	Nutrients for which the margin between the RDA (or measured intake) and UL is low (factor <5)
Moderate risk	Nutrients for which the UL is 5 to 100 times higher than the RDA (or measured intake)
Low risk	Nutrients for which a UL cannot be defined because up to now no adverse side effects have been identified despite intake 100 times higher than the RDA

3.3.2 Principles to derive maximum levels for vitamins and minerals in food supplements and fortified foods

Whereas SCF and other scientific bodies have defined a Tolerable Upper Intake Level (UL) for the daily intake of a nutrient from all food sources, BfR has derived a daily maximum level (TL) of a vitamin or mineral in individual products.

3.3.2.1 Theoretical foundations

In Part I "Minerals and Trace Elements", which has already been published on the Internet, we proposed a procedure for the derivation of daily maximum levels for individual products which is presented once again here in detail (BgVV, 2002).

This sequential procedure and the separate derivation of daily maximum levels for food supplements and fortified foods aims to take account of multiple exposure which may result

from the daily parallel consumption of both product categories (food supplements, fortified foods) and also of the parallel daily consumption of several products within a category (e.g. consumption of several food supplements per day). At the same time, this procedure aims to facilitate the flexible handling of multiple exposure and to reflect the specificities of food supplements and fortified foods. Differences between the two categories result from the fact that food supplements contain nutrients in dosed form (e.g. capsules or tablets) and must carry information about recommended daily intake along with a warning not to exceed the stipulated daily dose. In contrast, the consumption of fortified foods is not based on the amount of vitamins and minerals contained therein but is mainly determined by factors like hunger, thirst, appetite and availability. In contrast to the situation with food supplements, consumption recommendations are not usual or could not be complied with. In addition, appropriate consideration must be given to the fact that vitamins and/or minerals may be added to a wide range of processed foods.

3.3.2.1.1 Derivation of tolerable vitamin and mineral levels for additional intake through food supplements and fortified foods

The basic assumption is that the tolerable upper intake level of a vitamin or mineral (UL), derived by the EU Scientific Committee on Food (SCF) - that normally comprises intakes from all sources - is already used up to a certain degree through the normal consumption of solid and liquid foods. The resulting difference to the UL represents the respective residual amount (R) of vitamin and/or mineral intake which may be taken in altogether from all other intake sources if the UL is not to be exceeded. It, therefore, constitutes the amount available for additional intake from food supplements and fortified foods. In line with a precautionary approach, for the calculation of the residual amount (R) the highest percentile available from corresponding studies is used as the value for Dietary Intake by Normal Food (DINF). As a rule, these are data on the 95 or 97.5 percentile. This leads to the following formula:

Formula 1 ➔ R = UL – DINF

UL	=	Tolerable Upper Intake Level (SCF) usually referring to the daily total intake
DINF	=	Dietary Intake by Normal Food (upper percentile)
R	=	Residual or maximum amount for safe addition to foods including dietary supplements

3.3.2.1.2 Derivation of the total tolerable intake of a vitamin or mineral via food supplements or the total intake level for via fortified foods

The residual amount R calculated according to formula 1 constitutes the sum of the total tolerable intake of a vitamin or mineral from food supplements and fortified foods. The following applies:

Formula 2
Residual amount (R) = total tolerable intake via food supplements + total tolerable intake via fortified foods

or total tolerable intake via food supplements + total tolerable intake via fortified foods = UL – DINF

The percentage of this residual amount allocated to food supplements or fortified foods for additional intake is freely selectable. It may be between 0 and 100% whereby, however, the sum of the two percentages may not exceed 100%.

For the individual vitamins and minerals the distribution between the two food categories should be selected in such a way that the derived maximum daily levels for food supplements or fortified foods still reach significant sizes. In cases of conflict a decision should be taken in favour of the addition to food supplements. Nevertheless, in the case of

vitamins and minerals with large margins between the tolerable upper intake level and the 95 or 97.5 percentile of intake, it makes sense to divide the available (large) residual amount in equal parts between food supplements and fortified foods. By contrast, in the case of vitamins and minerals with small margins, e.g. zinc, it is recommended that the available (small) residual amount be allocated to the category of food supplements alone and therefore to be no fortification of conventional foods.

3.3.2.1.3 Derivation of maximum levels for individual food supplements (TL_{FS})

Based on the total tolerable intake level laid down for food supplements (see formula 2), the daily maximum level can be derived for individual products. In this context, consideration must be given to multiple exposure via the product category food supplements. No corresponding figures are available to estimate the possible multiple exposure. Although a scientifically based numerical value cannot be derived at the present time, it is justified from the precautionary angle to assume that vitamins and minerals under certain circumstances are taken in daily from two different food supplements. This is conceivable, for instance, in the case of the intentional intake of vitamins and minerals via multivitamin and mineral products and the additional intake of food supplements which are consumed and promoted for their content of other substances (herbs, extracts etc.) and which contain vitamins and minerals as well (thus leading to unintentional additional intake of these nutrients). In this context it is relevant that food supplements do indeed carry instructions not to exceed the recommended daily dose but no recommendation to pay attention to the corresponding contents of other food supplements consumed. By improving knowledge about the consumption of food supplements, a more reality-based factor can be indicated for taking into account possible multiple exposure which can be correspondingly adapted when deriving the daily maximum levels.

$$TL_{FS} = \frac{\text{Total tolerable intake via food supplements } ^{*)}}{2}$$

*) Total tolerable intake via food supplements = UL – (DINF + total tolerable intake via fortified foods)

3.3.2.1.4 Derivation of maximum levels for individual fortified foods (TL_{FF})

When deriving daily maximum levels for individual fortified foods (individual products), attention must also be paid to possible multiple exposure which may result from the addition of vitamins and minerals to a wide range of processed foods. As in the case of food supplements, no corresponding figures are, however, available. Not least because of this fact, various methods are feasible for the derivation of maximum levels for individual fortified foods:

a) Derivation of maximum levels for individual products based on food portions (numerical multiple exposure factor)

   Here the derivation is based on the number of portions of foods fortified with a specific vitamin or mineral which are consumed daily. In order to derive the maximum level per individual product, the total tolerable intake via fortified foods is divided by the number of portions of fortified foods consumed daily. The level obtained in this way may be contained in a normal portion of the food concerned. Here, too, from the precautionary angle, the assumption of a multiple exposure factor of 2 (i.e. consumption of 2 portions of a food fortified with the same nutrient per day) is justified. In the case of an extension of the fortification of foods and/or consumption of fortified foods, higher factors may be necessary or there may be a need for regular adjustment in line with market developments. The maximum level per portion could be derived as follows (multiple exposure factor = 2):

$$TL_{FF}/Portion = \frac{\text{Total tolerable intake via fortified foods }^{(**)}}{2}$$

(**) Total tolerable intake via fortified foods = UL – (DINF + total tolerable intake via food supplements)

b) Derivation of maximum levels for individual products based on energy content, adapted to the model by Flynn *et al.* (2003)

In line with the method used by Flynn *et al.*, the derivation is based on the 95 percentile of energy intake which was estimated for consumers in the European Union as 3,600 kcal. In the same way, a maximum fortification rate of conventional food is assumed to be 50% since vitamins and minerals can only be added to processed foods but not, however, to fresh foods like fruit, vegetables or meat. Their addition is also limited by a number of other factors (Flynn *et al.*, 2003).

For the derivation of the daily maximum level, referred to an energy content of 100 kcal, this leads to the following formula:

$$TL_{FF}/100\ kcal = \frac{\text{Total tolerable intake via fortified foods }^{(***)}}{36 * 0.5}$$

(***) Total tolerable intake via fortified foods = UL – (DINF + total tolerable intake via food supplements)

Although a multiple exposure factor can only be estimated at present, BfR favours the portion-based approach (Option a) since in the case of a reference to energy density (Option b) a special provision would be necessary for the groups of energy-reduced foods and low-energy drinks. Furthermore, Option a) offers the advantage of a uniform portion-based method both for food supplements and fortified foods.

3.3.2.2 Practical implementation

The method presented above was developed in order to guarantee the uniform derivation of maximum levels for the various vitamins and minerals. However, when considering individual vitamins and minerals, for the vast majority the method was not applicable or not fully applicable to the derivation of maximum levels in food supplements and/or fortified foods or it did not lead to viable results. The available data – or more appropriately the sparse data – normally meant that cases had to be considered on an individual basis.

The reasons which restricted or ruled out the application of the method or did not lead to viable results are the following:

- SCF did not derive a UL (e.g. vitamin B_1, B_2, pantothenic acid, biotin) or the work on the derivation of a UL by SCF or EFSA has not yet been concluded (e.g. iron) (when this report was published);
- not enough data are available on the dietary intake of vitamins/minerals or supply status;
- the therapeutic dose would be exceeded (e.g. vitamin K);
- BfR has well founded reservations about ULs which have been defined already (e.g. vitamin E).

3.4 Tabular overview of the results

Table 3 provides an overview of the classification of vitamins and minerals (including trace elements) in the risk or supply categories. Tables 4 and 5 present the maximum levels proposed by BfR for the use of vitamins and minerals in food supplements and fortified foods.

Table 3: Overview of the classification of vitamins and minerals in supply and risk categories

Nutrients	Risk category (Classification according to Table 2)	Supply category (Classification according to Table 1)
Vitamins		
Vitamin A	high	2/3
Beta-carotene	high *	3
Vitamin D	high	1
Vitamin E	moderate	2/3
Vitamin K	moderate	2
Vitamin B_1	low	3
Vitamin B_2	low	3
Vitamin C	moderate*	3/4
Niacin - Nicotinamide - Nicotinic acid	 low high	3/4 - -
Vitamin B_6	moderate	4
Folic acid	moderate *	1/2
Pantothenic acid	low	2
Biotin	low	2
Vitamin B_{12}	low	4
Minerals		
Sodium	high * (additional administration as NaCl)	4
Chloride	-	4
Potassium	high (FS)	2/3
Calcium	high	4 from 0-3 years after 1/3
Phosphorus	moderate	4
Magnesium	moderate *	2/3
Iron	high	1/2
Iodine	high	1
Fluoride	moderately high *	2
Zinc	high	2
Selenium	moderately high *	2
Copper	high	3
Manganese	high *	2/3
Chromium	low	2
Molybdenum	moderate	2

* Classification deviates from Table 2

Table 4: Proposed maximum levels for the use of vitamins and minerals in food supplements (FS) referred to the daily dose recommended by the manufacturer

Nutrients		Recommended daily intake for adults [1]	Proposal for maximum level in FS	Comments
Vitamins				
Vitamin A	µg	800	400 (only for adults)	for children aged between 4 and 10: 200 µg
Beta-carotene	mg	2-4 [2]	2	
Vitamin D	µg	5	5	for persons >65 years: 10 µg
Vitamin E (equivalents)	mg	11-15 [2]	15	
Vitamin K	µg	80 [2]	80	
Vitamin B_1	mg	1.3	4	
Vitamin B_2	mg	1.5	4.5	
Niacin	mg	17	17	no use of nicotinic acid
Vitamin B_6	mg	1.6	5.4	
Folate equivalents	µg	400	400 (as folic acid)	
Pantothenic acid	mg	6 [2]	18	
Biotin	µg	60 [2]	180	
Vitamin B_{12}	µg	3	3-9	
Vitamin C	mg	100	225	
Minerals				
Sodium	mg	550 [3]	0	
Chloride	mg	830 [3]	0	
Potassium	mg	2000 [3]	500	
Calcium	mg	1000-1200	500	
Phosphorus	mg	15 to <19 y: 1250 from 19 y: 700	250 (as phosphate)	
Magnesium	mg	15 to <19 y: 400/350 19 to <25 y: 400/310 25 to <65 y: 350/300 65 years and older: 350/300 (m/f)	250	where appropriate, break down into 2 single doses
Iron	mg	15 to <19 y: 12/15 19 to <51 y: 10/15 51 y and older: 10/10 (m/f)	0	
Iodine	µg	180-200	100	
Fluoride [4]	µg	15 to <19 y: 3.2/2.9 19 to 65 y and older: 3.8/3.1 (m/f)	0	
Zinc	mg	7 (f) 10 (m)	2.25	no supplements for children or adolescents under the age of 18
Selenium	µg	30-70	25-30	
Copper	µg	from 15 y: 1000-1500 [2]	0	
Manganese	mg	2-5 [2]	0	
Chromium	µg	30-100 [2]	60	
Molybdenum	µg	50-100 [2]	80	maximum level not suitable for children under the age of 11

* (D-A-CH, 2000)
1 Recommended intake in Germany for adolescents and adults from age 15 (D-A-CH, 2000)
2 Estimated values for adequate daily intake (D-A-CH, 2000)
3 Estimated values for minimum intake (D-A-CH, 2000)
4 Guidance values for upper intake for caries prevention (D-A-CH, 2000)

Table 5: Proposed maximum levels for the fortification of conventional foods with vitamins and minerals referred to the expected daily portion of a food

Nutrients		Proposal for maximum levels in fortified foods	Comments
Vitamins			
Vitamin A	µg	no fortification	Except: Margarine and mixed fat products (10 mg/kg)
Beta-carotene	mg	no fortification	
Vitamin D	µg	no fortification	Except: Margarine and mixed fat products (2.5 µg/100 g) Edible oils (20 µg/L)
Vitamin E (equivalents)	mg	15	Where appropriate, linking of vitamin E fortification to the polyene fatty acid content of the food
Vitamin K	µg	80	
Vitamin B_1	mg	1.3	
Vitamin B_2	mg	1.5	
Niacin	mg	17	No use of nicotinic acid
Vitamin B_6	mg	1.2-1.6	
Folic acid	µg	200	Where appropriate, reassessment in the case of fortification of flour
Pantothenic acid	mg	6	
Biotin	µg	60	
Vitamin B_{12}	µg	3	Where appropriate, limiting addition of the vitamin to specific food groups
Vitamin C	mg	100	
Minerals			
Sodium	mg	no fortification	Exception: drinks, which are directly intended to balance substantial losses in the healthy consumer (e.g. as a consequence of heavy sweating)
Chloride	mg	no fortification	
Potassium	mg	no fortification	Instead addition of potassium only for the purposes of replenishment, where appropriate parallel reduction of table salt content in processed foods
Calcium	mg	only dairy substitutes	Calcium amounts like in dairy products
Phosphorus	mg	no fortification	
Magnesium	mg	15-28 mg/100 kcal or 22.5 mg/100 ml, referred to ready-to-eat food	
Iron	mg	no fortification	
Iodine	µg	no direct fortification of foods	Restriction to iodised salt as the suitable carrier food
Fluoride	µg	only table salt	250 mg/kg
Zinc	mg	no fortification	
Selenium	µg	no fortification	
Copper	µg	no fortification	
Manganese	mg	no fortification	
Chromium	µg	no fortification	
Molybdenum	µg	no fortification	

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4 Risk Assessment of Vitamin A

4.1 Summary

The surveys available for Germany on vitamin A intake indicate that at least one quarter of the population does not meet the recommendations for adequate intake. In fact, this figure is probably even higher since in the surveys conducted up to now the conversion factor used to calculate vitamin A activity from beta-carotene intake was too low. Pregnant and lactating women are particular risk groups for inadequate vitamin A supply since these groups have higher requirements, On the other hand, the 97.5 percentile of adults already exceeds the UL for retinol from normal dietary intake. As the plasma retinol concentrations are not a reliable indicator for the assessment of the vitamin A status because of the strict homeostatic regulation of retinol in the organism, there are major uncertainties overall about the assessment of the supply of the population with this vitamin (supply category 2/3).

The safety margin (margin between recommended intake and UL) is low (<5) in the case of preformed vitamin A. In some age groups the 97.5 percentile of retinol intake is even higher than the UL. Furthermore, the results from various studies indicating that high chronic vitamin A intake from all sources can lead to an undesirable reduction of bone density must be taken seriously. Preformed vitamin A must, therefore, in the opinion of BfR be classified in the highest risk category.

Since, in most cases, food supplements are taken on the basis of the subjective feelings of an individual and not on the basis of a diagnosed deficiency, it would be appropriate for the protection of the already (more than) adequately supplied population groups, to refrain from offering any food supplements or fortified foods with preformed vitamin A. On the other hand, more than 25% of the population do not reach the recommended intake for vitamin A. BfR, therefore, recommends food supplements with maximum 400 µg retinol per daily dose for adults and with maximum 200 µg per daily dose for children aged between 4 and 10. BfR is of the opinion that, aside from in margarine and mixed fat products, preformed vitamin A should not be used to fortify foods. Instead, it should be recommended to the population that they eat foods which are rich in vitamin A like liver (products) more frequently.

Recommended intake	0.8-1.0 mg retinol equivalents (RE)/day			
	Vitamin A		**Retinol**	
Intake [mg RE/day or mg retinol/day] (NFCS, 1994)	**m**	**f**	**m**	**f**
Median	1.01	0.89	0.66	0.53
P 2.5	0.34	0.32	0.22	0.18
P 97.5	4.64	3.87	4.1	3.44
Tolerable upper intake level			3.0 mg/day	
Proposal for **maximum levels** in:				
food supplements	0.4 mg retinol/daily dose 0.2 mg retinol/daily dose (for children aged between 4 and 10)			
fortified foods	No fortification			

4.2 Nutrient description

4.2.1 Characterisation and identification

The term vitamin A (CAS No. 68-26-8) is the collective term for all fat soluble compounds which possess the full efficacy of all-trans-retinol and its esters. Furthermore, the vitamin A family includes approximately 50 carotinoids, of which around 12 are vitamin A active (Gaßmann, 1998). The biological activity of the individual vitamin A compounds is expressed

in International Units (IUs) (1 IU = 0.3 µg retinol). Furthermore, the following conversion factors apply: 1 mg retinol equivalent = 1 mg retinol = 1.5 mg all-trans-retinol acetate = 1.83 mg all-trans-retinyl palmitate.

Up to now, vitamin A and its derivatives were equated in Germany with additives (§ 2 para 2c Food and Other Commodities Act – LMBG), i.e. the addition of vitamin A to conventional foods was not permitted aside from a few exceptions (margarine and mixed fat products). According to European Directive 2002/46/EC vitamin A may be added to food supplements in the form of retinol, retinyl acetate, retinyl palmitate and as beta-carotene. The above-mentioned vitamin A forms are also listed in the Annex to the Proposal for a Regulation on the addition of vitamins and minerals and certain other substances to foods (COM(2003)671 final of 10.11.2003). Hence, they may be used in future to fortify conventional foods.

The following maximum level derivation refers solely to preformed vitamin A, i.e. retinol, all-trans-retinyl acetate and all-trans-retinyl palmitate. Maximum levels for beta-carotene are discussed in Chapter 5.

4.2.2 Metabolism, function, requirements

Metabolism:
In the case of sufficient fat intake, around 80% of dietary vitamin A is absorbed (Gerster, 1997). Retinyl esters, which are found in food, are hydrolysed in the intestines to retinol. In the presence of bile acid 70-90% are absorbed by enterocytes. After re-esterification, they are incorporated into chylomicrons and transported to the liver. Around 50-80% of total vitamin A is stored in the liver. 98% is to be found as esters in the Kupffer cells of the liver and is packaged in lipid droplets (Blomhoff, 1994). In healthy adults the average concentration of retinyl esters is 100 to 300 µg and in children 20 to 100 µg per g liver (Biesalski, 1989; Olson, 1987). The half-life of the retinyl esters stored in the liver is 50-100 days although this may be shorter in the case of heavy alcohol consumption. Retinyl esters can be released from the liver after renewed hydrolysis in the form of retinol. Retinol is bound in plasma to retinol-binding protein (RBP) and to transthyretin for transport in this form to the target cells where it is taken up by receptor mediation. In the cells retinol is mainly oxidised into retinaldehyde and partly into retinoic acid. Other metabolites are 13-cis-retinoic acid and its 4-oxo-metabolites. Furthermore, by means of isomerisation some of the all-trans-retinoic acid is rearranged as 9-cis-retinoic acid (Gerster, 1997). Retinol then undergoes cytochrome-P-450-dependent hydroxylation, glucuronidation and renal elimination.

Absorption, storage and release of retinol-binding protein is controlled in a strictly homeostatic manner which means that the retinol level in the plasma is relatively constant between 300 and 700 µg/L (1.05-2.45 µmol/L) aside from in conditions of extreme hypovitaminosis or hypervitaminosis A. The dietary intake of preformed vitamin A does not, therefore, normally correlate with the retinol concentration in the plasma (Blomhoff, 1994; Solomons, 2001).

Nutrient interactions:
Vitamin A and iron: Vitamin A influences haematopoieses and leads to the increased production or release of red blood cells (Garcia-Casal, 1998; Layrisse *et al.*, 1998). In the past it was assumed that vitamin A increases the bioavailability of inorganic iron. A more recent study was not able to confirm this (Walczyk *et al.*, 2003).

Vitamin A and zinc: The synthesis of RBP is zinc-dependent which means that retinol transport in the organism may be impaired in the event of zinc deficiency. Furthermore, a zinc deficiency may reduce the activity of zinc-dependent retinol dehydrogenase which impedes the conversion of retinol to retinaldehyde. However, there is no clear evidence of a

synergy between the two nutrients in the human organism or of their importance for health (Christian and West, 1998; Gerster, 1997).

Function:
Vitamin A and its metabolites are of relevance for oogenesis, spermatogenesis, placentary and embryonal development as well as for the proliferation and differentiation of epithelia. Growth, development and reproduction are also dependant on adequate vitamin A intake. Furthermore, vitamin A is essential for sight (as 11-cis-retinaldehyde) and for hearing, taste and smell (Gerster, 1997; Russel, 2000; Solomons, 2001).

Requirements:
The following table gives the recommended intakes for vitamin A for various age groups (DGE/ÖGE/SGE/SVE, 2000). The recommendations apply to total vitamin A intake (preformed vitamin A and vitamin A active carotinoids) and are given in retinol equivalents (µg RE):

Table 6: Recommended vitamin A intakes

Age (years)	Recommendation (µg RE)	
	m	f
1 - 3		600
4 - 6		700
7 - 9		800
10 - 12		900
13 - 14	1100	1000
15 - 18	1100	900
>19	1000	800
Pregnant women (from 4th month)		1100
Lactating women		1500

As reflected in the recommended intakes, there are higher requirements for vitamin A during pregnancy and lactation. Given the major importance of this vitamin for lung development and maturity, care should be taken particularly in the 2nd and 3rd trimesters of pregnancy to ensure adequate intake (Biesalski and Nohr, 2003). To cover the vitamin A requirements of infants, an intake of 400 µg (1.4 µmol) per day is deemed to be adequate for the first 6 months and an intake of 500 µg (1.7 µmol) per day for the period from the end of the first year of life (IOM, 2001).

4.2.3 Exposure (dietary and other sources, nutritional status)

Sources:
Food: Natural vitamin A sources are foods of animal origin, in particular liver, milk and dairy products as well as egg yolks, cheese and fish which contain the vitamin in the form of retinyl esters (mostly as retinyl palmitate). Yellow and green vegetables like carrots, spinach and tomatoes contribute, with their high level of beta-carotene, to vitamin A supply. Exposure to heat and light may lead to a mean loss of the vitamin of 20% in the case of a normal diet and careful preparation of foods (Bognar, 1995). The vitamin A level in human milk varies depending on the supply of the mother. In well-nourished women the vitamin A levels in colostrum are 151 µg (0.53 µmol) per 100 ml, in transition milk 88 µg (0.3 µmol) per 100 ml and in mature human milk 75 µg (0.3 µmol) per 100 ml (Lawrence, 1994 in: Ross and Harvey, 2003). According to the US National Research Council the vitamin A levels in human milk in well-nourished women in the USA and Europe are between 40 and 70 µg (0.14 and 0.24 µmol) per 100 ml (National Research Council, 1998). The relatively high vitamin A levels, which are also measured in the colostrum of less well-nourished women without

supplements fall rapidly within a month to less than 50% of the original level. Hence, early commencement of breast-feeding is of major importance for the infant's vitamin A supply.

Food supplements: In conjunction with the Federal Health Survey, Mensink *et al.* (1999) determined that in Germany 22% of women and 18% of men regularly take food supplements (FS). However, no information is available about how frequently vitamin A-containing food supplements are taken.

The evaluation of questionnaires in conjunction with the so-called "Baby Care Programme" revealed that out of the 1,000 pregnant women interviewed, 11% regularly take food supplements with vitamin A (Briese *et al.*, 2001).

Medicinal products: For the treatment of manifest vitamin A deficiency conditions, which cannot be remedied through food, medicinal products with 50,000 IU (or 16 mg RE) per capsule are administered, with the recommendation that children aged between 7 and 10 should take 1 capsule daily, adolescents between 11 and 17 1 to 2 capsules daily and adults (with the exception of women of childbearing age and during pregnancy) 1 to 3 capsules daily (Heyl Fachinformation, 2000).

Nutritional status:
Dietary intake: In the National Food Consumption Survey vitamin A intake (as retinol and as total vitamin A) was determined for approximately 20,000 people on the basis of 7-day food consumption protocols.

According to this, median *retinol* intake in the 1980s was between 0.39 (4-6 years) and 0.56 (≥65 years) mg/day for women. In the case of men the median intake levels were between 0.44 (4-6 years) and 0.69 (≥65 years) mg/day. For both the female and the male study groups, the 2.5 percentile for retinol intake, depending on age, was between 0.16 (4-6 years) and 0.23 (>65 years) mg/day. The 97.5 percentile of the female group reached, depending on age, between 2.17 (4-6 years) and 3.62 (>65 years) mg/day and that of the male group between 1.89 (4-6 years) and 3.91 (>65 years) mg/day (Adolf *et al.*, 1995).

The mean intake of *total vitamin A* (retinol plus vitamin A active carotinoids) was 0.95 mg retinol equivalents (RE) per day. The median values for the female group were 0.64 (4-6 years) to 0.91 (≥65 years) mg RE/day and for the male group 0.69 (4-6 years) up to 1.04 (≥65 years) mg RE/day. The 2.5 percentile of total vitamin A intake was between 0.25 (4-6 years) and 0.32 (≥65 years) mg RE/day for the females and the 97.5 percentile was between 2.47 (4-6 years) and 4.02 (≥65 years) mg RE/day. For the male persons the 2.5 percentile of total vitamin A intake was between 0.27 (4-6 years) and 0.36 (≥65 years) mg RE/day and the 97.5 percentile was between 2.25 (4-6 years) and 4.32 (≥65 years) mg RE/day (Adolf *et al.*, 1995). According to the National Food Consumption Survey approximately 25% of all age groups only meet 60-80% of the recommendations whereby the 13-18 year old age group had the lowest intake (Adolf *et al.*, 1995). When assessing the nutritional status it should, however, also be borne in mind that the vitamin A active carotinoids taken up in food only possess 50% of the retinol activity assumed up to now according to new findings. The conversion factor 6, which was used in the National Food Consumption Survey and in other nutrition surveys too to calculate the vitamin A activity of beta-carotene, has since undergone an upward correction. Today, it is assumed that 12 µg beta-carotene are equivalent to 1 µg retinol (RAE - Retinol Activity Equivalents), whereas factor 24 is assumed for all other vitamin A active carotinoids (IOM, 2001). If one were to apply the new conversion factors to the data from German food consumption surveys, this would lead to total vitamin A intake which is approximately 15% lower. The percentage of those who only reach 50% of the recommended intake, would increase considerably. If previously on average 87% of the requirements of total vitamin A were covered in children and adolescents (female, 4≤ 19

years), then after application of the conversion factor of 12 on average less than 70% of the needs would be covered.

According to Müller (1996), the median intake of beta-carotene, which plays an important role in Germany in vitamin A supply, is between 1.1 and 1.4 mg/day. Using a conversion factor of 12, this would correspond to a vitamin A activity of 92-117 µg RE. According to this, 64% of the population take in less than 2 mg beta-carotene per day. However, this does not include food supplements or fortified foods.

In the EPIC study, the following *total vitamin A* intakes per day were determined:

Men:	P 10 = 0.35 mg RE;	P 50 = 0.9 mg RE;	P 90 = 3.0 mg RE
Women:	P 10 = 0.3 mg RE;	P 50 = 0.8 mg RE,	P 90 = 2.5 mg RE.

Retinol intake amounted to between 0.3 (P 10) and 2.5 (P 90) mg/day, with a median of 0.7 mg/day for the men examined (40-64 years). In the case of women (35-64 years) retinol intakes of between 0.2 mg (P 10) and 1.3 (P 90) mg/day were determined. The median was 0.5 mg/day (Schulze *et al.*, 2001). 10% of the population examined in the EPIC study does not, therefore, reach the lowest intake threshold for retinol defined by SCF which is 300 µg for men and 250 µg for women per day. Even taking into account beta-carotene intake, this 10% of the population only reaches around 40% of the recommended vitamin A intake. However, it must also be assumed here that the proportion of individuals who do not meet the recommendations is probably under-estimated because a conversion factor of 6 was used to convert beta-carotene into vitamin A.

Neither the National Food Consumption Survey nor the EPIC study takes vitamin A intake from food supplements and fortified foods into account.

In the DONALD study, which describes the diet of children and adolescents aged between 1 and 18 in a longitudinal manner, the influence of fortified foods on vitamin A intake was examined besides vitamin A uptake from normal food. Total vitamin A intake is, therefore, most favourable in the case of 2-3 year olds (80-90% of recommended intake is achieved). After that, intake falls to approximately 65% of recommended intake up to age 7-9 and is lowest (55% (m) and 60% (f) of recommended intake) amongst 15-18 year olds (Kersting *et al.*, 2000). This supports the results of the National Food Consumption Survey. According to the DONALD study, foods fortified with vitamin A account for around 10 to 20% of vitamin A supply in the age groups of children and adolescents. This means that total intake levels of up to 90% of the recommended intake are reached (Kersting *et al.*, 1995; Sichert-Hellert and Kersting, 2001).

Plasma concentrations: According to the VERA Study, the median values for plasma retinol were 1.78 µmol/L [P 2.5 = 1.04 µmol/L; P 97.5 = 3.02 µmol/L] in the women's group and 2.04 µmol/L [P 2.5 = 1.22 µmol/L; P 97.5 = 3.29 µmol/L] in the men's group. Values ≤ 0.35 µmol/L were only measured for 0.1% of the random sample which, according to the WHO definition, points to an acute deficiency. In particular young men aged between 18-34 were shown to have low plasma concentrations particularly in winter and spring. Plasma values ≤ 0.7 µmol/L were determined for 0.4% of the random sample examined which points to a sub-clinical deficiency (Heseker *et al.*, 1991; 1992).

Even if the plasma values seem to indicate that the German population is generally adequately supplied with vitamin A, it must be borne in mind that the power of these data is restricted because of the homeostatic control described above. In order to make reliable statements about the supply status of the German population, clinical trials would have to be carried out (e.g. modified relative dose response test – MRDR). So far, tests of this kind have not been conducted in Germany.

Retinol concentration in human milk: In Germany mean retinol concentrations in human milk of 2.8 µmol/L were measured in a group of 40 women. For around 20% of the group the concentration was <1.6 µmol/L. This is less than optimal when it comes to covering the vitamin A requirements of infants through human milk (Biesalski, unpublished). The study results presented here reflect the situation in a small group and cannot be generalised. However, they can be seen as an important indication that the nutritional status of some young women in Germany is possibly too low given their current dietary habits and especially during life phases with higher requirements like pregnancy and lactation. So far, no other (representative) study has been conducted in Germany in which retinol levels in human milk were examined.

4.3 Risk characterisation

4.3.1 Hazard characterisation (NOAEL, LOAEL)

When assessing the risk of high chronic vitamin A intake, hepatic diseases, reduced bone mineral density and teratogenicity must be considered as possible negative effects.

a) *Hepatic diseases*: Both in animal experiments and in humans a clear link has been established between high vitamin A intake and liver damage. It is not just the level of vitamin A intake which plays a role but also the period during which the high amounts of this vitamin were taken. The lowest intake associated with cases of liver cirrhosis was 7,500 µg per day, taken over 6 years (IOM, 2001). Chronically high alcohol consumption increases susceptibility to liver toxicity through vitamin A but other factors, too, like hepatitis A, B and C, liver toxic medicines or existing hepatic disorders strengthen the vitamin A effect or do not permit any clear conclusions about the actual toxic dose.

b) *Bone mineral density*: *In vitro*, animal and human data indicate that a chronically high vitamin A intake has a negative impact on bone resorption (Binkley, 2000). In laboratory tests an increased risk of bone fragility and spontaneous fractures was observed for example in rats with hypervitaminosis A (Ilich and Kerstetter, 2000; Johansson *et al.*, 2002). It is suspected that the effect of retinoic acid on gene expression and interactions between retinoic acid and 1,25-dihydroxycholecaliferol are the cause of the damaging effects (Johansson and Melhus, 2001).

   A series of epidemiological studies indicate that in human beings, too, there is a link between high vitamin A uptake and a risk of reduced bone density and fractures (Feskanich *et al.*, 2002; Melhus *et al.*, 1998; Michaëlsson *et al.*, 2003; Promislow *et al.*, 2002). In other studies no link was found (Ballew *et al.*, 2001; Sowers and Wallace, 1990).

   Association between vitamin A intake and bone density: Melhus *et al.* (1998) observed a significant decrease in bone mineral density and a related elevated risk of hip fractures in post-menopausal women from an intake of 1500 µg vitamin A upwards per day. Serum retinol values were not measured in this study.

   In a prospective study by Feskanich *et al.* (2002) the risk of fracture increased two-fold when vitamin A intake (as retinol) was higher than 2000 µg per day compared to intake levels of less than 500 µg per day.

   Promislow *et al.* (2002) examined 388 men and 570 women (age: 55-92 years), who participated in the Rancho-Bernardo Study. They observed that bone density fell with increasing retinol intake (the retinol intake was determined retrospectively using Food Frequency Questionnaires from 1988-1992).

Association between serum retinol and bone density: In a prospective long-term study involving 2,322 Swedish men, who were approximately 50 at the start of the study, it was determined that the group with the highest retinol levels in serum (>2.64 µmol/L) had a 64% higher risk of fracture than those with mean levels (2.17-2.36 µmol/L). From serum levels of 3 µmol/L upwards, the risk increases exponentially and was seven times higher at a value of 3.6 µmol/L (Michaëlsson *et al.*, 2003).

In a group of 246 post-menopausal women aged between 55-80, no significant link was found between the taking of vitamin A in the form of supplements, the retinol concentrations in the serum and bone mass or fractures (Sowers and Wallace, 1990).

In a representative random sample of the Third National Health and Nutrition Examination Survey, 1988-1994 (NHANES III) (5,790 non-pregnant persons over the age of 20), no association was found between the level of retinyl ester concentration in the serum, measured on an empty stomach, and bone densities (Ballew *et al.*, 2001).

Opotowsky and Bilezikian (2004) analysed the data from 2,799 women (50-74 years), who participated in the first epidemiological follow-up to the National Health and Nutrition Examination Survey concerning the link between vitamin A serum concentrations and the incidence of hip fractures. It was shown that the risk of fractures was higher both in the lowest and in the highest quintile compared to the serum retinol concentrations in the mean range.

The study results available up to now do not permit any clear statements about the effect of vitamin A (from food, supplements or fortified foods) on bone mineral density or the incidence of fractures. Further prospective studies are needed in order to derive a dose-response relationship for vitamin A intake or the retinol plasma level in conjunction with possible bone damage. The studies in which the level of vitamin A intake was examined in conjunction with bone density nevertheless indicate that, for reasons of consumer health protection, retinol intakes of more than 1500-2000 µg per day should be avoided until more up-to-date scientific findings are available.

c) *Teratogenicity*: We know from animal studies that vitamin A in the form of retinoic acid is teratogenic if it is administered during organogenesis either as a high single dose or over a longer period (Dolk *et al.*, 1999; Rosa *et al.*, 1986). In order to examine whether this observation also applies to human beings, a series of case control studies was conducted (Mills *et al.*, 1997; Shaw *et al.*, 1997). However, the results are contradictory and some are not statistically significant. Furthermore, it would seem it is rather the taking of supplements than the consumption of liver (products) that is responsible for negative effects. In a human study by Buss *et al.* (1994) the risk of increased vitamin A intake was examined from different sources. In this context, test persons were given the same vitamin A amount (50 mg or 150 mg retinol) in the form of a liver meal or as a food supplement. After that, the plasma levels of the various retinoid metabolites were measured over a longer period and the plasma kinetics were compared after liver consumption or supplementation. The results show that after vitamin A supplementation – possibly because of oversaturation of the absorption and metabolic pathways – increased oxidation of retinol to all-trans-retinoic acid, 13-cis-retinoic acid and 4-oxo-retinoic acid isomers takes place although the plasma levels of the retinyl esters and retinol were similar to those after liver consumption. The relative bioavailability of vitamin A from the liver (measured using the AUC value) was 75% compared with supplementation; however, the oxidative metabolism was considerably reduced.

A prospective, non-randomised study by Rothman *et al.* (1995) is the only investigation conducted in a large study population (~22.500 persons) in which a link was

established between supplementation of moderate retinol amounts (from 3000 µg/day) prior to the 7th week of pregnancy and a higher risk of malformations in new-born babies. However, this study was repeatedly criticised because of its inadequate design and a lack of clarity in the data evaluation (Biesalski, 1996; Miller *et al.*, 1998). So far, the results could not be confirmed by other working groups (Mastroiacovo *et al.*, 1999).

Since controlled, randomised intervention studies cannot be carried out for ethical reasons, it was and will not be possible to determine a definitively safe maximum level for the chronic intake of preformed vitamin A for human beings. It can, however, be assumed that even in the case of intake of large amounts of vitamin A from natural foods like liver, the formation of retinoic acid with a teratogenic effect takes place in a strictly controlled manner and does not exceed the physiological degree. When it comes to supplementation with preformed vitamin A, it can be said that in intervention studies vitamin A in amounts of up to 2400 µg RE was administered without any signs of teratogenic damage.

4.3.2 Deficiency, possible risk groups

The first signs of vitamin A deficiency are impaired vision, dry and scaly skin and metaplasia. In later stages there may be loss of appetite, higher susceptibility to infection, particularly pneumonia, taste and hearing disorders, reduced fertility and malformations in babies and even death.

A vitamin A deficiency requiring treatment may occur in the case of maldigestion and malabsorption because of gastrointestinal diseases like, for instance, Crohn's disease and sprue, ileojejunal bypass, pancreatic disorders, parenteral alimentation over a longer period and as a consequence of alcohol abuse (SCF, 2002).

Vitamin A deficiency, in the real sense with the above-mentioned symptoms, does not occur amongst the healthy population in Germany. As already described in Chapter 4.2.3, vitamin A intakes below the recommended intake must, however, be expected in far in excess of 25% of the population. Risk groups for sub-optimal supply are in particular pregnant and lactating women in whom sufficient intake is rendered more difficult by the fact that their vitamin A requirements during these phases are higher. This is all the more the case since the former BgVV in 1995 recommended that pregnant women no longer eat liver since, in some cases, very high vitamin A contents were detected in liver linked to the feedstuffs given to the animals. However if pregnant and lactating women don't eat liver, which is by far the largest source of vitamin A, it is difficult for them to achieve vitamin A intake which covers their requirements. Shah *et al.* (1987) showed that inadequate vitamin A intake by pregnant women is associated with a low vitamin A liver store in the foetus. This, in turn, can lead to low birth weight and a higher risk of complications during labour.

Other risk groups for low vitamin A intake are groups in the population which practice an extremely one-sided form of nutrition or diet. In general, population groups in the lower income brackets have a lower vitamin A intake than those in the higher income brackets (Gerster, 1997).

4.3.3 Excessive intake, possible risk groups

The intake of high amounts of vitamin A produces a characteristic toxicity picture (hypervitaminosis A). The term hypervitaminosis A is used to describe plasma retinol levels of more than 1 mg/L (3.5 µmol/L). Acute hypervitaminosis A can occur from a one-off ingestion of approximately 500 mg RE in adults, 100 mg RE in children and 30 mg RE in infants. The symptoms are headache, extreme fatigue, nausea and papilloedemas. After 24 hours massive scaling of the skin occurs. Infants and young children may experience bulging

fontanelle. There is an elevated fibrinolysis time, lower Quick's value, higher GOT and GPT values. The symptoms disappear after 36 hours (Hathcock *et al.*, 1990). Chronic vitamin A intoxications have been described in adults after taking high-dose (25,000-100,000 IUs) supplements (Geubel *et al.*, 1991; Kowalski *et al.*, 1994) and frequently in children after repeated consumption of chicken liver meals (36,000-42,000 IUs) (Mahoney *et al.*, 1980; Carpenter *et al.*, 1987).

Although the 97.5 percentile of the adult population in Germany currently shows some retinol intakes which are far higher than the recommended intake, no information is available in the literature about the prevalence of hypervitaminosis A in Germany.

The studies available for Germany on the intake of vitamin A indicate that more than one quarter of the population does not meet the recommendations. In fact, this proportion is probably higher since in the surveys conducted up to now the conversion factor used to calculate vitamin A activity from beta-carotene intake was too low. Pregnant and lactating women are particular risk groups for inadequate vitamin A supply since these groups have higher requirements.

On the other hand, the 97.5 percentile of adults already exceeds the UL for retinol from normal dietary intake. Vitamin A intake in part of the population would also be higher than presented in food consumption surveys conducted up to now if the intake of food supplements and the consumption of fortified foods had been taken into account.

Furthermore, as the plasma retinol concentrations are not a reliable indicator for the assessment of the vitamin A supply status because of strict homeostatic regulation in the organism, there are major uncertainties about the assessment of the supply of the population with this vitamin (supply category 2/3).

4.4 Tolerable upper intake level for vitamin A

Based on the existing data on vitamin A and the teratogenicity risk, SCF has derived a UL of 3000 µg RE for women of child-bearing age. Since this value is 2.5 times lower than the lowest dose which led to hepatotoxic damage (7500 µg RE), the UL is considered to be sufficiently safe for adult men, too. Lower values were estimated for children and adolescents by means of extrapolation:

Age (years)	UL [µg RE/Tag]
1 - 3	800
4 - 6	1100
7 - 10	1500
11 - 14	2000
15 - 17	2600
Adults	3000

In its report SCF stresses that the influence of vitamin A on bone mineral density should not be under-estimated but that the data are not sufficient in order to derive a dose-response relationship or a UL. Given the knowledge currently available, SCF recommends that after menopause women restrict their intake of preformed vitamin A from all sources to on average 1500 µg per day (SCF, 2002).

The study results presented in Chapter 4.3.1 indicate that the effects on bone density must be taken into account not only for women after menopause but also for men of that age (Michaëlsson *et al.*, 2003). There is still a need for considerable research in this area. In the opinion of BfR both women after menopause and adult men should not take on average more than 1500 µg preformed vitamin A per day from all sources for precautionary reasons until more up-to-date scientific findings are available. This opinion is shared by the Expert

Group on Vitamins and Minerals of the United Kingdom (EVM) (Food Standards Agency, 2003).

4.4.1 Derivation of a maximum level for vitamin A in food supplements

Up to now in Germany a maximum level of 800 µg retinol per daily dose was accepted for food supplementation.

In other chapters of our report we sometimes used a formula for the derivation of maximum levels for micronutrients which is not applicable here as the 97.5 percentile of the population in Germany already ingests retinol from normal food in amounts which are higher than the UL. This would lead to a negative value in the numerator of the formula.

When deriving maximum levels for the use of preformed vitamin A in food supplements and fortified foods, the following must be borne in mind:

- More than a quarter of the German population does not achieve the recommended intake for vitamin A and around 10% have a retinol intake which is below the lowest intake threshold defined in Europe.
- The food consumption surveys show that vitamin A intake is lowest amongst young people aged between 13 and 18.
- Since a 10 to 50% higher vitamin A intake is recommended to pregnant and lactating women because of their increased requirements than to the general population, there is a special risk of inadequate vitamin A supply in these groups.
- A high intake of preformed vitamin A at the beginning of pregnancy is mainly linked to the risk of teratogenic effects when these high amounts of vitamin A are taken as supplements. A high vitamin A intake from normal, unfortified foods does not bear a risk of this kind according to the knowledge available today. Up to now, liver had high vitamin A concentrations because feed had been fortified with vitamin A.
- In the case of chronically high intake of preformed vitamin A (>1500 µg per day) from all sources, negative effects on bone density cannot be ruled out. The same possibly applies to very low intakes.

4.4.1.1 Possible management options

a) Maintaining the previous maximum level of 800 µg preformed vitamin A per daily dose
Advantages: None observed

Disadvantages: A large proportion of the population ingest more than 1500 µg per day in total in the case of additional intake of 800 µg retinol. Food supplements with this daily dose would not be suitable for children or adolescents because the UL for these age groups would largely be used up or would be exceeded in the higher percentiles (see Table 7 in Annex 4.7).

b) Limit to 400 µg preformed vitamin A per daily dose
Advantages: In the case of less well-nourished persons, this vitamin A level can make an effective contribution to vitamin A supply without the well-nourished groups in the population having to fear a health risk from excessive vitamin A intake. Aside from the 97.5 percentile, no group in the population exceeds an intake of 1500 µg retinol per day (see Table 8 in the Annex 4.7).

Disadvantages: Some of 4 to 10 year-old children would almost completely use up the UL for this age group through the additional ingestion of 400 µg retinol per day.

Persons, whose intake is well below the recommendations or whose requirements are greater, would not achieve the recommended intake either through the additional ingestion of retinol-containing food supplements with the proposed maximum level. For instance, for some of the women who are pregnant or lactating, this dose could still be too low to achieve overall vitamin A intake which covers their requirements (the intake from their other foods would have to be at least 700 and 1100 µg respectively for these groups).

c) Limit to 400 µg preformed vitamin A per daily dose for adults and to 200 µg for children aged between 4 and 10

Advantages: See Option b)

Disadvantages: The disadvantage described under b) would persist that persons, whose intake is well below the recommendations, could probably not achieve the recommended intake by taking retinol-containing food supplements at the proposed dose.

d) No use of retinol in food supplements

Advantages: There is no risk of excessive intake through the taking of retinol-containing food supplements by the well-supplied groups in the population.

Disadvantages: Persons who have increased requirements would not have an opportunity to improve their vitamin A supply through retinol-containing food supplements.

4.4.2 Derivation of a maximum level for vitamin A in fortified foods

In the case of vitamin A the safety margin between recommended intake and the level upwards of which health risks must be expected, is relatively low. Since the fortification of conventional foods can lead to excessive intake of vitamin A in the case of uncontrolled, possibly one-sided consumption of specific products and portions that vary greatly from one individual to another, no deviations should be made from the previous procedure of only permitting the addition of preformed vitamin A to conventional foods in exceptional cases (margarine and mixed fat products).

The French Expert Committee AFSSA also notes in its report on the fortification of foods with minerals and vitamins in January 2002 that retinol should not be used for the fortification of foods because of the low safety margin between normal dietary intake and the tolerable upper intake level for the general population (AFSSA, 2002).

The safety margin (margin between recommended intake and UL) is low (<5) in the case of preformed vitamin A. In some age groups the 97.5 percentile of retinol intake is even higher than the UL. Furthermore, the results from various studies indicating that high chronic vitamin A intake from all sources can lead to undesirable reduction of bone density must be taken seriously. Preformed vitamin A must, therefore, in the opinion of BfR be classified in the highest risk category.

Since, in most cases, food supplements are taken on the basis of the subjective feelings of an individual and not on the basis of a diagnosed deficiency, it would be appropriate for the protection of the already (more than) adequately supplied population groups, to refrain from

offering any food supplements or fortified foods with preformed vitamin A. On the other hand, more than 25% of the population do not reach the recommended intake for vitamin A.

BfR, therefore, recommends food supplements with maximum 400 µg retinol per daily dose for adults and maximum 200 µg per daily dose for children aged between 4 and 10 (Option c). BfR is of the opinion that, aside from in margarine and mixed fat products, preformed vitamin A should not be used to fortify foods. Instead the population should be encouraged to use the natural sources of vitamin A including liver (products).

4.5 Gaps in knowledge

- In order to be able to more reliably assess the vitamin A status of the German population, studies would be necessary to determine the nutritional status, (e.g. MRDR test, retinol concentration in human milk) in addition to representative food studies which also record the use of food supplements.
- Further prospective studies are needed to examine the link between the intake of high or low vitamin A amounts and/or the serum retinol level and bone density.

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4.7 Annex

The following tables give an overview of the effect of various food supplement doses on total daily intake of retinol. The data from the National Food Consumption Study served as the basis:

Table 7: Retinol intake from normal food and effects of supplementation with 800 µg retinol per day

	Average retinol intake							
Age and gender	**P 2.5** (µg)	**+ 800** µg	**P 50** (µg)	**+ 800** µg	**P 75** (µg)	**+ 800** µg	**P 97.5** (µg)	**+ 800** µg
Children m/f (4-10 years)	160	960	415	1215	630	1430	**2150**	**2950**
Adolescents m/f (11-18 years)	170	970	480	1280	800	**1600**	**3150**	**3950**
Women (>19 years)	170	970	540	1340	780	**1580**	**3360**	**4160**
Men (>19 years)	210	1010	675	1475	1000	**1800**	**3840**	**4640**

Table 7 shows that through the taking of a food supplement containing 800 µg retinol per daily dose in addition to intake from normal food, the upper level of 1500 µg RE per day is almost reached in the median group and is exceeded in the higher percentiles (values printed in bold). In the highest percentile normal food intake leads, in some cases, to an exceeding of the UL of 3000 µg/day.

Table 8: Retinol intake from normal food and effects of supplementation with 400 µg retinol per day

	Average retinol intake							
Age and gender	**P 2.5** (µg)	**+ 400** µg	**P 50** (µg)	**+ 400** µg	**P 75** (µg)	**+ 400** µg	**P 97.5** (µg)	**+ 400** µg
Children (4-10 years)	160	**560!**	415	815	630	1030	**2150**	**2550**
Adolescents (11-18 years)	170	**570!**	480	880	800	1200	**3150**	**3550**
Women (>19 years)	170	**570!**	540	940	780	1180	**3360**	**3760**
Men (>19 years)	210	**610!**	675	1075	1000	1400	**3840**	**4240**

Aside from the 97.5 percentile, the retinol intakes in all population groups are less than 1500 µg RE if the supplement dose is reduced to 400 µg retinol per daily portion. However, it is also shown that in the case of the 2.5 percentile the recommended intake is not reached even if a food supplement containing 400 µg retinol per day is taken.

5 Risk Assessment of Beta-carotene

5.1 Summary

β-carotene is the most important provitamin A. As a component of fruit and vegetables it makes a decisive contribution to vitamin A supply in Germany, particularly amongst groups of the population whose intake of preformed vitamin A does not cover their requirements. Up to now, however, there have only been unclear ideas about the desirable daily intake of β-carotene. Based on calculated intake levels and the β-carotene plasma concentrations resulting from a specific diet, which were interpreted in epidemiological studies as an indicator for the prophylactic effects of the carotinoids, an estimated value of 2-4 mg per day has been derived for desirable β-carotene intake. Taking into account the current conversion factor of 12, this corresponds to vitamin A activity of 167-333 µg RE.

The results of food consumption studies, from which the β-carotene intake of the German population was calculated, indicate that on average 2 mg per day are ingested. There are no indications of explicit β-carotene deficiencies in human beings (supply category 3).

Since the intake of isolated β-carotene in intervention studies involving heavy smokers led to an increase in the lung cancer rate and in Germany more than 18% of the adult population must be included in the risk group of smokers, there is a high risk of adverse health effects particularly for this group when taking β-carotene in food supplements and fortified foods. The effect of isolated β-carotene in non-smokers or the dose at which a negative effect is to be expected has not yet been definitively ascertained. Until the submission of more up-to-date scientific findings, BfR is of the opinion that the intake of isolated β-carotene should only be undertaken on a very limited scale. This means that extreme caution should be exercised when using β-carotene in foods for technological purposes. Furthermore, BfR recommends orienting the maximum level for β-carotene in food supplements towards the lower estimated value range for desirable intake, i.e. 2 mg per daily dose. Conventional foods should not be fortified with β-carotene in the opinion of BfR.

Estimated values for adequate intake	2-4 mg/day	
Intake [mg/day] (Schulze *et al*., 2001)	**m**	**f**
Median	2.0	1.9
P 10	0.7	0.6
P 90	5.9	7.6
Tolerable upper intake level	not defined	
Proposed **maximum levels** in: food supplements	2 mg/daily dose	
fortified foods	no fortification	

5.2 Nutrient description

5.2.1 Characterisation and identification

β-carotene (CAS No. 7235-40-7) belongs to the group of carotinoids which consist of 8 isoprenoid units. Given their chemical structure carotinoids can be broken down into oxygen-free carotenes, e.g. α-carotene, β-carotene, lycopene and oxygen-containing xanthophylls, e.g. lutein, zeaxanthin, β-cryptoxanthin.

Of the 650 known carotinoids around 50 can be converted into vitamin A whereby β-carotene is the most important. In human serum and human milk 34 carotinoids have been identified

up to now including 13 geometric all-trans-isomers. The most frequently detected are lutein, cryptoxanthin, zeaxanthin, α-carotene, β-carotene and lycopene (Pelz *et al.*, 1998; Watzl and Bub, 2001).

According to Annex 2 of European Directive 2002/46/EC β-carotene may currently be added to food supplements as a source of vitamin A. In the Annex to the Proposal for a Regulation on the addition of vitamins and minerals and certain other substances to foods (COM(2003) 671 final of 10.11.2003), β-carotene is also envisaged as a source of provitamin A for the fortification of conventional foods. Furthermore, β-carotene is a dye and according to Annex 1 of Directive 94/36/EC may be used in a number of foods either at the maximum level of 100 mg/kg or quantum satis for colouring purposes.

5.2.2 Metabolism, function, requirements

Metabolism: β-carotene is absorbed, after release from the food matrix, in the small intestine together with other lipids by passive diffusion. Some of the β-carotene is cleaved in the enterocytes either at a central double bond and converted into retinol or cleaved at eccentric double bonds to apo-carotenals. After re-esterification, the retinyl esters together with the non-cleaved β-carotene and lipids are incorporated into chylomicrons and released into blood by the intestinal lymph. Bound to the lipoproteins, β-carotene is transported in the plasma whereby 58-73% of the β-carotene is bound to LDL, 17-26% to HDL and 10-16% to VLDL (Parker, 1996; Solomons, 2001; Wolf, 2001). The highest concentrations of β-carotene are found in the liver, adrenal glands, kidneys and ovaries (Stahl *et al.*, 1992). The total body content of β-carotene is between 100 and 150 mg. In the case of a normal mixed diet the β-carotene concentrations in the serum reach 200 to 400 µg/L (0.4-0.75 µmol/L). β-carotene can pass through the placenta and reach human milk.

Bioavailability: the bioavailability of β-carotene depends on:

- the form of the carotinoid (all-trans β-carotene is absorbed more easily than the cis-isomer)
- the amount of β-carotene taken up with food
- the food matrix in which the β-carotene is bound
- the supply status of human beings
- genetic factors (Castenmiller and West, 1998; Parker *et al.*, 1999; Parker, 1996).

Depending on the fat content of food the absorption rate of β-carotene from foods of plant origin is between 30 and 60%. Furthermore, the bioavailability of β-carotene is influenced by whether it is present in the food matrix in crystalline form, esterified or emulsified in fat. Furthermore, availability depends on the way in which the food was prepared, on mechanical breakdown in the upper digestive tract and the pH value of the stomach. β-carotene is absorbed far more easily from processed or cooked foods than from raw vegetables. Furthermore, absorption is inhibited by roughage (Edwards *et al.*, 2002). In isolated form (from supplements) β-carotene is more easily absorbed than in native form (from fruit and vegetables) (van het Hof *et al.*, 2000). This is reflected in a far higher increase in the β-carotene plasma level after taking supplements compared with the intake of the same amounts from normal food.

Given the many different factors which influence the bioavailability of β-carotene, FAO and WHO (1967) set a conversion factor of 6 for the vitamin A activity of β-carotene in 1967. According to this, the intake of 6 mg β-carotene was necessary to achieve vitamin A activity corresponding to the intake of 1 mg preformed vitamin A. As already outlined in Chapter 1, it is assumed – on the basis of more recent findings - that 12 µg β-carotene are equivalent to 1 µg retinol (RAE - Retinol Activity Equivalents), whereas for all other vitamin A active

carotinoids the factor 24 is assumed (IOM, 2001). De Pee *et al.* (1998) even take a factor of 26 as the basis for β-carotene when it is taken up from green leafy vegetables and carrots.

When it comes to the intake of carotinoid mixtures, the individual carotinoids compete and interact and impede or promote each other (Elmadfa and König, 2002). For instance, in the ATBC Study, supplementation with 20 mg β-carotene also led to an increase in α-carotene, β-cryptoxanthin and lutein serum levels.

Function: The only proven function of β-carotene in human beings up to now is its vitamin A activity. In particular for population groups, who have very low levels of preformed vitamin A, fruit and vegetables rich in β-carotene play a major role when it comes to covering vitamin A requirements. Other carotinoids with this vitamin A function are α-carotene, β-cryptoxanthin, 13-cis-β-carotene and β-apo-8-carotenal.

Physiological effects: β-carotene is antioxidative. It is able to scavenge oxygen radicals and other oxidants (by quenching the singlet oxygen and inhibiting lipid peroxidation) and, in this way, to protect for instance the cell membranes from oxidative damage. Furthermore, immunostimulating effects have been described. In intervention studies it was observed that β-carotene at a dose of up to 25 mg/day in men over 65 years of age increased the activity of natural killer cells. In the 51-64 year-old men, the expression of adhesion molecules and the *ex vivo* secretion of the tumour necrosis factor α were increased (Bayer and Schmidt, 1991; Watzl and Bub, 2001). According to *in vitro* studies various carotinoids specifically stimulate communication between cells by means of the gap junctions[1], by increasing the expression of mRNA for connexin. Since it is assumed that a disturbed signal transfer via the gap junctions leads to uncontrolled cell growth, β-carotene could help to improve intercellular communication and possibly protect against cancer (Watzl and Bub, 2001).

Requirements: There are no clear ideas up to now about the desirable daily intake of β-carotene. Based on the results from animal, cell culture and epidemiological studies, a protective effect of β-carotene with regard to the risk of the onset of chronic diseases has been derived (Erdman Jr., 1999; Pryor *et al.*, 2000; Slattery *et al.*, 2000; Thurnham and Northrop-Clewes, 1999). In epidemiological studies β-carotene plasma concentrations >0.4 µmol/L were associated with a lower risk of the onset of chronic disease. However, this observation does not suffice in order to define β-carotene requirements as the protective effect could have been triggered by other substances in fruit and vegetables or through specific behaviour characteristics which go hand in hand with increased consumption of fruit and vegetables. In controlled, large-scale intervention studies no positive or, in the case of smokers, unexpectedly negative effects on the onset of lung cancer were identified in conjunction with the administration of β-carotene supplements (20-50 mg/day) (Heinonen and Albanes, 1994; Hennekens *et al.*, 1996; Kritchevsky, 1999; Omenn *et al.*, 1996). Against this backdrop a favourable diet with a high level of β-carotene-containing fruits and vegetables rather than the β-carotene itself seems to be responsible for the preventive effect observed in epidemiological studies. Based on the calculated intake levels of β-carotene from normal food, an estimated value of 2-4 mg per day was derived by D-A-CH as desirable β-carotene intake (Biesalski *et al.*, 1997; DGE/ÖGE/SGE/SVE, 2000; Gaßmann, 1998).

If one looks at the only proven function for β-carotene in humans, i.e. its vitamin A activity, then the intake of β-carotene on the level of the estimated value range taking into account the current conversion factor 12 corresponds to vitamin A activity of 167-333 µg retinol equivalents, i.e. 15-30% of recommended vitamin A intake.

[1] Gap junctions are canal-like links between two cells which consist of one protein connexin.

5.2.3 Exposure (dietary and other sources, nutritional status)

Sources:
Foods: The main sources of β-carotene are yellow and green (leafy) vegetables and yellow fruits, in particular carrots, spinach and pumpkin as well as broccoli, grapefruit, mangos, peaches and tomato (juice). β-carotene is relatively heat stable but sensitive to exposure to light and oxygen.

Dye: In Germany β-carotene is used in roughly 5% of all foods as a dye whereby on average amounts of between 1 and 5 mg/kg or mg/L are added to solid food and drinks. German manufacturers estimate that the intake of β-carotene as a dye is approximately 0.5 mg per person/day (BLL, 2001). SCF assumes that the amount of β-carotene ingested as an additive is on average 1-2 mg per day (SCF, 2000a; b).

Fortified foods: According to market sales figures, 736 million litres of multivitamin or ACE drinks were sold in Germany in 2000. This corresponds to a mean intake of 4 litre multivitamin drinks and 3 litre ACE drinks per capita/year. In the case of ACE milk products consumption only amounted to 0.18% of total consumption of dairy products (26.9 kg/year). A consumer survey by La Roche involving approximately 1,000 people revealed that 21% of the interviewees regularly drink multivitamin beverages and around 5% regularly drink ACE beverages (BLL, 2001). According to a survey by Tennant *et al.* (2004) β-carotene fortified drinks with concentrations of <1 mg/100 ml (30%) or 1-2 mg/100 ml (58%) are the ones most frequently on sale on the market in Germany. Drinks with β-carotene levels between 2 and 2.5 mg/100 ml or >2.5 mg/100 ml accounted for 9% and 3% of the fortified drinks.

Food supplements: In food supplements either synthesised or all-trans-β-carotene is used or a mixture of all-trans and 9-cis-isomers extracted from algae, mushrooms or other foods of plant origin. According to a recent survey there are food supplements with an average 13.3 mg β-carotene per daily dose on the market; hence the minimum dose is 0.5 and the maximum is 100 mg per daily dose (Tennant *et al.*, 2004). According to the above-mentioned consumer survey from 2000 10% regularly take multivitamin supplements, 2% of which are food supplements with ACE vitamins (BLL, 2001).

Medicinal products: For the treatment of erythropoietic protoporphyria (EPP) the Food and Drug Administration (FDA) recommends a maximum dose of 300 mg β-carotene per day for adults (Anstey, 2002). In Germany β-carotene is used to treat EEP, polymorphous light dermatitis and pigment disorders (e.g. white spots disease, so-called vitiligo, dark spots on the skin, so-called hyperpigmentations) depending on sunlight exposure at daily doses of 50-200 mg for adults and 50-125 mg for school children (Hermal-Fachinformation, 2001). Within the framework of a graduated plan procedure that is on level II (as per June 2003), BfArM intends:

(1) medicinal products containing β-carotene as the active substance and in which the recommended maximum daily intake level of 20 mg is exceeded to carry a warning that this medicinal product may not be taken by smokers.

(2) medicinal products containing the active substance β-carotene and for which the recommended maximum daily intake level is between 2 and 20 mg to carry a warning that this medicinal product should not be taken regularly by smokers over a longer period.

Nutritional status:
Intake: In the 1980s the mean total carotinoid intake was 5.39 mg/day for men and 5.27 mg/day for women according to the National Food Consumption Study (NFCS) (Heseker *et al.*, 1994). Pelz *et al.* (1998) later calculated from the NFCS data mean β-carotene intake:

based on these calculations men and women both ingested on average 1.81 mg β-carotene per day. According to the NVS data, 52% of the ingested β-carotene stems from fresh vegetables and 10% from juices and soft drinks. Müller (1996) indicates a median β-carotene intake between 1.1 and 1.4 mg/day for the German population. However, this information is based solely on consumption data without taking into account differences in the bioavailability of different foods and forms of preparation. For instance, the bioavailability of β-carotene from drinks is far better than that from the solid food matrix which means that juices and soft drinks, despite their lower share in total consumption, probably contribute to a higher degree to β-carotene supply. Heseker *et al.* (1994) indicate that the German population, according to NFCS, covers 25-30% of its total vitamin A intake through β-carotene-containing foods. All the same, the contribution of β-carotene to vitamin A supply falls to around 12-15% if the conversion factor 12 is taken as the basis.

According to the EPIC Study the men examined there ingested on average 2 mg and the women 1.9 mg β-carotene per day. Nevertheless, these values fluctuated in men between 0.7 mg (P 10) and 5.9 mg (P 90) and in women between 0.6 mg (P 10) and 7.6 mg (P 90) per day (Schulze *et al.*, 2001).

Up to now, none of the consumption studies took β-carotene intake through the consumption of food supplements and fortified foods into account.

Plasma concentrations: The average plasma value of β-carotene is between 0.4 and 0.75 µmol/L in healthy individuals. According to Biesalski *et al.* (1997) it should not exceed 3 µmol/L; Woutersen *et al.* (1999) consider a β-carotene plasma value of ≤ 2 µmol/L to be safe.

In the VERA Study β-carotene plasma values were measured between 0.1 (P 2.5) and 1.56 µmol/L (P 97.5) in men and between 0.16 (P 2.5) and 2.19 µmol/L (P 97.5) in women. The lowest values were obtained for men aged between 35-44. In the group of women the plasma values are the lowest in the 25-44 age group. Around 75% of the study group reached a plasma value of 0.5 µmol/L (Biesalski *et al.*, 1997). β-carotene concentrations below 0.18 µmol/L were measured in 10.7% of the male and 3.4% of the female study group (Heseker *et al.*, 1992).

The values varied considerably within the groups and the β-carotene concentrations of the female population are on average 40% higher than those of the men. Other factors which may influence the level of the plasma values are, for instance, age and health condition but also smoking and alcohol consumption.

β-carotene is the most important provitamin A and it makes an important contribution to covering vitamin A requirements particularly in individuals whose vitamin A intake (as preformed vitamin) is low. Furthermore, it was observed in epidemiological studies that the onset of chronic diseases is reduced by eating large amounts of fruit and vegetables. This could be attributable, amongst other things, to the related intake of β-carotene from these sources. However, intervention studies have not been able to confirm the protective effect of isolated β-carotene administration up to now. Based on the epidemiological observations, a desirable plasma value for β-carotene of >0.4 µmol/L was indicated as the guidance value for adequate intake. This can be achieved by β-carotene intake on the level of the estimated value range of 2-4 mg per day. The existing studies indicate that in Germany on average 2 mg β-carotene is ingested daily from normal food. There are no signs of explicit β-carotene deficiencies in human beings (supply category 3).

5.3 Risk characterisation

5.3.1 Hazard characterisation (NOAEL, LOAEL)

Amounts of 20-180 mg β-carotene/day (ingested over several years as a high dose medicine against erythropoietic protoporphyria) did not trigger any toxic reactions or any unusually high retinol plasma levels in human beings (Mathews-Roth, 1990). Nor are there any reports of teratogenic effects of β-carotene in humans. The only observations concern aurantiasis of the skin (hypercarotinaemia) at a daily intake of 25-30 mg β-carotene and from a total carotinoid concentration in the serum of around 4000 µg/L (7.5 µmol/L). These side effects could be reversed after discontinuation of treatment (Bayer and Schmidt, 1991).

Both in the ATBC and in the CARET Study, the lung cancer rate and the number of fatalities caused by cardiovascular disease in the case of existing myocardial infarction increased amongst heavy smokers and asbestos workers who were given 20 mg isolated β-carotene daily, in combination with vitamins A and E or on its own. In the Physician's Health Study, neither a positive nor a negative effect was observed after supplementation with 50 mg β-carotene or aspirin per day over 13 years. In the Heart Protection Study (2002), in which over a period of 5 years 20 mg β-carotene was administered in a placebo-controlled manner together with 600 mg vitamin E and 250 mg vitamin C per day for the purposes of secondary prevention, there was a slight but statistically significant increase in total cholesterol, LDL cholesterol and triglycerides in the plasma of the treated group. Furthermore, in this study neither positive nor negative effects could be detected on mortality caused by vascular or coronary heart disease, stokes or on the incidence of myocardial infarction, the cancer rate or other chronic diseases (Heart Protection Study Collaborative Group, 2002)

The plasma levels (>3 µmol/L) considered critical by Biesalski *et al.* (1997) were reached or exceeded in both the ATBC and CARET studies whereas the values in the Physicians' Health Study and also in the Heart Protection Study were below 3 µmol/L. (study design of ATBC, CARET, PHS, HPS: see Figure 1).

Figure 3: (according to Heinonen and Albanes, 1994; Omenn *et al.*, 1996; Heart Protection Study Collaboration Group, 2002)

	The α-Tocopherol, β-Carotene Cancer Prevention Trial (ATBC) **National Cancer Institute and the National Public Health Institute of Finland**
Collective	**29,133 male smokers in Finland (50-69 years)**
Supplement	**50 mg tocopherol, 20 mg β-carotene or both (placebo controlled)**
β-carotene in plasma • at the start • after 3 years	 **Median = 0.3 µmol/L** **Median = 5.6 µmol/L**
Duration	**5-8 years**
Result	**18% more pulmonary carcinomas in the β-carotene group,** **8% more fatalities in the β-carotene group**
	The β-Carotene Cancer and Retinol Efficiency Trial (CARET) National Cancer Institute (USA)
Collective	18,314 participants (50-69 years), smokers or former smokers
Supplement	750 µg retinol and 30 mg β-carotene (placebo controlled)
β-carotene in plasma • placebo group • β-carotene group	 x = 0.34 µmol/L x = 4.2 µmol/L
Duration	4 years (prematurely abandoned after 21 months)

Result	28% more pulmonary carcinomas, 17% more fatalities
	The Physicians' Health Study (USA)
Collective	22,071 male participants (physicians) (40-84 years)
Supplement	alternately either 50 mg β-carotene or aspirin (placebo controlled)
β-carotene in plasma	
• placebo group	x = 0.56 µmol/L
• β-carotene group	x = 2.24 µmol/L
Duration	13 years
Result	No effect of β-carotene supplementation on the cancer rate in general or on any of the especially examined types of cancer (lung, prostate or colonic cancer)
	Heart Protection Study (UK)
Collective	20,536 participants (patients with coronary heart diseases) (40-80 years)
Supplement	20 mg β-carotene, 600 mg vitamin E, 250 mg vitamin C (placebo controlled)
β-carotene in plasma	
• placebo group	x = 0.32 µmol/L
• β-carotene group	x = 1.22 µmol/L
Duration	5 years
Result	No effect of β-carotene supplementation on mortality caused by vascular or coronary heart diseases, strokes or on the incidence of myocardial infarctions, cancer rate or other chronic diseases.

In 22,269 people of the ATBC collective who had been free from coronary heart diseases at the beginning an elevated albeit not significant, incidence of angina pectoris was observed in the group supplemented with β-carotene (Rapola *et al.*, 1996). Furthermore, in conjunction with the ATBC Study it was observed that in 28,519 people, who had not suffered a stroke prior to the commencement of the study, supplementation with β-carotene increased the risk of intracerebral haemorrhage whereby the underlying mechanism is not clear (Leppälä *et al.*, 2000). The incidence of strokes was not influenced by β-carotene supplements.

Another placebo-controlled study, AREDS[2], examined whether in 3,640 participants (55-80 years old) with, in some cases, advanced eye diseases, supplementation with antioxidants and/or zinc [(A): 500 mg vitamin C, 400 IU vitamin E, 15 mg β-carotene; (B): 80 mg zinc and 2 mg copper; (C): a combination of vitamins and zinc or (D): placebo] can halt the onset or progression of cataracts and/or age-related macular degeneration. At the start of the study 8% of the participants were smokers and 49% former smokers. Once a slight but non-significant increase in mortality in the antioxidant group had been observed soon after the start of the study, the smokers were taken off β-carotene supplementation. In the further course of the study no influence of smoking habits could be confirmed on the observed trend towards elevated mortality. Overall, no reliable conclusions can be drawn from the ARED Study concerning the safety of β-carotene for the general population. No positive effect of β-carotene administration could be determined (Age-Related Eye Disease Study Research Group, 2001).

A meta-analysis, including eight[3] large (>1,000) randomised studies, to analyse the effect of the administration of β-carotene supplements (15-50 mg/day over a period of 1.4 to 12 years)

[2] AREDS = Age-Related Eye Disease Study
[3] ATBC, CARET, HPS, SCP, AREDS, NSCP, PHS, WHS

on health revealed a slight but significant increase in overall mortality in the supplemented group and a slight increase in mortality as a consequence of cardiovascular disease (Vivekananthan *et al.*, 2003).

In the SU.VI.MAX-Study (SUpplementation en VItamines et Mineraux AntioXidants), another double-blind placebo-controlled intervention study with more than 10,000 test persons aged between 35 and 60, a preparation with 6 mg β-carotene, 120 mg vitamin C, 30 mg vitamin E, 100 µg selenium and 20 mg zinc was administered daily over a period of on average 7.5 years. From the results published so far from this study, no negative or positive effects of the administered nutrient combination can be derived (Malvy *et al.*, 2001; Zureik *et al.*, 2004).

In another double-blind placebo-controlled study with 864 participants, whose colonrectal adenoma had been removed and who were free of polyps, the effect of supplementation with 25 mg β-carotene, alone or in combination with vitamin C (1 g/day) and vitamin E (400 mg/day) was examined. In the group that neither smoked nor drank alcohol, the ingestion of β-carotene was associated with a lower risk of the recurrence of adenomas whereas in the group of smokers and alcohol drinkers the risk of adenoma was slightly increased and doubled when smokers drank more than one glass of alcohol per day (Baron *et al.*, 2003). It is again clear that lifestyle – in this case the consumption of alcohol in addition to the smoking of cigarettes – can have a negative impact on the physiological effect of β-carotene on cancer. The power of this study is not sufficient in order to derive general statements about the safety of β-carotene. It is known that β-carotene – depending on the matrix, composition and isomer portions of a carotinoid mixture – may be absorbed to differing degrees and have various effects. At present, however, it is still not clear what relevance this has for the negative results of the intervention studies or which mechanism is actually responsible for these observed effects.

From animal experiments with ferrets, the most suitable test model in terms of metabolism and the kinetics of β-carotene, it is known that high doses of β-carotene lead to increased activity of P450 enzymes. This, in turn, leads to a reduction of the retinoic acid level and disruptions of the retinoid signal pathways. This then triggers a cascade of effects which, in the final instance, lead to the observed cell proliferation in the lung. In animal experiments it was, however, also observed that the negative effects occur in relationship to the dose of β-carotene but independently of exposure to cigarette smoke (Russell, 2004).

For instance, Wang *et al.* (1999) showed that a dose of 2.4 mg/kg bw/day administered over a period of 6 months, without the additional influence of tobacco smoke, triggered proliferative changes and keratinised metaplasia of the pulmonary mucosa. Because of the ferret's absorption rate which is approximately five times lower, this dose corresponded to an intake of around 30 mg β-carotene/day in man. The pathological change which was deemed to be pre-carcinogenic (Wolf, 2002) was accompanied by a statistically significantly lower level of retinoic acid in the pulmonary tissue and a reduction of gene expression of the retinoic acid receptor RARβ. The dose of approximately 0.43 mg/kg bw/day tested in another study did not show any pathological changes any more; however, it only corresponded to a dose of 6 mg β-carotene/day in man (Liu *et al.*, 2000).

The study results up to now show that supplementation with β-carotene is only safe if β-carotene plasma values of 2-3 µmol/L are not exceeded. Furthermore, they showed that the intake of high dose β-carotene supplements can lead to competitive inhibition of other protective food components (Neuhouser *et al.*, 2003; Russell, 1998).

Since no dose-response relationship can be derived from the results of the β-carotene studies conducted up to now, neither a NOAEL or a LOAEL can be defined for this substance (SCF, 2000a; b).

5.3.2 Deficiency, possible risk groups

There is no evidence of clinical deficiency in man which can be directly attributed to low β-carotene intake. Only in groups in the population with marginal vitamin A intake can a low β-carotene intake have an additional negative effect on vitamin A status.

5.3.3 Excessive intake, possible risk groups

People who regularly take β-carotene-containing supplements and drink ACE or multivitamin juices as well, could reach or exceed the critical intake threshold of 20 mg isolated β-carotene per day. For Germany, however, there are no reliable data which permit an estimation of intake of β-carotene from food supplements or fortified foods. In line with the survey by Tennant *et al.* (2004), intakes of 2-4 mg/day average are expected in conjunction with the ingestion of β-carotene from fortified beverages. In the higher percentiles the authors are of the opinion that the intake of β-carotene from fortified drinks may amount to between 10 and 20 mg β-carotene/day.

5.4 Tolerable upper intake level for β-carotene

Up to now there are no indications from animal experiments that high doses of β-carotene have a teratogenic effect. However, no experience from epidemiological studies in human beings about the intake of high β-carotene announced during pregnancy is available. Since β-carotene may be converted in the human metabolism to all-trans-retinoic acid, safety studies would have to be conducted in future about the intake of β-carotene during pregnancy, particularly because the absorption of β-carotene from supplements is clearly far higher than from normal foods (Schulze *et al.*, 2001).

Given the negative results of large-scale controlled studies with β-carotene supplements, the Scientific Committee on Food of the European Union revoked in 2000 the ADI value for the acceptable daily intake of 5 mg β-carotene/kg bodyweight and advised that caution be exercised when using β-carotene in an isolated form. Since no dose-response relationships for β-carotene can be derived either from human studies or on the basis of suitable animal models and since there is no information on the specific effects of individual β-carotene isomers, no scientifically validated UL can currently be defined for β-carotene intake (SCF, 2000b).

Based on the estimate that β-carotene is taken up from normal food in amounts between 2 and 5 mg and as a dye between 1 and 2 mg per day, SCF derives a basic value for daily intake from both sources which is between 3 and 7 mg (up to maximum 10 mg) per day. It notes that the margin between the intake level, which was deemed to be desirable and safe up to now based on results from epidemiological studies (intake from normal food), and the intake level which has led to negative effects in the ATBC Study is very low. SCF is, therefore, of the opinion that caution should be exercised in conjunction with β-carotene supplementation and fortification (SCF, 2000b). However, all studies up to now indicate that the intake of isolated β-carotene was linked to negative effects but not the uptake from normal food (IOM, 2000; Männistö *et al.*, 2004).

Around 18% of the adult population in Germany belongs to the group of heavy smokers. They, therefore, constitute a possible risk group for negative health effects through the intake of isolated β-carotene. According to a report by the Institute for Therapy Research, there are 13.1 million smokers in Germany in the age group 18-69. That is 23% of the age group (the age group encompasses a total of 57.2 million people – as per 2000) who smoke 6 or more cigarettes a day, 6.3 million of this age group (= 11%) smoke 20 or more cigarettes a day whereas 4.3 million (7.5%) are nicotine dependent according to the DSM-IV (Diagnostic and

Statistical Manual of Mental Disorders - Fourth Edition) (Institut für Therapieforschung, 2002).

Since there is no known dose-response relationship for the intake of isolated β-carotene and, furthermore, it has not yet been clarified whether the risk of adverse effects could also apply to non-smokers, BfR is of the opinion that, for reasons of precautionary consumer health protection, β-carotene should not be used at all or only on a very limited level in food supplements and fortified foods.

In its report "Safe Upper Levels for Vitamins and Minerals" (2003) the UK Expert Group on Vitamins and Minerals (EVM) recommended in conjunction with the use of isolated β-carotene that "normal consumers" should not ingest more than 7 mg isolated β-carotene per day. Furthermore, EVM suggests that smokers and asbestos workers should, in principal refrain, from taking isolated β-carotene (Food Standards Agency, 2003).

5.4.1 Derivation of a maximum level for β-carotene in food supplements and fortified foods

Since, up to now, no UL could be defined for β-carotene, a maximum safe level for use in food supplements and fortified foods cannot be calculated with the help of the formula applied elsewhere.

Proposals for maximum levels are, therefore based on the following considerations:

- In Germany 2 mg β-carotene are ingested on average from natural sources daily. Based on manufacturers' information the intake from dyes is between 0.5 and maximum 2 mg per day.
- The claimed health-promoting benefit of additional β-carotene administration has been shown to be untenable in controlled studies.
- A physiological need for β-carotene supplementation only exists for the purposes of improving the vitamin A supply of the population.
- For the derivation of maximum levels for β-carotene in food supplements and fortified foods, the contribution from normal food can be ignored because previous observations indicate that a diet rich in fruit and vegetables can be recommended without any concerns or volume constraints.
- The intake of 20 mg β-carotene in the form of supplements led to an elevated lung cancer rate amongst heavy smokers and asbestos workers in intervention studies.
- It has not yet been clarified whether β-carotene in isolated form can also trigger negative effects in non-smokers. Until the mechanism for the onset of lung cancer has been clarified in conjunction with β-carotene supplementation, it cannot be ruled out that other forms of exposure in conjunction with the ingestion of β-carotene supplements cause an effect similar to the one observed in smokers.

5.4.1.1 Possible management options for the use of β-carotene in food supplements

a) Limiting the maximum level in food supplements to 2 mg per daily dose, as already recommended in the past by BgVV/BfR

   This amount corresponds to the lower estimated value of desirable intake of β-carotene. The taking of two food supplements and an intake of 1-2 mg β-carotene as dye/day would mean ingestion of maximum 6 mg β-carotene/day in addition to intake from normal food.

Advantages: This amount was largely accepted in recent years by the manufacturers of food supplements. There are no known negative health effects up to now from the use of this amount of β-carotene. There does not seem to be any need for a specific warning for risk groups.

Disadvantages: None identifiable

b) Limiting the maximum level in food supplements to 2-4 mg per daily dose

This amount is oriented to the estimated value range of desirable β-carotene intake defined by D-A-CH. Taking into account intake of 1-2 mg β-carotene as dye/day and assuming that consumers may take not just one but two food supplements a day, maximum 10 mg isolated β-carotene per day would be ingested from various sources in addition to normal food.

Advantages: None identifiable

Disadvantages: We do not know from what amounts below 20 mg β-carotene/day negative health effects may occur. Therefore, it cannot be stated with any reliability whether a total amount of isolated β-carotene of 10 mg/day is sufficiently safe.

5.4.1.2 Possible management options for the use of β-carotene in fortified foods

a) Limiting β-carotene addition to fortified foods to 2 mg per 100 g or 100 ml, as proposed in the BMVEL draft ordinance (BMVEL- File ref.: 222-8161-4)

Advantages: Constraints would be imposed on the uncontrolled β-carotene fortification of foods practised up to now. This would reduce the risk of the ingestion of high levels of β-carotene which would be advantageous in particular for the risk group of smokers.

Disadvantages: In the case of consumers, who take food supplements with β-carotene and individuals who regularly eat fortified foods, there is a risk that high levels of β-carotene would be ingested. Merely drinking 0.5 litre of a fortified soft drink would lead to ingestion of 10 mg β-carotene. The products would need to carry information or comprehensive risk communication would be needed in order to draw consumers' attention to the health risks related to β-carotene.

b) No fortification of foods with β-carotene

Advantages: The sole use of β-carotene as a dye and in food supplements would restrict the intake of this substance to such an extent that no risk to health is to be expected.

Disadvantages: None identifiable

Since the intake of isolated β-carotene in intervention studies involving heavy smokers led to an increase in the lung cancer rate and in Germany more than 18% of the adult population must be included in the risk group smokers, there is a high risk of adverse health effects particularly for this group when taking β-carotene in food supplements and fortified foods. The effect of isolated β-carotene in non-smokers or the dose at which a negative effect is to be expected has not yet been definitively ascertained. Until the submission of more up-to-date scientific findings, BfR is of the opinion that the intake of isolated β-carotene should only be undertaken on a very limited scale. This means that extreme caution should be exercised when using β-carotene in foods for technological purposes. Furthermore, BfR recommends orienting the maximum level for β-carotene in food supplements towards the lower estimated

value range for desirable intake, i.e. 2 mg per daily dose (Option a). Conventional foods should not be fortified with β-carotene in the opinion of BfR (Option b).

5.5 Gaps in knowledge

- There are no representative data on the uptake of β-carotene from food supplements or fortified foods.
- There are gaps in knowledge concerning the mechanism of action of isolated β-carotene (and its isomers) and possible interactions amongst carotinoids themselves and with other fat soluble vitamins, food components, medicinal products or xenobiotics. It seems advisable to examine interactions of this kind in order to guarantee consumer safety when consuming food supplements and fortified foods. To do this, further studies are necessary in ferrets involving different doses of β-carotene.

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6 Risk Assessment of Vitamin D

6.1 Summary

The data available for the Federal Republic of Germany on the vitamin D status indicate that there is a risk of a clinical deficiency or store depletion for specific age groups, particularly pregnant women, lactating women, infants and young children as well as older people, especially when they are only rarely exposed to sunlight. Dark-skinned migrants are another risk group (supply category 1). According to BfR estimates, the use of vitamin D in food supplements or for the purposes of food fortification is linked to a high food safety risk (cf. Table 2).

It is recommended that no food supplements with vitamin D be authorised for infants or young children under the age of two whilst retaining medication-based rickets prophylaxis.

For reasons of preventive health protection we recommend the setting of the recommended maximum safe level of 5 µg in food supplements for children up to the age of 10, adolescents and adults (up to and under age 65) and of 10 µg for older people (65 years and above). For fortified foods a limited extension of the offering is recommended, where appropriate, e.g. in edible oils with a maximum level of 20 µg/L taking into account national eating habits and changes in dietary behaviour.

Recommended intake	5 µg/day (10 µg/day adults >65 years)	
Intake [µg/day] (NFCS, 1994)	**m**	**f**
Median	4.02	3.12
P 2.5	1.17	1.0
P 97.5	16.8	11.9
Tolerable upper intake level	50 µg/day (adolescents, adults) 25 µg/day (children 2-10 years)	
Proposal for **maximum levels** in:		
food supplements	5 µg/daily dose (children <10 years, adolescents, adults <65 years) 10 µg/daily dose (adults >65 years)	
fortified foods	limited extension of offering (e.g. edible oils: 20 µg/L)	

6.2 Nutrient description

6.2.1 Characterisation and identification

Vitamin D is the generic term for a series of biologically active *calciferols*. A distinction is made between synthetic *ergocalciferol (vitamin D_2)* (CAS No. 50-14-6) and *cholecalciferol (vitamin D_3)* (CAS No. 67-97-0) which occurs in foods of animal origin. Vitamin D levels are normally stated in weight units. One International Unit (IU) vitamin D corresponds to 0.025 µg vitamin D or 1 µg vitamin D corresponds to 40 IU.

Vitamin D may only be added to foods in the form of ergocalciferol (vitamin D_2), cholecalciferol (vitamin D_3) and cholecalciferol-cholesterol (vitamin D_3-Cholesterol) (Ordinance on requirements to be met by food additives and marketing of food additives authorised for technological purposes - Additives Marketing Ordinance, ZVerkV, Annex 2, List 11), whereby in Germany explicit authorisation is required because they are equated with additives. Of these compounds, only cholecalciferol and ergocalciferol are listed in EU Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutritional uses and in EC Directive 2002/46/EU for food supplements. Furthermore, the latter are included in the "Proposal for a Regulation of the European

Parliament and Council on the addition of vitamins and minerals and of certain other substances to foods" of 10.11.2003 (COM (2003) 671 final). According to the new EC drinking milk regulation 2597/97 a Member State may be permitted to fortify drinking milk amongst other things with vitamins from milk.

Vitamin D is relatively stable in foods. There are scarcely any losses through storage or preparation. It is heat stable up to 180°C. However, up to 40% of supplemented vitamin D may be lost in fortified milk exposed to light (Faulkner *et al.*, 2000; Renken and Warthesen, 1993).

6.2.2 Metabolism, function, requirements

Metabolism: Vitamin D is not a vitamin in the real sense of the word since dietary intake is only of essential importance in the case of inadequate endogenous synthesis in the skin under the influence of sunlight. The actual biological substance, calcitriol (1,25 dihydroxy-cholecalciferol, $1,25(OH)_2D_3$) (CAS No. 32222-06-3), can be considered a hormone.

Vitamin D is absorbed in the small intestine by passive diffusion by chylomicrons of the lymph system. Bile acid and edible fats promote absorption. Ergosterol from vegetable sterin in food is converted by ultraviolet B (UV-B) radiation in the skin to vitamin D_2 (Bässler *et al.*, 2002). Vitamin D_3 taken up from food as well as endogenous vitamin D_3 from the skin is transported in blood by a specific vitamin D binding protein (DBP) to the liver where it is converted by mitochondrial CYP27 hydroxylase to 25-OH-cholecalciferol (calcidiol, $25(OH)D_3$) (Jones *et al.*, 1998; Okuda, 1994). $25(OH)D_3$, also bound to DBP, is transported to the kidneys. In the proximal tubule area calcidiol is converted to the two biologically active metabolites $1,25(OH)D_3$ (calcitriol) and $24R,25(OH)_2D_3$. Normally these two vitamin D hormones are only formed in the kidneys but during pregnancy the placenta can also excrete significant amounts of $1,25(OH)_2D_3$ into the blood (Care, 1997). The concentration of circulating $1,25(OH)_2D_3$ is controlled in a fine-meshed manner by the plasma content of parathormone (PTH) and the vitamin D status. The lowering of the calcium level then leads to the release of parathormone and increased synthesis of $1,25(OH)_2D_3$. Elevated concentrations of $1,25(OH)_2D_3$ inhibit the activity of renal $25(OH)D_3$-1α hydroxylase (negative feedback) (Jones *et al.*, 1998). In infants and young children this regulation is not so extensive which means that they react in a more sensitive manner than adults (Stahl and Clairmont, 1997).

Vitamin D and its metabolites are eliminated by the gallbladder and only to a limited degree by the kidneys. The elimination half-life of vitamin D_3 is 4.5 days at a concentration of 9×10^{-8} mol/L, that of $25(OH)D_3$ is 31 days at the same concentration and that of $1,25(OH)_2D_3$ 1-5 hours at 10^{-10} mol/L (Bässler *et al.*, 2002). Non-converted vitamin D_3 is stored in fatty tissue and has a long biological half-life (Horst and Reinhardt, 1997; Norman, 2001). With the help of a compartment model it was calculated that during the winter months around 85 µg stored cholecalciferol is converted daily to $25(OH)D_3$. In order to maintain the serum concentrations of 25(OH)D (~ 70 nmol/L) assumed for the summer during the winter period, too, at least 12.5 µg cholecalciferol would have to be taken up daily in addition to the contribution from food and fatty tissue store (Heaney *et al.*, 2003a).

Functions: In cell tissue or in the target cell $1,25(OH)_2D_3$ and $24R,25\ (OH)_2D_3$ have, by means of vitamin D receptors (VDR) in the nucleus, in some cases, a synergistic effect with vitamin A and other hormones when it comes to regulating the gene expression of specific proteins and cell proliferation and maturation (Brown *et al.*, 1999; Jones *et al.*, 1998). Clarification of the molecular mechanism of action has led to better understanding of the functions in the organism which are not only linked to calcium or phosphate homeostasis like, for instance, increased calcium absorption through induction of the calcium-binding protein in the small intestine mucosa but also to other effects like, for instance, differentiation and

maturation of cells of the immune system, bone marrow, of chondrocytes during callus formation after fractures, epidermis and on protein synthesis in muscles which leads, in the final instance, to improved muscle power (Bässler *et al.*, 2002; Bischoff-Ferrari *et al.*, 2003; Carlberg, 1995; DeLuca and Zierold, 1998; Jones *et al.*, 1998; Kato, 2000; Norman, 2001; SCOGS, 1978; Stahl and Clairmont, 1997).

Requirements: Because of biosynthesis and the relatively large store capacity which is enough for 2 to 4 months' supply, no exact details can be given about human requirements. In our latitudes (between 37°N and 60°N) a healthy adult can cover his requirements if he spends enough time in the sun (10-15 minutes/day) and exposes a sufficient area of skin (30%) to optimum UV-B radiation (air pollution, winter months!). An increase in skin pigmentation, ageing and the use of sun protection creams reduce the formation of cholecalciferol in the skin (Fuller and Casparian, 2001; Holick, 1985; 1995; Lips, 2001; MacLaughlin and Need *et al.*, 1993). Only in the case of infants, a lack of sunshine and in older people, does intake of vitamin D from food play an important role. DGE recommends daily intakes of 10 µg for infants and older adults (65 years and above) on precautionary grounds and of 5 µg for children, adolescents and adults including pregnant and lactating women (D-A-CH, 2000). The population reference intakes for Europe are as follows: infants 6-11 months 10-25 µg; young children 1-3 years 10 µg; children 4-10 years 0-10 µg; 11-17 years 0-15 µg; adults 18-64 years 0-10 µg; adults 65 and older 10 µg; pregnancy 10 µg and lactation 10 µg (SCF, 1993).

For the first time, considerably higher recommendations have been made for older people whereas, according to DGE, there is no need to increase vitamin D intake during pregnancy and lactation beyond the age-based recommendation (D-A-CH, 2000). More recent studies provide grounds for rethinking the prior recommendations for pregnant and lactating women since sufficient serum concentrations of circulating 25 (OH)D could only be achieved at daily doses of 25 µg and higher not only in ethnic, non-European minorities (Datta *et al.*, 2002; Hollis and Wagner, 2004; Mallet *et al.*, 1986).

6.2.3 Exposure (dietary and other sources, nutritional status)

Sources: *Endogenous* vitamin D_3 (cholecalciferol) is formed from 7-dehydrocholesterol (Bässler *et al.*, 2002; Holick, 1995). 7-dehydrocholesterol is a precursor in cholesterol biosynthesis.

Food: In foodstuffs vitamin D_3 is mainly found in food of animal origin and, more particularly, in fatty fish (1-27 µg/100 g), cod liver oil (300 µg/100 g), liver, eggs, butter, cheese and milk (1-2 µg/100 g). Fortified foods like, for instance, margarine and mixed fat products contain up to 2.5 µg/100 g and dairy products up to a total of 12.5 µg/kg calculated as calciferol. Foods of plant origin, aside from a few exceptions, do not contain almost any preformed vitamin D_2, but they do contain its provitamin (ergosterol) (Bässler *et al.*, 2002; Gaßmann, 1998). Furthermore, the addition of 20 µg vitamin D/L is permitted for the manufacture and placing on the market of edible oil fortified with vitamins A and D as supplemented food by means of exemptions pursuant to § 37 Food and Other Commodities Act (LMBG).

Infant formula and foods for special medical purposes contain at least 1 µg and 0.5 µg vitamin D/100 kcal. Vitamin D is also found in food supplements (maximum 5 µg per recommended daily portion. Human milk contains less vitamin D (0.1 to 1.2 µg/L).

Medicinal products: Pharmaceutical forms with daily doses of 10-12.5 µg (400-500 IU) are used as medicinal products to prevent rickets and at daily doses of 20-125 µg (800-5000 IU) to treat rickets and osteomalacia. A maximum daily dose of more than 25 µg (>1000 IU) is only available on prescription whereas preparations with a maximum daily dose of more than 10-25 µg (>400-1000 IU) are pharmacy only. Only preparations with a maximum daily dose

of up to 10 µg (400 IU) are over the counter products (BfArM, 2001). A joint panel of the WHO Collaborating Centre for Public Health Aspects of Rheumatic Diseases (Liège, Belgium) and the WHO Collaborating Centre for Osteoporosis Prevention (Geneva, Switzerland) has explicitly recommended that the treatment and prevention of osteoporosis should be done with doses of 800 IU (20 µg) vitamin D per day under medical supervision in order to guarantee optimum efficacy and safety. The experts, therefore, considered vitamin D preparations of this kind as medicinal products within the intendment of Directive 2001/83/EC (Boonen *et al.*, 2004).

Nutritional status: The dietary intake of vitamin D only correlates weakly with the 25(OH)D concentrations in the serum. Better predictions can be made using UV-B radiation and time spent outside. In this context, relatively short exposure to the sun of 3 times 15 minutes per week suffices in order to achieve the necessary vitamin D level (Booth *et al.*, 1997; Holick, 1995; Vieth, 1990). Other factors which influence vitamin D status in man are type of skin and polymorphisms in vitamin D receptors (Ames *et al.*, 1999; Berger *et al.*, 2003; Brown *et al.*, 1999; Clemens *et al.*, 1982; Eisman, 1998; Ferrari *et al.*, 1995).

Dietary intake: In line with the reassessment in the National Food Consumption Study (NVS), average vitamin D intake in the Federal Republic of Germany is 4.1 and 4.5 µg/day respectively in women and men. By way of comparison, it is around 50% lower: 1.8 to 2.0 µg/day in children (4 to 10 years). In individuals with reduced synthesis ability, daily intake is 5.1 µg (51-65 years) and 4.2 µg (>65 years) respectively. In the age groups of males up to under 25 and in all age groups of women, average daily intake of vitamin D is generally considerably lower than the intake recommended by DGE (DGE, 1996). The previous assessment determined (n=1134) for women and (n=854) for men as the median (2.5-97.5 percentile) 3.12 (1.00-11.9) and 4.02 (1.17-16.8) µg vitamin D/day respectively (Heseker *et al.*, 1992). In a nationwide survey of the food situation of older people (65 years), not even half of the recommended intake of 10 µg per day was reached by around 60% of men and 70% of women (DGE, 2000). These intake levels do not take into account endogenous synthesis following UV-B radiation of the skin which, depending on the season, can make a large contribution to covering requirements. Supply bottlenecks may only arise in the case of people who are housebound and do not have any corresponding UV-B exposure. This only applied to 2% of the older interviewees (DGE, 2000). There is inadequate vitamin D intake amongst infants and young children which must be corrected through vitamin D supplements in conjunction with rickets prophylaxis (Hövels *et al.*, 1984; Kruse, 2000). Infants take in at least around 5 µg and at most up to 20 µg per day from supplements (10 µg/day), human milk (low level), infant and follow-on formula (at 400 ml between 4 and 8 µg/day) and, where appropriate, baby foods.

Biomarkers: In conjunction with the NFCS/VERA Study, the vitamin D supply of a representative random sample (n=574) of Germans aged 18 and over was measured and assessed using the plasma concentrations of 25-hydroxy-cholecalciferol (25(OH)D_3). This biomarker is mainly endogenous and, therefore, an indication of the average length of UV-B radiation of the skin. Plasma concentrations of 25 to 130 nmol/L are considered to be normal values. Measured values below 10 nmol/L are clearly too low. The median (2.5-97.5 percentile) for both genders was 146 (10-571) nmol 25(OH)D_3/L (Heseker *et al.* 1991; 1992). There are regional differences. The vitamin status of the population in the south is significantly better than in other regions. In northern Germany the prevalence of low measured values for vitamin D supply is the highest. However there are very marked seasonal fluctuations of 25(OH)D concentrations in the plasma. In winter and particularly in spring a far less favourable vitamin D supply is observed. In the spring more than 10% of men and women are found to have low measured values (<10 nmol/L) whereas vitamin D supply in summer and, more particularly, in autumn is not a problem. Overall, an inadequate vitamin D supply status was determined in 5% of Germans using this parameter. Plasma concentrations in heavy smokers (>30 cigarettes/day) are 45% lower than those of male and

female non-smokers. Women who stated that they took a vitamin preparation daily had significantly higher measured values (Heseker *et al.*, 1991; 1992).

SCF has indicated a limit value for sub-clinical vitamin D deficiency of <27.5 nmol/L (SCF, 2002). This value should be complied with in order to prevent rickets and osteomalacia (FNB, 1997). Very little information is available about the necessary 25(OH)D level in order to maintain normal calcium metabolism and peak bone mass in adolescents and younger adults. Given the inverse relationship between 25(OH)D_3 concentrations and the concentration of parathormone (PTH), some authors do, however, classify serum concentrations of <40-50 nmol/L as inadequate, particularly in the case of older people with bone mass losses. Others considered measured values of more than 75-100 nmol/L as desirable for optimum vitamin D supply and balanced calcium homeostasis where the PTH is suppressed to a minimum in comparison with the 25 (OH)D level (Chapuy *et al.*, 1997; Gennari, 2001; Haden *et al.*, 1999; Harkness and Cromer, 2004; Heaney, 2003; Heaney *et al.*, 2003b; Lips, 2004; McKenna and Freaney, 1998; Szulc *et al.*, 2003; Vieth, 1999; Vieth *et al.*, 2001). In a more recent study from the USA a higher prevalence of sub-clinical vitamin D deficiency conditions in the general population of 35% could be determined because of the setting of a higher limit value (<37.5 nmol 25(OH)D/L). Hence, in Germany, too, there may be a higher prevalence of marginal vitamin D deficiencies in the general population (MacFarlane *et al.*, 2004).

6.3 Risk characterisation

6.3.1 Hazard characterisation (NOAEL, LOAEL)

The hazard potential of hypervitaminosis D and vitamin D toxicity lies in hypercalcaemia as the direct consequence of vitamin D-dependent, elevated intestinal calcium absorption and elevated bone absorption (Barger-Lux *et al.*, 1995). However, elevated 25(OH)D concentrations (>130 nmol/L), hypercalciuria and normal calcium values in the serum with suppressed PTH status were also found in patients with vitamin D intoxication. This means that elevated calcium elimination in the urine can be an early sign of hypercalcaemia which is possibly linked to a higher risk of kidney stone formation (Adams and Lee, 1997).

A **NOAEL** (No observed adverse effect level) of 45 µg/day was determined on the basis of growth retardation in infants, proven hypercalcaemia and the 25(OH)D concentrations in the serum by the Food and Nutrition Board (FNB). Based on earlier studies, growth retardation was observed in 35 hospitalised infants who were given 45 to 112 µg vitamin D/day (FNB, 1997; Jeans and Stearns, 1938). However, in a comparable study no negative effect could be determined on the growth of 13 infants, who were given 35 to 54 µg vitamin D/day (Fomon *et al.*, 1966). A reduction of hypercalcaemia was observed from 1960 in the United Kingdom in infants after vitamin D intake was reduced from >100 µg/day to between 18 and 34 µg/day (BPA, 1956; 1964). More recent studies showed that the upper reference intakes of 25 (OH)D concentration in serum (130-150 nmol/L) had already been exceeded by some infants at intakes of approximately 10 µg vitamin D_3/day and hypercalcaemia had occurred (Hesse *et al.*, 1990; 1993). At additional intake levels of 25 µg vitamin D_2/day, the average 25(OH)D concentration in breast-fed infants was close to the upper reference intakes (130-150 nmol/L), particularly if they had also been exposed to sunlight (Ala-Houhala, 1985). The Scientific Committee on Food (SCF) stresses in this context that there are no systematic studies which can be referred to in order to establish a NOAEL for infants who had been given more than 10 µg vitamin D_3 in addition to the intake of vitamin D from human milk (SCF, 2002).

In healthy 21-60 year-old adults (n=30), a LOAEL (Lowest Observed Adverse Effect Level) at a dose of 95 µg (3800 IU) was determined after 3 months daily supplementation of 10, 20, 30, 60 and 95 µg vitamin D which led to hypercalcaemia (>2.75 mmol/L or 11 mg/dl).

A dose of 60 µg (2400 IU) could be determined as the **NOAEL** at which an increase in the calcium level in the serum could already be observed which was, however, in the normal range (FNB, 1997; Narang *et al.*, 1984). New studies involving 23-56 year-old adults (n=63), showed, however, that supplementation of vitamin D_3 at a dose of 100 µg/day over a period of 6 months led to a plateau of the average 25(OH)D concentration of 96 nmol/L. The average calcium concentration (<2.45 mmol/L) still remained constant in the serum. This means that this level can also be considered safe (Vieth, 1999; 2001). In studies involving older 60-65 year-old test persons, a lower dose of 50 µg vitamin D/day was, however, determined at which hypercalcaemia (serum calcium >2.75 mmol/L) occurred in 2 of the 63 test persons after 6 months (Johnson *et al.*, 1980). Dawson-Hughes *et al.* (1997) gave 70 year-old test persons 17.5 µg/day supplements over 3 years which led to 25(OH)D concentrations of 112 nmol/L. Hence, other authors doubt that a dose of 100 µg/day can also be considered safe in the longer term (Muskiet *et al.*, 2001).

Based on the same data for adults, the Scientific Committee on Food (SCF) considers a dose of 100 µg vitamin D/day and a serum level of 200 nmol 25(OH)D/L as the NOAEL (SCF, 2002).

6.3.2 Deficiency, possible risk groups

6.3.2.1 Deficiency

A vitamin D deficiency in humans normally occurs when there are insufficient amounts of vitamin D in the intermediary metabolism, when its active metabolites are formed and when, at the same time, there is inadequate dietary intake. The causes for a lower vitamin D metabolite level (<25 nmol 25(OH)D/L) as an expression of a vitamin D deficiency are therefore:

- Inadequate dietary vitamin D_3 intake (vegetarians) (Lamberg-Allardt *et al.*, 1993; Outila *et al.*, 2000);
- Inadequate UV-B exposure (winter months, people who are confined to bed for longer periods or spend little time outside or suffer from a sunlight deficiency or use extensive sun protection cream) (Fuller and Casparian, 2001; Glerup et al., 2000; Gloth et al., 1995; Thomas et al., 1998);
- Malabsorption and maldigestion, e.g. through chronic intestinal diseases (Lark et al., 2001; Lo et al., 1985; Mawer,1997);
- Intermediary hydroxylation defects
  - reduced 25-hydroxylase activity in the case of liver cirrhosis (Masuda et al., 1989; Shiomi et al., 1999)
  - accelerated turnover of 25-OH-D3 through medicinal product interactions, e.g. anti-epileptic agents and barbiturates (Bässler et al., 2002; Bouillon et al., 1975)
  - reduced 1-hydroxylase activity and vitamin D resistance in the case of renal insufficiency (Dusso, 2003)
  - post-menopausal osteoporosis (30% less 1.25(OH)2D3 compared with the controls).

Vitamin D deficiency leads to a calcium imbalance. The first signs of a vitamin D deficiency are low calcium and phosphate concentrations, elevated alkaline phosphotase activity in the serum and secondary hyperparathyroidism. Later there is evidence of inadequate mineralisation of the skeleton (rickets in children prior to closure of the epiphysis or osteomalacia in adults), painful bones, severe bone deformations and changes in muscle

metabolism and respiratory functions as well as reduced immune function (Bernecker, 2004; Deluca and Cantorna, 2001; Horst and Reinhardt, 1997; Norman, 2001; Wharton and Bishop, 2003).

The incidence of vitamin D deficiency rickets in Germany is estimated to be at least 400 cases per year amongst children and is, therefore, by no means rare. It occurs more frequently amongst dark-skinned immigrant children from developing countries who have not received any vitamin D prophylaxis (Fitzpatrik *et al.*, 2000; Hövels *et al.*, 1983; Kruse, 2000; Mughal *et al.*, 1999). Children whose parents feed them a macrobiotic diet are also particularly at risk (Dagnelie *et al.*, 1990; Hautvast, 1990). Human milk only has a low vitamin D content (0.63 µg/L), which is by no means sufficient to cover the requirements of exclusively breastfed infants (American Academy of Paediatrics, 2003). In the case of infants and young children regular vitamin D rickets prophylaxis is necessary in Germany. Independently of vitamin D production through UV-B light in the skin and vitamin D intake from human milk or infant formula (basic vitamin supplementation) the Deutsche Gesellschaft für Kinderheilkunde (German Paediatric Society) recommends, for the purposes of medication-based prophylaxis in breastfed and non-breastfed infants, the daily administration of a vitamin D tablet of 10-12.5 µg (400-500 IU) from the end of the 1st week of life up to the end of the 1st year of life. The prophylaxis can be continued in the 2nd year of life in the winter months (D-A-CH, 2000; Deutsche Gesellschaft für Sozialpädiatrie, 1982; Hövels *et al.*, 1984; Kruse, 2000).

In its Guidelines for the prevention of rickets and vitamin D deficiency the American Academy of Paediatrics (2003) recommends additional daily supplementation of 5 µg vitamin D for children and adolescents who do not drink at least 500 ml of milk fortified with vitamin D daily or the taking of a multivitamin tablet daily which contains at least 5 µg vitamin D.

Osteomalacia is a mineralisation disorder affecting adult bones which – normally as a consequence of inadequate vitamin D supply and/or chronic calcium deficiency - involves softening of the bones with corresponding changes in the skeleton. In contrast to osteoporosis, there is only a reduction in the mineral content of the bone matrix which is newly formed in conjunction with the rearrangement process but not of the entire bone matrix (Demiaux *et al.*, 1992; Parfitt *et al.*, 2004). The prevalence of osteomalacia increases, as dose osteoporosis, with age. In the case of patients aged 90 and over with a femoral neck fracture, osteomalacia was diagnosed in 29% of cases and osteoporosis in 71% of cases (Hordon and Peacock, 1990). Serum levels of 25 (OH) D are, as a rule, considerably lower. Chemical laboratory tests normally establish hypocalcaemia and hypophosphataemia and an increase in PTH and alkaline phosphatase. Specific markers of the rate of bone formation like osteocalcin are generally elevated and point to increased osteoid synthesis and turnover (Bernecker, 2004; Demiaux *et al.*, 1992). Clinically manifest cases of osteomalacia have also been increasingly observed amongst dark-skinned immigrants and, above all, amongst younger multiparous Turkish women in Germany and other European countries (Datta *et al.*, 2002; Grover and Morley, 2001; Offermann, 1978).

Osteoporosis cannot be equated with vitamin D deficiency. It is also caused by disruptions of the calcium balance and bone metabolism and is, therefore, dependent on vitamin D status. However, it is conditioned by several factors and in its primary form it is genetically determined by altered vitamin D receptors (Gaßmann, 1998; Heaney, 2003; Riggs *et al.*, 1995). Rising life expectancy also increases the incidence of osteoporosis. It is estimated that it affects around 4-6 million people in Germany. There are approximately 87,000 cases of femoral neck fractures every year. When it comes to the prevalence of spinal column deformities and incidence rates of femoral neck fractures amongst 50-79 year-old men and women as a consequence of osteoporosis, there is a north-south divide in Europe. The mean prevalence in this age group is 12%. The highest rates are to be found in Scandinavia and the lowest in the Mediterranean (Wiesner, 1998). A prospective study evaluated the food

habits of 72,337 post-menopausal women over a period of 18 years. Women who daily take at least 12.5 µg vitamin D had a 37% lower risk of femoral fracture than women who took less than 3.5 µg vitamin D (Feskanich *et al.*, 2003). Lower vitamin D hormone levels were frequently measured in older people during the winter months (Gloth *et al.*, 1995; Lips, 2001; McKenna, 1992; van der Wielen *et al.*, 1995; Webb *et al.*, 1990). A vitamin D deficiency also leads to reduced muscle strength and impaired control of muscle activity. This is what leads to the higher inclination of older people to fall with the risk of femoral neck fractures (Allain and Dhesi, 2003; Bischoff-Ferrari *et al.*, 2003). Vitamin D and calcium are useful for the tertiary prevention of osteoporosis particularly amongst residents in nursing homes (Chapuy *et al.*, 1994; Großklaus, 1994; Lips, 2001; O'Brien, 1998; Reid, 1996). To achieve sufficient calcium homeostasis and maintain normal parathormone levels, higher vitamin D intakes via supplements or fortified foods are necessary to improve the vitamin D supply of older people particularly in the winter months (Chapuy *et al.*, 2002; McKenna and Freaney, 1998; Meier *et al.*, 2004; Nieves, 2003; Sahota, 2000; Simon *et al.*, 2002; Trivedi *et al.*, 2003).

The data available in the Federal Republic of Germany on the supply status of vitamin D indicate that there is a risk of a clinically manifest deficiency or store depletion for specific age groups, above all pregnant and lactating women, infants and young children as well as older persons particularly when they are only rarely exposed to the sun. Another risk group are dark-skinned immigrants (supply category 1).

6.3.3 Excessive intake, possible risk groups

6.3.3.1 Excessive intake

Excessive levels of vitamin D cannot normally be taken up from normal food sources which means that reports about vitamin D intoxications are rare. Nor is hypervitaminosis possible through lengthier UV-B radiation because provitamin in the skin is converted on into the inactive isomers lumisterol and tachysterol. The vitamin D_3 itself is photodegraded into 5,6-trans-cholecalciferol and inactive suprasterols. Furthermore, 1,25(OH)2D_3 formation is controlled (Holick, 1995). There is, however, the possibility of hypervitaminosis in individuals who ingest excessive amounts of vitamin D-containing supplements (Adams and Lee, 1997; Kato, 2000; SCOGS, 1978). There are, however, also reports about intoxications in the USA following the uncontrolled fortification of drinking milk (Giunta, 1998; Jacobus *et al.*, 1992).

Hypervitaminosis is generally characterised by an increase in the 25-(OH)-vitamin D concentrations in the plasma to values of 400 to 1250 nmol/L (160-500 ng/ml). However, lower increases have also been linked to a toxic effect (FNB, 1997; Food Standards Agency, 2001). In the initial stages the intoxication manifests no symptoms. Depending on the dose and length of treatment for severe and ongoing hypercalcaemia and hypercalciuria, the **symptoms** of vitamin D intoxication include cardiac arrhythmia, weakness, tiredness, headaches, nausea, vomiting and disturbances of consciousness. The early renal symptoms of hypercalcaemia are polyuria and polydipsia as a consequence of the reduced concentration ability of the kidneys. In the case of lengthier hypercalcaemia, metastatic calcifications mainly in the kidneys (with kidney stones) down to renal failure have been observed. In other organs too, particularly in the blood vessels, heart, lung, pancreas, skin, bones and teeth there are also calcifications (Davies and Adams, 1978; Giunta, 1998; Seelig, 1969). The intoxication symptoms are more severe when calcitriol is administered directly as a medicinal product since the physiological mechanisms of control are then bypassed. In individual cases fatal courses have been described in conjunction with overdoses of vitamin D-containing medicinal products (BfArM, 2001; BGA, 1988; Chesney, 1989; Norman, 2001).

Up to now it was assumed that there are no differences in the efficacy of vitamin D_2 and vitamin D_3 although the latter led to a more effective increase in the 25-(OH) vitamin D concentrations in plasma. There are, however, differences in the toxicity of the two vitamins.

One explanation for the lower toxicity of vitamin D_2 is that the vitamin D-binding protein has a weaker affinity for vitamin D_2 metabolites than for 25-(OH) vitamin D_3 and 1,25-(OH)2-D_3. This means that, after high doses of vitamin D_2, there is a lower increase in 25-(OH) vitamin D_2 in plasma and a lower formation of biologically active 1,25-$(OH)_2$-D_2 (SCGS, 1978; Vieth *et al.*, 2001).

In the **majority of adults** intoxication symptoms do not appear until daily doses of more than 50,000 IU = 1.25 mg vitamin D_3 per day. All the same, symptoms of vitamin D intoxication after longer administration of 250-1250 µg vitamin D_3 µg per day have been observed whereas in the case of the short-term administration (7 weeks) of 250 µg per day to healthy adults no effects on calcium and phosphate concentrations in the serum and urine could be identified. Nor were the 25(OH)D concentrations higher than in the case of daily whole body UV-B radiation. There are, however, individuals with pathological oversensitivity; they already manifest intoxication symptoms at 250 µg (incidence 1:50,000) (CSHP, 1995; Gaßmann, 1998; Schwartzman and Franck, 1987; Strubelt, 1992).

6.3.3.2 Possible risk groups

Pregnant women should only take daily doses of more than 12.5 µg (500 IU)/day after strict medical indication. Overdoses of vitamin D during pregnancy must be prevented since long-lasting hypercalcaemia can lead to physical and mental retardation, supravalvular aortic stenosis and retinopathy in the child (BfArM, 2001). However, there is uncertainty about from which dose upwards the teratogenic effect of vitamin D observed in animal experiments and in pregnant women affects the foetus (Ariyuki, 1987; Food Standards Agency, 2001; Friedman and Roberts, 1966; Hollis and Wagner, 2004; Horii *et al.*, 1992; NAS, 1990; Norman *et al.*, 2002; SCOGS, 1978; Toda *et al.*, 1985; Zane, 1976).

In the 1950s and 1960s there were more frequent cases of chronic vitamin D intoxication after doses of more than 8,000 IU = 200 µg vitamin D_3 per day in **infants and small children** as a consequence of high-dose vitamin D prophylaxis which was still common at the time. A smaller percentage of infants already developed hypercalcaemia with nephrocalcinosis at a moderate dose of vitamin D (between 1,000 and 2,000 IU or 25 and 50 µg) (Hesse and Jahreis, 1990; Markestad *et al.*, 1987; Strubelt, 1992). The doses administered today of 10-12 µg (400-500 IU) vitamin D/day for medication-based rickets prophylaxis are very probably safe. Only an extremely small group of infants, who seem to suffer from a rare, congenital disruption of vitamin D metabolism (infantile hypercalcaemia or Williams-Beuren syndrome), could be at risk through the administration of vitamin D in the recommended range (Deutsche Gesellschaft für Sozialpädiatrie, 1982).

6.4 Tolerable upper intake level for vitamin D

Based on the **NOAEL** of 45 µg/day and an uncertainty factor of 1.8 for **infants** (0-1 year), FNB has set a **Tolerable Upper Intake Level (UL)** of **25 µg** (1000 IU) vitamin D/day, i.e. the total daily intake which probably does not lead to any health-impairing effects (FNB, 1997).

The Scientific Committee on Food has also set a UL of 25 µg vitamin D/day for infants aged 0-24 months (SCF, 2002). Infants may not be given more than 25 µg vitamin D per day without a specific indication and regular controls of the calcium concentration in plasma and calcium elimination in urine (D-A-CH, 2000).

According to the French AFSSA Expert Committee on Human Nutrition a tolerable upper intake level of 50 µg/day still seems to be acceptable for infants. In France no side effects were observed in conjunction with prescription-only daily doses of 25-50 µg (1000-2000 IU) vitamin D for rickets prophylaxis in infants although an LOAEL of 100 µg/day for children below 2 years of age would only result in a **UL** of 10 µg/day (AFSSA, 2002; CSHP, 1995).

For **children** (1-18 years), **adults** (>18 years) and for **pregnant and lactating women** (14-50 years), the **UL** is **50 µg (2000 IU) vitamin D/day** (FNB, 1997) according to FNB. The Nordic Council of Ministers (Food) has also suggested an upper safe intake level of 50 µg/day for vitamin D (Nordic Council, 2001).

According to the D-A-CH reference intakes as well, a daily vitamin D intake of up to 50 µg can be considered safe for adults. In the case of lengthier intake of 95 µg/day, cases of hypercalcaemia (>11 mg/dl = 2.75 mmol/L) have been reported (D-A-CH, 2000).

In contrast to FNB, SCF has set a **UL** of **25 µg (1000 IU) vitamin D/day** on precautionary grounds for **children** aged 2-10 years taking into account their lower bodyweight. For **adolescents** aged between 11-17 and **adults** including **pregnant and lactating women**, SCF has derived a **UL** of **50 µg vitamin D/day**. According to this Committee, higher doses for older adults should be administered under medical supervision in order to achieve optimum serum concentrations of 25(OH)D for the purposes of the best possible bone mineralisation (SCF, 2002).

The French AFSSA Expert Committee on Human Nutrition has, however, proposed a **UL** of 25 µg vitamin D/day for adults (AFSSA, 2002).

The Expert Group on Vitamins and Minerals in the United Kingdom (EVM) has specified in its draft report that a "Safe Upper Level (SUL)" for longer-term intake of the entire population cannot be established on the basis of the available data from studies in humans and animals. Nevertheless, the EVM expert did feel that an intake of 25 µg/day, as a so-called guidance level, was possibly necessary in order to prevent a deficiency in some groups. Higher doses, like for instance 45 µg/day, would be well tolerated without any side effects but should, in their opinion, only be administered in the short-term under medical supervision in order to remedy a vitamin D deficiency. Given the difficulties of recording total exposure to vitamin D, it was not, however, possible to establish a guidance value for total intake (Food Standards Agency, 2003).

One critical comment which should be made here is that the parallel assessment and elaboration of upper levels for the safe intake of vitamins and minerals in the form of Safe Upper Levels or Tolerable Intake Levels and the so-called Guidance Levels will probably lead to conflict when different ULs are derived (BfR, 2002). Furthermore, different critical endpoints (biomarkers) were taken as the basis for the establishment of the NOAEL or LOAEL (see chapter 2.1). In the studies by Narang *et al.* (1984) on dose-response relationships with additional vitamin D supplements, there are no data on vitamin D status prior to treatment, the vitamin D compound administered or exposure to the sun. The moderate hypercalcaemia (>2.75 mmol/L) reported by them at an additional intake of 95 µg/day could not be confirmed in the studies by Vieth *et al.* (2001). Heaney *et al.* (2003a) even considered vitamin D intakes of up to 250 µg/day over 5 months as safe.

In many studies to determine vitamin D toxicity based on elevated 25(OH)D concentrations in the serum, the supplements were not administered for more than 4 weeks. Since the half-life of 25(OH)D is, however 1 to 2 months, a steady state cannot be expected given the short observation time (Muskiet *et al.*, 2001; Vieth, 1999). Despite these reservations, BfR considers a **UL** of 25 µg/day for children aged between 2 and 10 and a UL of 50 µg/day for adolescents and adults to be an upper safe daily intake level for vitamin D.

Since overall the margin for vitamin D between recommended intake and the tolerable upper intake level (UL) is very small (AFSSA, 2002; Nordic Council, 2001; CSHP, 1995), BfR is of the opinion that the lowest UL of 25 µg/day for children should be taken as the basis for the derivations of maximum levels of vitamin D in food supplements and fortified foods on the

grounds of preventive health protection since this age group normally has the same range of food as adults.

6.4.1 Derivation of a maximum level for vitamin D in food supplements

Up to now we have been of the opinion that, for reasons of preventive health protection, food supplements should not contain more than one-fold the daily intake of vitamin D recommended by DGE and have suggested a maximum level of 5 µg (BgVV, 1998). A defined maximum level for vitamin D in food supplements can now be derived on the basis of the quantitative risk assessment for infants, children and adults using the proposed formula (see chapter 3.2). However, there are derived upper levels of varying quality for the upper safe level of intake or tolerable upper intake level or guidance level, which means that there is a certain degree of uncertainty (AFSSA, 2002; CSHP, 1995; FNB, 1997; Food Standards Agency, 2003; Nordic Council, 2001; SCF, 2002). Taking into account practice up to now, a total of 5 different options are proposed.

6.4.1.1 Possible management options

a) No supplementation with vitamin D whilst maintaining medication-based rickets prophylaxis for infants and young children into the 2nd year of their life.

   As a dose of 10-12.5 µg (400-500 IU) was already laid down as a tolerable intake level (TL) within the framework of medication-based vitamin D rickets prophylaxis, no food supplements are necessary for these age groups (Hesse *et al.*, 1994; Hövels *et al.* 1983; 1984; Kruse, 2000; Manz, 1994).

   Advantages: Since 1939 the easily managed medication-based rickets prophylaxis for infants and young children has proved its worth in Germany. There are similar programmes in most European countries.

   Disadvantages: None

b) Continuation of existing practice from 4th year of life, i.e. "one-fold rule", which means that the one-fold recommended daily dose (5 µg) should not be exceeded in food supplements (BgVV, 1998).

   Advantages: No side effects of any kind have been reported for this range. The upper level is oriented towards nutritional-physiological requirements.

   Disadvantages: The proposed maximum level is not based on a science-based risk assessment.

c) Setting of a maximum level for food supplements (TL_{FS}) of 17 µg based on the UL of SCF for adolescents and adults

   If the proposed calculation based on the UL of SCF (2002) or FNB (1997) is used to determine the maximum tolerable level (TL_{FS}) of vitamin D in individual food supplements, this leads to the following value for adolescents and adults (and in the opinion of FNB also for children from age 3 upwards):

$$\frac{50\ \mu g\ ^{*}\ [UL] - 16.8\ \mu g\ ^{**}\ [DINF]}{2\ [MEF]} = 16.6\ \mu g\ [TL_{FS}]$$

* SCF, 2002
**97.5 percentile (Heseker *et al.*, 1992)

Legend:

UL	=	Tolerable Upper Intake Level (SCF) usually referring to the daily total intake
DINF	=	Dietary Intake by Normal Food (95. or 97.5 percentile)
MEF	=	Estimated Number of Consumed Products
TL	=	Tolerable Level in a single dietary supplement or fortified food

In this calculation the available residual amount was allocated to the food supplements (see chapter 3.2). The multi-exposure factor (MEF) was fixed at 2 as dietary exposure through product categories in Germany is limited at the present time. As data from countries with a higher proportion of supplement users confirm, there are indeed realistic grounds for fixing the MEF at 4 if more vitamin D-containing products were to be offered on the market in Germany (AFSSA, 2002; Nowson and Margerison, 2002; Tangpricha *et al.*, 2003).

Advantages: The derived maximum level applies to the addition of a vitamin to food supplements (individual products). Thanks to the multi-exposure factor it also takes other exposure sources into account.

Disadvantages: When laying down a tolerable intake level of more than 12.5 µg, specific warnings more particularly for pregnant women would have to be prescribed for food supplements in a similar way to the marketing of medicinal products (BfArM, 2001). Furthermore, at a recommended maximum level of 16 µg, all groups in the population would take in additional vitamin D which goes beyond their requirements. Bearing in mind the derived maximum level, since, up to 17 µg would be ingested in the recommended daily doses from food supplements leading to total vitamin D intake (normal food + fortified foods) of on average 21.0 up to 33.8 µg per day in the 97.5 percentile, there would still be a sufficient safety margin for adolescents and adults but not for children up to age 10 (UL = 25 µg/day). A corresponding warning for children would, therefore, be absolutely essential.

d) Setting the maximum level for food supplements (TL_{FS}) of 8.0 µg based on the UL of SCF for children aged between 3 and 10

If the proposed calculation using the UL of SCF (2002) derived for children is used to determine the maximum tolerable level (TL_{FSM}) of vitamin D, this leads to the following value:

$$\frac{25\ \mu g\ ^{*}\ [UL] - 9.13\ \mu g\ ^{**}\ [DINF]}{2\ [MEF]} = 7.94\ \mu g\ [TL_{FS}]$$

* SCF, 2002
**97.5 percentile (Adolf *et al.*, 1995)

Legend

UL	=	Tolerable Upper Intake Level (SCF) usually referring to the daily total intake
DINF	=	Dietary Intake by Normal Food (95. or 97.5 percentile)
MEF	=	Estimated Number of Consumed Products
TL	=	Tolerable Level in a single dietary supplement or fortified food

In this calculation the available residual amount was allocated to the food supplements (see chapter 3.2). The multi-exposure factor (MEF) was fixed at 2 as dietary exposure

through product categories in Germany is limited at the present time. The highest 97.5 percentile of intake of this age group is 9.13 µg vitamin D (Adolf *et al.*, 1995).

Advantages: The proposed maximum level takes into account the special sensitivity of children up to the age of 10. No warnings are necessary.

Disadvantages: Different individual products and instructions on use are required for children and adults. Since the residual amount was allocated to food supplements, there is scarcely any possibility of extending fortification to conventional foods.

e) Setting the recommended maximum level for food supplements (TL_{FS}) at a uniform 5 µg for children up to the age of 10, adolescents and adults and at 10 µg for older adults (65 years and older)

For reasons of preventive health protection the tolerable upper intake level (TL_{FS}) in a single product for children, adolescents and adults should be set at a uniform 5 µg. Since a higher recommended maximum daily level can be accepted in older adults (>65 years), an indication should be given on the label for reasons of simplicity that up to 2 individual products may be consumed per day.

Advantages: These two age-related maximum levels permit a larger safety margin. Children and pregnant women are not exposed to a higher risk. Older people can cover their higher requirements through corresponding instructions on the packaging.

The proposed uniform maximum level for children (3-10 years), adolescents and adults takes a sufficient safety margin to the UL of 25 µg/day derived by SCF for children into account. A uniform dose of 5 µg in a single product cannot lead to confusion. Even in the event that two different vitamin D-containing food supplements with this maximum level should be taken in an uncontrolled manner, there is still a sufficient safety margin for children and pregnant women. Hence, warnings are not necessary when setting age-related maximum levels, particularly not for pregnant women. This vitamin D level is also recommended by the American Academy of Paediatrics (2003) in its new guidelines for children and adolescents.

Disadvantages: None

6.4.2 Derivation of a maximum level for vitamin D in fortified foods

Up to now, the fortification of foods in Germany has been handled in a very restrictive manner. Vitamin D may only be added to butter substitutes, mixed fat products (a total of 25 µg vitamin D/kg), edible oils (20 µg vitamin D/L), dairy products (up to a total of 12.5 µg/kg calculated as calciferol), certain foods for special dietary purposes and food supplements subject to compliance with maximum levels. A common feature of all these foods is that they are consumed in predictable or recommended amounts. Other European countries, too, restrict the addition of vitamin D to oils and margarines, low fat milk, children's milk, cereal flakes and flour (Austria, Finland, Greece, Iceland, Netherlands, Norway, Sweden, United Kingdom) or do not permit it in conventional foods (Denmark, Italy, Luxembourg, Spain) aside from the purposes of restoring the original level (France), normally subject to compliance with prescribed upper levels (SCOOP, 1997). According to the EC Drinking Milk Regulation 2597/97 a Member State may permit drinking milk, amongst other things, to be fortified with vitamins from milk. However, no maximum levels have been set up to now. The natural vitamin D content of drinking milk (minimum 3.5% fat) is roughly 0.1 µg/L. Up to now, BgVV had not supported the fortification of milk with vitamins.

The harmonisation of food provisions is necessary to meet the requirements of the European Union. In accordance with the criteria in a Proposal for a Regulation of the European Parliament and Council on the addition of vitamins and minerals and of certain other substances to foods of 10.11.2003 (COM (2003) 671 final) in conjunction with Article 16 of this Regulation, maximum levels for vitamins and minerals are also to be drawn up by the European Commission with the support of the Standing Committee on the Food Chain and Animal Health (SCFA). There are difficulties when it comes to taking into account the different food habits in the individual Member States, particularly with respect to geographic and ethnical differences in Europe.

6.4.2.1 Possible management options

a) Continuation of existing practice, i.e. further fortification of conventional foods should not be permitted because of the low safety margin between recommended intake and the tolerable upper intake level (UL) of 50 and 25 µg vitamin D/day respectively (AFSSA, 2002; CSHP, 1995; FNB, 1997; Nordic Council, 2001; SCF, 2002).

   Advantages: Restricting fortification to a few foods offers the best possible protection against potential overdose and also makes a major contribution to rickets prophylaxis (Bässler *et al.*, 2002).

   Disadvantages: Different maximum levels for the fortification of margarine and mixed fat products may constitute trade barriers. Changes to eating habits are not taken into account (e.g. drop in consumption of margarines in favour of edible oils). The needs of "low consumers" and/or other risk groups (e.g. senior citizens, residents in homes) are not sufficiently taken into account.

b) Extension of fortification to specific foods coupled with the setting of maximum levels

   A maximum level of 20 µg vitamin D/L has already been set for the vitamin supplementation of edible oils in conjunction with the exemptions pursuant to § 37 Foods and other Commodities Act (LMBG). This reflects the fact that eating habits have changed in Germany since many consumers are increasingly using vegetable oils instead of butter or margarine.

   If the offering were to be extended to other foods and food categories, the residual amount available would have to be reallocated.

   Advantages: A limited extension of the offering of fortified foods is conceivable taking into account national eating habits and changes in eating behaviour.

   Disadvantages: There is a higher risk of potential overdose particularly amongst individuals who take several food supplements including cod-liver oil and have a high consumption of fatty fish, eggs and/or fortified foods.

According to BfR, there is a high health risk of adverse effects when vitamin D is used in food supplements or for the purposes of food fortification.

For infants and young children up to the age of 2 there is a medication-based rickets prophylaxis which means that no food supplements are necessary for this age group (Option a).

After weighing up all advantages and disadvantages of the above-mentioned options, we recommend the setting of a recommended maximum safe level in food supplements of 5 µg for children up to the age of 10, adolescents and adults (up to under 65) and of 10 µg (Option e) for older persons (65 and above). For fortified foods a restricted extension of the offering is recommended, where appropriate, for instance in edible oils with a maximum level of 20 µg/L taking into account national eating habits and changes in eating behaviour (Option a) in combination with Option b).

6.5 Gaps in knowledge

- There are no comparative epidemiological studies on the prevalence of vitamin D deficiency conditions in Europe in conjunction with the setting of uniform threshold values or limit values of 25 (OH)D concentrations in the serum or plasma to assess the vitamin D status of the population and/or individual risk groups.
- There are no reliable studies on dose-response relationships in conjunction with the additional intake of vitamin D taking into account all sources of exposure (food, UV-B light), particularly with regard to geographical and ethnical differences in Europe.
- There are no up-to-date data for adults or children on the intake of vitamin D taking into account all sources of exposure (food + food supplements + fortified foods + UV-B light).
- In order to assess the storage of vitamin D in the body, methods must be developed to improve the assessment of vitamin D requirements in the absence of sunlight, particularly during the winter months in all age groups.
- Identification of risk groups as a consequence of a possible extension of the fortification of conventional foods.

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7 Risk Assessment of Vitamin E

7.1 Summary

The calculations available in the Federal Republic of Germany for vitamin E intake indicate that on average some women clearly fail to reach the reference intakes. The biochemical studies undertaken to estimate vitamin E supply do not, however, provide any indication of deficiency conditions (supply category 2/3).

No exact information is available about vitamin E requirements. Whereas specific requirements for vitamin E are still in the recommendations of the Deutsche Gesellschaft für Ernährung (DGE) (German Nutrition Society) from 1991, the new reference intakes from 2000 merely mention estimated values for adequate intake.

Compared with other fat soluble vitamins, vitamin E is described as relatively untoxic when taken orally. Given the higher intake levels in the course of a growing use of vitamin E in food supplements and for the purposes of food fortification, doses may, however, be reached which are linked to an elevated risk of bleeding, particularly amongst consumers with blood-clotting disorders or as a consequence of interaction with certain medicinal products. Hence, BfR is of the opinion that the use of vitamin E is linked to a moderate health risk (cf. Table 2).

Taking into account the results of the ATBC Study and the ongoing gaps in knowledge, BfR recommends, on the grounds of preventive health protection, that the maximum admissible level in food supplements be restricted to one-fold the reference intake of maximum 15 mg vitamin E per daily dose. This amount is oriented towards nutritional-physiological requirements and is based on the estimated value indicated by DGE in 2000 for adequate intake by male adolescents and adults in the 15 to 25 age group. At this maximum level no adverse effects and no risks for healthy children or adolescents are to be expected according to the current level of knowledge. Based on the information available at present, there are no signs of an elevated risk of bleeding even amongst individuals who take thrombocyte aggregation inhibitors or cumarins.

In the case of fortified foods, purpose-specific vitamin addition in the expected recommended daily intake should not exceed one-fold the estimated value of maximum 15 mg. Furthermore, given the dependence of vitamin E requirements on the amount of polyene fatty acids ingested, it also appears reasonable to limit vitamin supplementation to specific food groups.

Estimated values for adequate intake	maximal 15 mg α-tocopherol equivalents/day	
Intake [mg/day] (NFCS, 1994)	**m**	**f**
Median	14.6	12.3
P 2.5	6.77	5.22
P 97.5	33.1	27.9
Tolerable upper intake level	300 mg/day (SCF)	
Proposal for **maximum levels** in:		
food supplements	15 mg/daily dose	
fortified foods	15 mg/daily portion where appropriate restriction to specific foods groups and link to the polyene fatty acid level	

7.2 Nutrient description

7.2.1 Characterisation and identification

Vitamin E is the *generic term* for all naturally occurring and synthetic tocol and tocotrienol derivatives which, in qualitative terms, exhibit the biological activity of the natural occurring and most efficacious stereoisomer "*RRR-alpha-tocopherol*" (old designation: "*D-α-tocopherol*") (CAS No.: 59-02-9, MG 430,69). Tocopherols are fat soluble substances which consist of a chroman ring with different substitutes on which the different vitamin E activity[*)] is based (Bässler *et al.*, 2002; DGE/ÖGE/SGE/SVE, 2000). The eight naturally occurring substances with vitamin E action include 4 tocopherols (α-, β-, γ-, δ-) with one saturated and 4 tocotrienols (α-, β-, γ-, δ-) with one unsaturated side chain. In order to reflect the various compounds with vitamin E activity, RRR-α-tocopherol (D-α-tocopherol) is used as the reference substance and vitamin E activity is stated in milligram D-α-tocopherol equivalents (Bässler *et al.*, 2002; DGE/ÖGE/SGE/SVE, 2000; von Bruchhausen *et al.*, 1994).

Free tocopherols are heat stable, non-soluble in water and easily oxidisable. The esters of tocopherols (e.g. acetate or succinate) are far more resistant to atmospheric oxygen, heat and ultraviolet light. Hence, they have no antioxidative properties but have biological action of a similar nature (N.N., 1999).

Commercial forms of alpha-tocopherol include natural RRR-α-tocopherol, the α-tocopherol esters and the fully synthetic form, a mixture of 8 stereoisomers (*all-rac-α-tocopherol*) (Bässler *et al.*, 2002; von Bruchhausen *et al.*, 1994). In Germany, besides the free forms, the ester compounds α- and β-tocopheryl acetate as well as α- and β-tocopherylsuccinate, classified as additives, are generally authorised for addition to foods (Ordinance on vitaminised foods, Ordinance on foods for special dietary purposes - DiätVO). In Annex 9 of the DiätVO, the following vitamin E compounds are listed:

➢ D- α-tocopherol	(new: RRR-α-tocopherol)
➢ DL-α-tocopherol	(new: all-rac-α-tocopherol)
➢ D-α-tocopheryl acetate	(new: RRR-α-tocopheryl acetate)
➢ DL-α-tocopheryl acetate	(new: all-rac-α-tocopheryl acetate)

In the Commission Directives 2000/15/EC (of 15 February 2001 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses) and 2002/46/EC (of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements), *D-α-tocopheryl acid succinate* is also listed. No adequate information is available on the bioavailability of the various compounds.

The following tocopherols are authorised as antioxidants for technological purposes in foods (see references, Food Additives Marketing Ordinance – ZVerkV):

E 306 (extracts with high tocopherol content)	E 307 (α-tocopherol)
E 308 (γ-tocopherol)	E 309 (δ-tocopherol)

7.2.2 Metabolism, function, requirements

Metabolism: Vitamin E taken up from food is absorbed together with fats in the small intestine whereby gallbladder and pancreas functions are essential for normal absorption. Only free tocopherol is absorbed. Tocopheryl acetate is hydrolysed in the small intestine by

[*)] Vitamin E activity of a tocopherol derivative is given in RRR-α-tocopherol equivalents (TE) whereby the following conversion factors apply in practice: 1 mg RRR-α-tocopherol equivalent = 1mg RRR-α-tocopherol = 1.1 mg RRR-α-tocopheryl acetate = 2.0 mg RRR-β-tocopherol = 4.0 mg RRR-γ-tocopherol = 100 mg RRR-δ-tocopherol = 3.3 mg RRR-α-tocotrienol = 1.49 mg all-rac-α-tocopheryl acetate = 1.49 International Units (IU).

pancreatic lipases and/or esterases of the intestinal mucosa. The main site of absorption is the upper small intestine. The absorption mechanism is based on passive diffusion. In the physiological range absorption is around 25-60% and decreases in the higher dose range (Bässler *et al.*, 2002; N.N., 1999). At doses of 12 mg, 24 mg and 200 mg vitamin E absorption rates of approximately 54%, 30% and 10% were observed in conjunction with average fat intake (DGE/ÖGE/SGE/SVE, 2000). Absorption also depends on the type of edible fat ingested at the same time: this is improved through medium-chain, saturated fatty acids and reduced through long-chain, polyunsaturated fatty acids.

In terms of absorption there are no significant differences between α- and γ-tocopherol. The latter is mainly eliminated with bile which explains the relatively low plasma level of γ-tocopherols although this is common in food. β- and δ-tocopherols are only absorbed to a minor degree (FNB, 2000). More recent discussions focus on the fact that γ-tocopherol may also play a special role and could have properties which α-tocopherol does not (Jiang *et al.*, 2001). In conjunction with α-tocopherol supplementation, a decrease in the γ-tocopherol concentrations in plasma and tissue was observed whereas an increase in both parameters was determined in conjunction with the administration of γ-tocopherol. Furthermore, it is known that the synthetic all-rac form only possesses around half the bioavailability of naturally occurring vitamin E (Burton *et al.*, 1998; Gaßmann, 1997).

Since the largest share of absorbed α-tocopherols is transported away via the lymphatic system and incorporated into lipoproteins, there is a high correlation between the α-tocopherol concentration and total fat content in the plasma. Transport in the cells and cell membranes is mediated by lipoprotein lipase and an alpha-tocopherol binding protein. Metabolism mainly takes place in the liver. The details of this process have still to be clarified (Bässler *et al.*, 2002; DGE/ÖGE/SGE/SVE, 2000; N.N., 1999).

Normal plasma concentrations of α-tocopherol range from 0.5-2.5 mg/100 ml plasma. The vitamin E content consists to almost 90% of RRR-α-tocopherol. There is no specific storage organ; vitamin E can, however, be detected in most body tissues. The total amount in the body is 2.0-4.0 g. The highest levels are found in fatty tissue, liver, adrenal glands and muscle tissue. In the plasma, liver, kidney and spleen tocopherol undergoes rapid turnover (half-life 5-7 days), whereas this turnover is slower in fatty tissue. An approximate ten-fold increase in tocopherol intake is necessary to double the plasma concentration. Most of the vitamin E ingested orally is eliminated in faeces and only around 1% in urine (Bässler *et al.*, 2002; DGE/ÖGE/SGE/SVE, 2000; von Bruchhausen *et al.*, 1994; N.N., 1999).

Interactions: Very high doses can interfere with the absorption of other fat soluble vitamins. This impedes the absorption of iron and strengthens the effect of vitamin K antagonists (for instance anticoagulants like cumarin). Vitamin E can intervene in eicosanoid synthesis and prolong bleeding time particularly in the case of parallel treatment with thrombocyte aggregation inhibitors (like e.g. acetyl salicylic acid). Cholestyramine can inhibit the absorption of α-tocopherol through binding and inhibiting the reabsorption of bile (BGA, 1994; DGE/ÖGE/SGE/SVE, 2000; N.N., 1999).

Functions: Tocopherols have diverse effects and their mechanism has not yet been fully clarified. In addition to antioxidant effects, direct membrane effects, anti-inflammatory, immunomodulatory and antithrombotic effects, impact on protein synthesis and functions in the neuromuscular system are also being discussed. It is assumed that peroxidative damage plays a role in the development of various degenerative diseases and that vitamin E, because of its antioxidative properties, takes on special importance in the prevention of these diseases. All the same, none of the large, randomised, placebo-controlled intervention studies carried out in the meantime reported positive results (Heart Protection Study Collaborative Group, 2002; Morris and Carson, 2003; Vivekananthan *et al.*, 2003). Also in respect of the discussed immunomodulatory and anti-inflammatory effects, key evidence is

missing. For instance, supplementation with 200 mg vitamin E in older individuals compared to placebo over a period of up to 15 months did not influence the incidence of acute infections of the upper respiratory tract. It was far more the case that a severe course of the disease was observed in conjunction with supplementation (Graat *et al.*, 2002). In the ATBC Study a higher risk of the onset of the common cold was observed in conjunction with vitamin supplementation in individuals who did a lot of physical exercise. At 10% this risk was not significant compared to placebo in the vitamin E group but it was significantly higher at 21% in the vitamin E plus beta-carotene group (Hemilä *et al.*, 2003).

It is known that, beside antioxidative effects, vitamin E can theoretically also develop prooxidative effects. There is a functional relationship between vitamin E and nutrients which are also involved in antioxidative/prooxidative processes like vitamin C, beta-carotene, sulphur-containing amino acids or selenium (Bässler *et al.*, 2002; Chow, 2001; Volkert and Schlierf, 1995).

Requirements: The *vitamin E requirements* of human beings are not exactly known. Because of the close relationship between vitamin E and unsaturated fatty acids, the intake of unsaturated fatty acids is to be taken into account when formulating reference intakes. For the protection of 1 g linolic acid, the amount of 0.4 mg TE is considered to be adequate (Bässler *et al.*, 2002; DGE/ÖGE/SGE/SVE, 2000; SCF, 1992). DGE believes that for adults a basic requirement of 4 mg α-tocopherol equivalents (TE) per day is necessary to protect against peroxidation (DGE/ÖGE/SGE/SVE, 2000). The following estimated values were derived for adequate intake:

Table 9: Estimated values for adequate vitamin E intake

Age (years)	Estimated values of DGE for adequate intake (mg α-TE/day) (DGE/ÖGE/SGE/SVE, 2000)	
	m	f
Children		
1 up to under 4	6	5
4 up to under 7	8	8
7 up to under 10	10	9
10 up to under 13	13	11
13 up to under 15	14	12
Adolescents and adults		
15 up to under 25	15	12
25 up to under 51	14	12
51 up to under 65	13	12
65 and older	12	11
Pregnant women	-	13
Lactating women	-	17

The DGE recommendations from 1991 (DGE, 1991) still contained recommendations for adequate intake which envisaged a uniform value of 12 mg tocopherol equivalents/day for adolescents and adults. SCF (1992) refers vitamin E requirements to the intake of polyunsaturated fatty acids (PUFA) from food. It gives a uniform value for all age and gender groups: **vitamin E requirements (mg α-tocopherol equivalent) = 0.4 x (g PUFA)**.

In principle, it should be borne in mind that an increasing intake of polyunsaturated fatty acids also increases the requirements for vitamin E which is needed to protect the fatty acids from peroxidation. Other recommendations envisage that the necessary vitamin E amount should be adjusted in foods in line with the saturation degree of the fatty acids, i.e. placed in relation to the number of double bonds. For instance, SCF (1996) issued the following recommendation for calculating the necessary vitamin E level in infant formula:

Fatty acids	Number of double bonds	Vitamin E requirements **) (mg/g fatty acid)
Linoleic acid (18:2,ω-6)	2	0.5
α-linolenic acid (18:3,ω-3)	3	0.75
Arachidonic acid (20:4,ω-6)	4	1
Eicosapentaenoic acid (20:5,ω-3)	5	1.25
Docosahexaenoic acid (22:6,ω-3)	6	1.5

7.2.3 Exposure (dietary and other sources, nutritional status)

Sources: Tocopherols are only synthesised in plants. All the same, they reach the animal organism via the food chain and thus also become components of foods of animal origin (Bässler *et al.*, 2002). The vitamin E levels in foods of animal origin are far lower than in vegetable products. The highest levels are to be found in cereal seeds and vegetable oils. Other sources are leafy vegetables, animal organs, milk and butter. In wheat germ, sunflower and olive oil RRR-α-tocopherol accounts for the main share (49-100%) of vitamin E whereas γ-tocopherol is the main component in soya, corn and palm oils.

Since the free vitamin E compounds are less stable, they are largely to be found as fatty acid esters in foods, particularly those of animal origin. The more stable forms of ester are mainly used for therapeutic purposes or as food supplements (Gaßmann, 1997). D-α-tocopheryl acetate (RRR-α-tocopheryl acetate) and DL-α-tocopheryl acetate (all-rac-α-tocopheryl acetate) are the compounds which are seemingly used the most frequently in supplements, for fortification and in medicinal products (FNB, 2000).

Medicinal products: In the monograph of the Federal Health Institute from 1994 (BGA, 1994), oral doses in the range of 10-100 mg/day for adults and 10 mg/day for children are recommended for prophylaxis. The drug inventory "Red List" (BPI, 2004) indicates that vitamin E in an amount of 30 mg α-tocopherol acetate per capsule and day is offered in an oral medicinal product ("E-Vicotrat 30 mg") with the indication "prevention and treatment of a vitamin E deficiency".

Nutritional status:
Dietary intake: The *National Food Consumption Study* (*NFCS*), in its revised form (DGE, 1996), gives 10.9-12.1 mg α-TE as the mean daily intake in men depending on age and 9.3-11.1 mg α-TE/day in women. Whereas men on average met the DGE recommendations to 100%, the mean average intake in all age groups of female persons was described as inadequate at 86%. The highest intake levels, measured at the 97.5 percentile, were 33 mg in men over the age of 65 (VERA-Schriftenreihe, 1995).

The nutrition survey (Mensink *et al.*, 1999) carried out as a complement to the *Federal Health Survey 1998* produced the following results: For around half the men vitamin E intake was below that of the DGE recommendations (median around 100%, 25 percentile around 80%, 75% percentile around 130%). The majority of women, however, did not meet the recommendations (median around 80%, 25 percentile around 60%, 75 percentile around 105%).

In the *EPIC Study* (Schulze *et al.*, 2001) conducted in Heidelberg and Potsdam between 1996 and 1998, the average vitamin E intake of men was 14.3-15.0 mg TE and of women 11.3-12.3 TE mg/day. The 10 and 90 percentiles determined for men were 5.1 and 26.1 mg TE and for women 4.5 and 22.4 mg TE/day. On average 65-86% of vitamin E was ingested in the form of α-tocopherol. According to this, the intake of alpha-tocopherols would be larger than the intake of tocopherol equivalents amongst the German population.

**) D-α-tocopherol

No reliable information is available about the proportion and level of vitamin E intake from fortified foods and food supplements.

Vitamin E plasma concentrations: In order to assess supply, the α-tocopherol concentrations were determined in blood plasma using the HPLC method in a representative random sample of over 18 year-olds in the VERA Study (VERA-Schriftenreihe, 1992). Here a reference value of 17.7 µmol/L (750 µg/dl) was used to assess the vitamin E status. Dependence on blood lipids turned out to be a special problem for the assessment. The statistical mean for the plasma level was 30.6 µmol α-TE/L. The median was 29.1 µmol α-TE/L and the range of the 2.5 to 97.5 percentiles extended from 17.6 to 53.5 µmol α-TE/L. The plasma concentrations were below the limit value in only 2.8% of the random sample. From the results it was concluded that vitamin E supply is largely secured in the Federal Republic of Germany.

7.3 Risk characterisation

7.3.1 Hazard characterisation (LOAEL, NOAEL)

Compared with the other fat soluble vitamins A and D, vitamin E is relatively untoxic when taken orally. Even after the administration of high doses of vitamin E over a period of years, there are no known cases of hypervitaminosis. Nor are there any indications of adverse effects which could be attributed to the increased intake of vitamin E which occurs naturally in foods (BGA, 1994; DGE/ÖGE/SGE/SVE, 2000; FNB, 2000). In animal experiments FNB (FNB, 2000) derived an **LOAEL** (Lowest observed adverse effect level) of 500 mg/kg/bw/day. By contrast, the EU Scientific Committee on Food (SCF, 2003), based on the study by Meydani *et al.* (1998), established a **NOAEL** (No observed adverse effect level) of 540 mg/day (see also Chapter 7.4). In conjunction with the administration of higher vitamin E amounts in human beings, the discussion primarily focuses on the following risks:

7.3.1.1 Elevated risk of bleeding

a) *Inhibition of thrombocyte aggregation, vitamin K antagonism*: Some authors stated that no disadvantageous effects were identified in conjunction with the administration of 600 mg α-tocopherol/day over 3 years. At the present time, it is unclear what relevance these effects could have for the healthy population (FNB, 2000).

   Jandak and co-workers (1989) noted that vitamin E can inhibit *thrombocyte aggregation*. In 6 healthy test persons the 2-week administration of 200 IU (= 134 mg α-TE) vitamin E (as D-α-tocopherylacetate) per day led to a 75% reduction in thrombocyte aggregation; the intake of 400 IU (= 268 mg α-TE) vitamin E led to an 82% reduction.

   In principle, the uncontrolled intake of larger amounts of vitamin E in the case of an existing vitamin K deficiency or in conjunction with anti-coagulation treatment can be linked to an elevated risk of bleeding (Garewal and Diplock, 1995). Corrigan and Ulfers (1981) examined 12 heart patients who were treated with cumarins and given 100-400 IU α-tocopherol/day (= 67-268 mg α-TE) over a period of 4 weeks. A potentiation of the coagulation-inhibiting effect of cumarin was observed and it was recommended that individuals with threshold vitamin K status should avoid uncontrolled vitamin E intake.

b) *Increased risk of haemorrhagic strokes*: In the **ATBC Study** (Alpha-Tocopherol-Beta-Carotene cancer prevention study) (ATBC Study Group, 1994) a 50% higher mortality caused by haemorrhagic stokes was observed amongst smokers in conjunction with the administration of 50 mg alpha-tocopherol (as all-rac-α-tocopheryl acetate) daily over a period of 6 years compared with the control group. Although a link is

conceivable between vitamin E and a higher risk of haemorrhagic complications, the value of this finding is still a subject of controversial discussion (FNB, 2000). Some experts (FSA, 2001) discuss whether vitamin E supplements should not be recommended, for the time being, to smokers.

In an evaluation of a randomised sub-population in the ATBC Study, involving 409 men there was a report of the increased incidence of gingival bleeding in the alpha-tocopherol group (Liede *et al.*, 1998). From the results the authors concluded that supplementation with 50 mg alpha-tocopherol/day can already increase the risk of clinically relevant bleeding to a higher degree than when acetyl salicylic acid is administered alone. The effect was the most manifest in the case of the combination of vitamin E with the thrombocyte aggregation inhibitor acetyl salicylic acid.

7.3.1.2 Intermanagement with vitamin C

There is still uncertainty about the mode of action and optimum dose of antioxidative nutrients. There are indications that disruptions of the vitamin E and vitamin C balance may even have the secondary effect of worsening antioxidant status (Biesalski, 1995; Herbert, 1994).

Brown *et al.*, (1997) examined 100 healthy men (50 smokers with more than 10 cigarettes/day and 50 non-smokers) over 20 weeks who were either given 70, 140, 560 or 1050 mg D-α-tocopherol acetate or a placebo. In all test persons (smokers and non-smokers) given vitamin E, a reduction in the vitamin C level in the plasma was observed which was explained by increased intake and utilisation of vitamin C for the regeneration of the tocopheroxyl radical formed when scavenging radicals in the erythrocytes. From the results the authors concluded that there was a different bioavailability in smokers and non-smokers. Nevertheless, the conclusion was also drawn that a supplement with 70-140 mg D-α-tocopherol/day is sufficient in both smokers and non-smokers to protect against radical-mediated peroxidation in erythrocytes *in vitro* and that higher vitamin E amounts should be combined with adequate vitamin C amounts. The authors recommended that caution should be exercised until the presentation of more detailed knowledge about the exact pathomechanism and interaction between vitamin E and vitamin C during long-term intake.

7.3.1.3 Other adverse effects

From case reports and uncontrolled studies there are reports of various adverse effects (Kappus and Diplock, 1992; Meyers *et al.*, 1996). In conjunction with the administration of doses in a range of 800 mg or more per day, there were isolated cases of stomach and intestinal disorders. In the case of longer administration of doses above 400 mg vitamin E per day, the thyroid hormone level may fall. Other reported side effects like thrombophlebitis, high blood pressure, weak muscles, tiredness and headaches could not be confirmed (BGA, 1994).

7.3.2 Deficiency, possible risk groups

7.3.2.1 Deficiency

An isolated vitamin E deficiency is rare in human beings. It does not occur primarily as the consequence of a food-related deficiency since sufficient amounts of vitamin E are contained in a mixed diet (Bässler *et al.*, 2002; BGA, 1994; DGE/ÖGE/SGE/SVE, 2000). DGE correspondingly notes that sufficient vitamin E intake is possible "without supplements". A deficiency condition may, however, occur during long-term disorders which normally go hand in hand with a fat malassimilation, e.g. (Gaßmann, 1997; N.N., 1999):

- Absorption disorders (e.g. in the case of sprue, short bowel syndrome, cystic fibrosis, chronic pancreatitis, cholestase);
- Transport disruptions (e.g. in the case of A-β-lipoproteinaemia).

Because of body reserves, it takes 1-2 years for clinical symptoms to be observed in adults with a vitamin E deficiency. The lower standard limits are 0.5 mg α-tocopherol/100 ml plasma corresponding to 11.6 µmol/L or 0.8 µg/mg total lipids (Bässler *et al.*, 2002). The typical signs of a vitamin E deficiency are:

- Increase in lipidoxidation products in the plasma;
- Shortening of the erythrocyte life span and elevated inclination to haemolysis;
- Myopathies (weak muscles) and increase in creatine kinase in the plasma and creatinuria as an expression of damage to the muscle membranes;
- Neuropathies with disruptions of depth sensitivity, areflexia and ataxias, encephalopathy;
- In premature babies: haemolysis, retrolental fibroplasia, bronchopulmonal dysplasia, ventricle bleeding.

7.3.2.2 Possible risk groups for deficiency

Vitamin E deficiency normally only occurs in conjunction with congenital or acquired diseases. A purely nutritional vitamin E deficiency is rare. The possible risk groups for deficiency under discussion are:

a) Vitamin E requirements depend on the amount of polyunsaturated fatty acids taken up from food. The higher their share the more vitamin E is required. As foods that are rich in polyene fatty acids are normally also sources of vitamin E, they normally cover the need they generate. However, in the case of *long-term, one-sided eating habits* (e.g. through increased fish consumption with high polyene fatty acid share), meeting requirements may be critical (Bässler *et al.*, 2002).

b) As infants have a low tocopherol store, they need a relatively high intake during the first year of life. In the case of *premature babies*, supply is particularly critical (Bässler *et al.*, 2002).

The calculations of vitamin E intake for the Federal Republic indicate that on average some women seemingly do not achieve the reference intake. The biochemical studies undertaken to estimate vitamin E supply do not, however, provide any indication of deficiencies (supply category 2/3).

7.3.3 Excessive intake, possible risk groups

7.3.3.1 Excessive intake

For Germany there are no indications of the occurrence of hypervitaminosis. The highest intake levels determined in the National Food Consumption Study (NFCS) were measured at the 97.5 percentile: 33 mg for over 65 year-old men. In the VERA Study a plasma concentration of 53.5 µmol α-TE/L was determined at the 97.5 percentile.

7.3.3.2 Possible risk groups in the case of growing vitamin E supplementation

- Potential risk groups in conjunction with the growing use of vitamin E in foods are patients with blood-clotting disorders caused by vitamin K deficiency or as a consequence of treatment with vitamin K antagonists and thrombocyte aggregation inhibitors.
- Given the results of the ATBC Study, it cannot currently be ruled out that smokers could be exposed to a higher risk of developing haemorrhagic complications. With regard to the antioxidative/prooxidative effects, which vitamin E may have in relation to other nutrients, there is a need for clarification of the extent to which, for instance, marginal level of vitamin C could lead to a higher risk.

7.4 Tolerable upper intake level for vitamin E

In 1992 large doses were generally described by SCF as harmless. Regarding the possible occurrence of gastrointestinal orders, it was however recommended that an amount of **2000 mg** α-tocopherol/day should not be exceeded.

The **Tolerable Upper Intake Level (UL)**[♦] derived by FNB only refers to supplements since up to now no adverse effects have been described which can be attributed to an elevated intake of vitamin E occurring naturally in foods. The higher risk of bleeding was selected as the critical endpoint. The results of the ATBC Study were not considered to be convincing enough for them to be included in the derivation. Based on the LOAEL of 500 mg/kg/bw/day, taken from animal experiments, a reference bodyweight of 68.5 kg and an unsafety factor of 36 a UL of **1000 mg/day for adults** was derived which refers to the eight stereoisomers of **α-tocopherol from supplements**. Bearing in mind their lower bodyweight, the following values were laid down for children and adolescents: children (1-3 years) 200 mg, children (4-8 years) 300 mg, children (9-13 years) 600 mg, adolescents (14-18 years) 800 mg. The same ULs are recommended for pregnant and lactating women as for non-pregnant and non-lactating women.

The UL derived by FNB has been criticised. Horwitt (2001) is of the opinion that, given the widespread use of acetyl salicylic acid, fears of a higher tendency to bleeding are justified and that, based on the precautionary principle, this should prompt a reassessment and setting of a lower UL. For these reasons alone, it does not make sense to use a formula which takes into account the UL laid down by FNB for the derivation of a safe and tolerable maximum level of vitamin E in a food.

It has also been shown that the recommendations for an upper safe daily intake level or an upper intake level from the various bodies fluctuate considerably. Bearing in mind the results of the ATBC Study and differentiation to the doses administered for therapeutic purposes, the French body (CSHPF) 1995 suggested an amount of **40 mg** vitamin E per day as the "safety dose for dietary consumption". DGE (DGE/ÖGE/SGE/SVE, 2000) describes an amount of **200 mg** α-TE/day as the upper intake level without adverse effects for adults.

The Expert Group on Vitamins and Minerals (EVM) in the United Kingdom proposed a "safe upper level" of **540 mg vitamin E**, which only refers to supplements (corresponding to approximately 12 mg vitamin E/kg bw with a reference weight of 60 kg). The recommendation stems from a study by Meydani *et al.* (1998) in which over 65-year-old

♦ The Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals (FNB, 2000).

persons were given 800 IU all-rac-α-tocopherol♠ daily over a period of 4 months in a double-blind, placebo-controlled study without any adverse effects being observed.

In April 2003 the deliberations concerning a UL were also concluded in the EU Scientific Committee on Food EU (SCF). A UL for adults of 270 mg vitamin E/day was derived which was rounded up to 300 mg/day (SCF, 2003). In parallel to the UK Expert Group on Vitamins and Minerals (FSA, 2003), the study by Meydani *et al.* (1998) was taken as the basis for deriving the UL. A value of 540 mg vitamin E/day and an uncertainty factor of 2 were taken as the basis for the NOAEL. A higher risk of bleeding was chosen as the critical effect. Based on these data the following ULs for vitamin E were derived for children and adolescents: 120 mg/day (4-6 years); 160 mg (7-10 years); 220 mg (11-14 years); 260 mg (15-17 years).

The FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 1987) has derived an **ADI value** (acceptable daily intake = safe dose of a food additive which can be ingested daily throughout life without any adverse effects on health) of **0.15-2 mg/kg bodyweight** for the addition of tocopherol(s) to foods (for technological purposes). These values were justified by the Committee in the following way:

"α-tocopherol may be an essential nutrient. The U.S. National Academy of Sciences/National Research Council has recommended a dietary allowance of 0.15 mg/kg bw/day. However, excessive intakes of α-tocopherol produce adverse clinical and biochemical effects, and self-medication with large doses of vitamin E preparations could present a hazard. The previous-allocated ADI was amended to include a lower value, which reflects the fact that α-tocopherol may be an essential nutrient. The upper value, which represents the maximum value for the ADI, is based on clinical experience in man."

For an adult weighing 60 kg, this leads to a value of maximum 120 mg vitamin E per day. Taking into account the reference weight of 68.5 kg for adults laid down by FNB, this leads to a maximum value of 137 mg/day.

No data are available for Germany about the level of α-tocopherol intake as an additive. However, it cannot be ruled out that a major share of this ADI value is already used up by the use of tocopherol as an additive.

One major reason for the relatively low vitamin E toxicity is thought to be declining absorption kinetics. When deriving maximum levels, account should, therefore, also be taken of (nutritional)-physiological aspects. The following points are of relevance for vitamin E:

Estimated values for adequate intake: Human vitamin E requirements can still not be determined with the desirable degree of accuracy which means that DGE (DGE/ÖGE/SGE/SVE, 2000) is only able to give estimated values but not make any recommendations. However, these estimated values derived as a rule from the diet of healthy, adequately fed groups of individuals give good indications, according to the definition, for adequate and safe intake.

The reference intakes given for vitamin E are also amounts which are assumed to protect almost all individuals in the respective group in the population from food-related health damage, to enable them to perform fully and, moreover, are intended to create a certain body reserve. Daily nutrient intake on the level of the estimated values, which is achieved from normal food in central Europe, means that inadequate supply is very unlikely.

♠ Note: Taking into account a conversion factor: 1 D-α-tocopherol equivalent = 1 mg D-α-tocopherol = 0.74 x D,L-α-tocopherol (= all-rac-α-tocopherol), 800 IU all-rac-α-tocopherol would correspond to an amount of around 538 mg D-α-tocopherol. The information from the study (800 IU = 727 mg vitamin E) is not comprehensible.

Specificities of vitamin E metabolism:

- Given the body's reserves, a deficiency can only normally be expected after long-term insufficient dietary intake.
- Tocopherol requirements very much depend on the intake level of polyene fatty acids.
- The bioavailability of vitamin E falls as the size of the daily dose increases. The amount which can be absorbed by a healthy person is limited. This can lead to the conclusion that the intake of large amounts of α-tocopherol does not bring any additional benefits for the healthy population and the risk of an imbalance (e.g. worsening of antioxidant status, lowering of γ-tocopherol concentrations) can be increased. People suffering from diseases are not included in this consideration.

7.4.1 Derivation of a maximum level for vitamin E in food supplements

These comments show that there are different recommendations and estimations for an upper safe intake level. It should be noted that there are still considerable gaps in knowledge about vitamin E and even the vitamin E requirements of human beings are not exactly known. Furthermore, vitamin E has some special features like, for instance, dependence of requirements on polyene fatty acid intake or declining absorption rate as intake grows. The different activity of the various vitamers should be taken into account.

Based on the available data the supply situation of the German population can largely be deemed to be secured. Given the widespread use of tocopherols for technological purposes, it can be assumed that the actual intake of vitamin E is even higher. However, no data are available for Germany about the intake level of vitamin E as an additive.

In other chapters in our report maximum levels for some micronutrients for use in food supplements were calculated using the following formula:

$$TL = \frac{UL - DINF}{MEF}$$

Legend

UL	=	Tolerable Upper Intake Level (SCF) usually referring to the daily total intake
DINF	=	Dietary Intake by Normal Food (95. or 97.5 percentile)
MEF	=	Estimated Number of Consumed Products
TL	=	Tolerable Level in a single dietary supplement or fortified food

It was already mentioned that the UL set by FNB, which moreover refers to supplements only, is considered to be too high given the risks described. Hence, it should not be used in the formula to derive a safe and tolerable maximum level in food supplements.

It is also noticeable that the Expert Group on Vitamins and Minerals of the United Kingdom (FSA, 2003) and the Scientific Committee on Food (2003) have derived different maximum levels although the study by Meydani *et al.* (1998) was used in both cases as the foundation. A safe upper level of 540 mg d-α-tocopherol equivalents/day in the form of supplemented vitamin E from the United Kingdom (FSA, 2003) is set against a UL for an upper intake level for adults of 300 mg vitamin E/day by SCF (2003).

In the opinion of BfR the study by Meydani *et al.* (1998), which sought to examine the effects of different vitamin E doses, amongst other things, on the immune system, bleeding time, blood fats, antioxidant status as well as kidney and liver parameters in 88 healthy over 65 year-olds, has some weaknesses. The power of the 4-arm study is limited because of the

relatively low random sample size (n=19-25 persons/group), a study length of only 4 months and selection of exclusively older test persons. BfR, therefore, doubts that the choice of the study as the sole basis for the derivation of tolerable maximum levels is sufficient.

BfR recommends that, in line with the precautionary principle, the experiences from the ATBC Study, which refer to a higher bleeding risk, particularly in conjunction with parallel use of widespread acetyl salicylic acid, be taken into account (ATBC Study Group, 1994; Liede *et al.*, 1998).

Up to now BgVV had proposed a maximum value of **36 mg** for the daily intake of vitamin E from food supplements (BgVV, 1998).

7.4.1.1 Possible management options

Taking into account the available data and the above-mentioned comments, the following management options are proposed:

a) Continuation of existing practice with an upper level of maximum 36 mg vitamin E (α-TE) in food supplements per daily dose

   Advantages: We do not know of any side effects from practice so far. The value is oriented towards the nutritional-physiological requirements and is based on "three-fold" the DGE recommendations from 1991.

   Disadvantages: The safety margin to the vitamin E level of 50 mg/day, at which a higher bleeding risk was described in conjunction with intake over many years, used in the ATBC study is relatively small. Hence, a health risk for consumers cannot in principle be ruled out. This applies in particular to individuals who take thrombocyte aggregation inhibitors or anticoagulants of the cumarin type.

b) Restricting the maximum level to one-fold the reference intakes with an upper level of maximum 15 mg vitamin E (α-TE) in food supplements per daily dose

   Advantages: This amount is oriented towards the nutritional-physiological requirements and is based on the estimated value given by DGE in 2000 for adequate intake for male adolescents and adults aged between 15 and 25. At this maximum level no side effects and no risks for children or adolescents are to be expected. This amount corresponds to 30% of the vitamin E dose used in the ATBC Study. Based on the current level of knowledge, there is no evidence of an elevated risk of bleeding for people taking thrombocyte aggregation inhibitors or anticoagulants of the cumarin type either.

   Disadvantages: There are no identifiable disadvantages.

c) Withdrawal of previous procedure with maximum levels of more than 36 mg up to maximum 1000 mg vitamin E for adults in food supplements per daily dose (corresponding to the FNB tolerable upper intake level)

   Advantages: There are no known advantages.

   Disadvantages: Taking into account the existing data situation a higher risk of bleeding cannot be ruled out especially in the case of parallel intake of the widely used thrombocyte aggregation inhibitor and analgetic, acetylsalicylicacid. There is no guarantee of demarcation to medicinal products and to doses administered for therapeutic purposes. The ADI derived by the Joint FAO/WHO Committee on Food

Additives (JECFA) (FAO/WHO, 1987) is exceeded several times over without this discrepancy being understandable.

7.4.2 Derivation of a maximum level for vitamin E in fortified foods

In line with the comments of the Working group of food chemistry experts of the federal states and BgVV (Arbeitskreis Lebensmittelchemischer Sachverständiger der Länder und des BgVV - ALS, 1988; 1998), an appropriate vitamin addition to the recommended daily portion should not exceed the three-fold amount of recommended daily vitamin intake (corresponding to **36 mg**). Given the existing gaps in knowledge and in order to prevent a cumulation of high vitamin doses from various products, BfR is of the opinion that in fortified foods an appropriate vitamin addition in the expected daily portion should not exceed one-fold the estimated value (**15 mg**).

Since the supply situation of the German population can largely be deemed to be secured, there is no initial need for fortification on nutritional-physiological grounds. Furthermore, uncontrolled fortifications should be avoided in order to protect against imbalances. Given the dependence of vitamin E requirements on the amount of ingested polyene fatty acids, the fortification of polyene fatty acid-containing but low-tocopherol foods could make sense. The different biological activity of the various vitamers should be taken into account.

7.4.2.1 Possible management options

Bearing in mind the above comments, the following management options seem appropriate:

a) Continuation of existing practice with an upper level of maximum 36 mg vitamin E (α-TE) per recommended daily dose

   Advantages: We do not know of any side effects from practice so far. The value is oriented towards nutritional-physiological requirements and is based on "threefold" the DGE recommendations from 1991.

   Disadvantages: As a rule, fortified foods are consumed in an uncontrolled manner without any fixed daily portion. This means that, depending on choice of food and food habits, vitamin E intake could reach a scale at which a higher risk of bleeding has already been described. For that reason, a health risk for consumers cannot, in principle, be ruled out. This applies in particular to persons who take thrombocyte aggregation inhibitors or anticoagulants of the cumarin type.

b) Limiting the maximum level to one-fold the reference intakes with an upper level of maximum 15 mg vitamin E (α-TE) per recommended daily dose

   Advantages: This amount is oriented towards nutritional-physiological requirements and is based on the estimated value for adequate intake indicated by DGE in 2000. There are no concrete indications of specific risks or disadvantageous interactions with specific medicinal products.

   Disadvantages: There are no identifiable disadvantages.

c) Limiting fortification to specific food groups:

   Dependence of fortification on the polyene fatty acid content of the food

   Advantages: Since the supply situation of the German population can be deemed to be largely secured, there is no initial need for fortification on nutritional-physiological

grounds. Uncontrolled fortification, which may bring with it a risk of imbalance, could still be prevented. A linking of vitamin E fortification to the polyene fatty acid content of the food makes sense from the nutritional-physiological angle. Bearing in mind the current supply situation in Germany, no disadvantages or risks would be expected in conjunction with preventive health consumer protection.

Disadvantages: There are no identifiable disadvantages; however, further special regulations would be necessary.

Increasing use of vitamin E in food supplements and for the purposes of food fortification, can, however, lead to doses that can be linked to a higher risk of bleeding, particularly amongst consumers with blood coagulation disorders or through interaction with specific medicinal products. Hence, BfR is of the opinion that the use of vitamin E is linked with a moderate risk of adverse reactions.

Of the above management options, Option b) (15 mg/daily dose) is recommended for food supplements and Option b) (15 mg/recommended daily intake) in combination with Option c) (limiting fortification to specific food groups) for fortified foods.

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8 Risk Assessment of Vitamin K

8.1 Summary

For the Federal Republic of Germany there are no indications of an inadequate vitamin K status with the exception of newborn babies and breastfed infants. However, no representative consumption data or validated biomarkers are available (supply category 2). In the opinion of BfR there is a generally low health risk of adverse effects in conjunction with the use of vitamin K in foods supplements or for the purposes of food fortification. However, patients taking certain medication (anticoagulants) are considered to be a risk group because of the possible life-threatening complications following uncontrolled vitamin K intake, particularly at levels above 250 µg/day. A level of 100 µg/day is considered to be safe in respect of adverse health interactions with these blood-clotting inhibitors.

BfR, therefore, recommends for reasons of preventive health protection a maximum level of 80 µg for food supplements referred to the daily dose recommended by the manufacturer and 80 µg for fortified foods in the recommended daily portion. No health risks for consumers are to be expected from these vitamin K levels which are oriented towards nutritional-physiological requirements. There is no need for a warning for specific risk groups. Otherwise, food supplements and fortified foods with a higher dose would have to carry a warning because of possible interactions with specific medicinal products. So far there is no evidence of any benefit of higher doses in healthy people.

Estimated values for appropriate intake	60-80 µg/day	
Intake [µg/day]	**m**	**f**
Median	? *	? *
P 2.5	? *	? *
P 97.5	? *	? *
	* no representative intake data for the Federal Republic of Germany	
Tolerable upper intake level	not defined; inadequate database	
Proposal for **maximum levels** in:		
food supplements	80 µg/daily dose	
fortified foods	80 µg/daily portion	

8.2 Nutrient description

8.2.1 Characterisation and identification

Vitamin K (CAS No. 12001-79-5) is not a single substance but encompasses a group of substances with 2-methyl-1.4-naphthoquinone as the common basic structure. There are two natural sources of vitamin K: *phylloquinone* which occurs in green plants (vitamin K_1) (CAS No. 84-80-0) and *menaquinone* formed by intestinal bacteria (vitamin K_2) (CAS No. 11032-49-8) with side chains (MK-n) of different lengths. Synthetic *menadione* (vitamin K_3) (CAS No. 58-27-5) is also biologically active as is its *menadiol* (vitamin K_4) (CAS No. 481-85-6) converted to 1.4-dibutyrate. All these compounds are fat soluble; only the salts of menadione are water soluble (Gaßmann, 1999).

Only phylloquinone (vitamin K_1) and farnoquinone (vitamin K_2) may be added to foods (Additives Marketing Ordinance - ZVerkV, Annex 2, List 11), whereby it is mainly phylloquinone that is used. When estimating the risk-benefit relationship, the use of menadione (vitamin K_3 and vitamin K-analogs) can no longer be defended because of the

reported side effects (BGA, 1989). Aside from vitamin K_1, the other vitamin K active compounds menadione sodium bisulphite (CAS No. 130-37-0), menadione pyrimidine bisulphite (CAS No. 14451-99-1) and menadione nicotinamide bisulphite (CAS No. 73581-79-0) used in animal feedstuffs according to valid feedstuff law do not constitute any risk to the target type of animal or any threat to the health of the user or consumer (BfR, 2004). EU Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods and Directive 2002/46/EC for food supplements only list phylloquinone and phytomenadione out of the above compounds. This also applies according to a proposal for a Regulation of the European Parliament and Council of 10.11.2003 (COM (2003) 671 final) on the addition of vitamins and minerals to foods for the purposes of fortification.

8.2.2 Metabolism, function, requirements

Metabolism: The fat soluble vitamin K_1 is mainly absorbed in the jejunum and ileum in the presence of bile acids and pancreatic lipase through micellisation in an active process. Edible fat promotes absorption. The bioavailability of phylloquinone from vegetables is not more than 20% compared to free phylloquinone from supplements (FNB, 2002; Garber *et al.*, 1999; Schurgers *et al.*, 2004). Vitamin K bound to lipoproteins and, in particular to the VLDL fraction, is transported in blood. Vitamin K is mainly accumulated in the liver and, to a lesser degree, in the adrenal glands, lung, bone marrow, kidneys and lymph nodes. The role of a specific menaquinone (MK-4) is not clear that can be formed both from menadione and also from phylloquinone in the organism. The level of MK-4 in the pancreas, salivary glands, brain and sternum is higher than that of phylloquinone. Normally, around 40-50% of the absorbed amount of vitamin K_1 is excreted through bile partly as conjugated and water soluble degradation products in faeces and around 20% in urine. The total body pool is small (70-100 µg and 155-200 nmol) and has a fast turnover (around 24 hours) (Ferland, 2001; FNB, 2002). The mechanism of absorption and use of bacterially formed menaquinones (vitamin K_2) in the human organism are not clear. An indirect indication of the absorption of bacterial menaquinones is their level in the human liver. However, their contribution to the vitamin K supply of man is questionable and probably low (Conly *et al.*, 1994; Ichihashi *et al.*, 1992; Schurgers *et al.*, 1999; Shearer, 1995; Suttie, 1995).

Functions: Vitamin K_1 is efficacious as a coenzyme in the synthesis of the biologically active form of a series of proteins which are involved above all in regulating blood coagulation and bone mineralisation. It is required for the carboxylation of specific glutaminic acid residues in a series of proteins to γ-carboxyglutaminic acid (Gla) residues. Through post-transnational modification of precursors, coagulation factor II (prothrombin), factors VII, IX and X, the plasma proteins C, S and Z and the 3 Gla proteins are formed in the osteoblasts of the bone: osteocalcin, MGP (matrix-Gla protein) and protein S as well as other, less well characterised proteins in the kidneys (nephrocalcin), spleen, pancreas and other tissues. Most information is available about the function of the coagulation factors and osteocalcin whereas the physiological importance of other calcium-binding proteins is less clear (Berkner, 2000; Booth and Mayer, 1997; Ferland, 1998; Gaßmann, 1999). Without vitamin K these proteins are present as inefficacious carboxyl precursors, formerly referred to as PIVKA (Protein induced by vitamin K absence or antagonist). During the carboxylation reaction hydroquinone is oxidised at the same time into vitamin K-2,3-epoxide which is converted back into native vitamin K (quinone), by epoxide reductase. Vitamin K antagonists, like for instance cumarins, can inhibit this reaction which leads to reduced synthesis of coagulation factors and, by extension, to a prolongation of coagulation time (thrombosis or heart attack prophylaxis).

Requirements: Since healthy people do not suffer from a food-related vitamin K deficiency and there are no sound experimental studies into vitamin K requirements, there are only estimated values for appropriate intake of vitamin K. Based on the plasma thrombin level for all age groups beyond neonate age, an adequate daily vitamin K intake of 1 µg/kg bodyweight is recommended. Newborn babies have higher requirements until the intestinal

flora has been colonised. It is, therefore, recommended that for infants up to the 4th month 4 and then 10 µg/day be considered the adequate intake. For children aged between 1 and 15 15-50 µg/day are considered adequate. From age 15 upwards 60-65 µg/day are considered to be adequate for women and 70-80 µg/day for men. It is not felt that there is an additional requirement during pregnancy or lactation (D-A-CH, 2000; SCF, 1993). The epidemiologically proven relations between vitamin K supply status and bone density and an increased risk of osteoporosis and/or arteriosclerosis are not sufficiently proven scientifically for them to be included in the estimation of requirements. To this end, further studies with validated biomarkers are needed in order to prove a possibly higher requirement for optimum bone growth (Binkley *et al.*, 2000; Booth *et al.*, 2003 a; b; Booth and Suttie, 1998; Feskanich *et al.*, 1999; Jie *et al.*, 1996; Schaafsma *et al.*, 2000; Weber, 2001). However, the Adequate Intakes (AI) in the new American recommendations of 90-120 µg per day for women and men are higher than the new D-A-CH reference intakes (FNB, 2002).

8.2.3 Exposure (dietary and other sources, nutritional status)

Sources, incidence:
Foods: Green vegetables are rich in vitamin K_1 (100-750 µg/100 g). Other sources are fats and oils (50-200 µg/100 g), liver, muscle meat, milk and dairy products, eggs (0.5-15 µg/100 g), cereals and fruit (0.1-3 µg/100 g). Foods of animal origin mainly contain MK-4 which is formed through the conversion of menadione or phylloquinone in the organism (Schurgers *et al.*, 1999).

Infant formula and foods for special medical purposes contain at least 4 µg and 3.5 µg vitamin K_1/100 kcal. Some food supplements also contain vitamin K (approx. 30 µg per recommended daily portion).

Another source is *menaquinone (vitamin K_2)* formed by various gram-positive bacteria in the intestines with differing lengths of isoprene residues (MK-n). Most menaquinones contain 6-10 isoprene residues (Basu and Dickerson, 1996; Bentley and Meganathan, 1982).

Medicinal products: Vitamin K-containing medicinal products are only available in pharmacies. They are sold in a fixed combination as multivitamin products with an oral dose of 50-150 µg/day for the prophylaxis and treatment of deficiencies when the vitamin requirements cannot be covered through an appropriate diet. Vitamin K need not necessarily be present in a fixed multivitamin combination for therapeutic use as higher doses and targeted monitoring are necessary for the therapeutic indication of this vitamin (BfArM, 1995; BGA, 1989). Individual vitamins are available for therapeutic use whereby oral doses of 1-5 mg are used in infants and adults to treat minor vitamin K deficiency bleeding. 1-10 mg vitamin K_1 is administered intravenously to treat life-threatening bleeding (Bässler *et al.*, 2002; BGA, 1989).

Nutritional status:
No representative data are available in Germany about the uptake of vitamin K_1 from food. One of the reasons is the lack of sufficient data about the vitamin K content in foods (Jakob and Elmadfa, 2000). It is difficult to monitor the total vitamin K amount taken in by the body since, aside from the amounts ingested from food, vitamin K_2 formed by intestinal bacteria can also be partially absorbed in the colon by passive diffusion (Suttie, 1995).

In the Rotterdam Study average intake of vitamin K_1 or long-chain menaquinones (MK-n) of 249 and 21 µg/day was calculated for older people (n=5435) in the Netherlands (Schurgers *et al.*, 1999). Data from 11 different studies in the USA show that the average vitamin K_1 intake of young adults is approximately 80 µg/day and that of older adults (>55 years) approximately 150 µg/day (Booth and Suttie, 1998).

In the United Kingdom vitamin K_1 intake of 68 µg/person/day was estimated using the provisional vitamin K_1 content of foods (Food Standards Agency, 2001). In a national survey involving older British people (>65 years), an average intake of vitamin K_1 of 65 µg/day was also estimated using food tables. 60% of total intake came from vegetables and only 0.5% from food supplements. 59% of participants had an intake which was below 1 µg/kg bodyweight which is currently considered to be the recommended intake level. In this context, the vitamin K_1 intake of older people from Scotland or from northern England was lower than that of people who live in south England. This was attributable above all to the differing levels of vegetable consumption. The plasma concentrations of phylloquinone correlated positively with phylloquinone intake. The concentration of this biomarker was also dependent on the season whereby lower values were measured in the autumn and winter than in spring and summer. There were differences in the weighted geometric mean between older people who lived at home and older people who lived in residential or nursing homes (0.36 versus 0.24 nmol/L, $p<0.001$) (Thane *et al.*, 2002a; b).

8.3 Risk characterisation

8.3.1 Hazard characterisation (NOAEL, LOAEL)

Up to now, no impairment of health caused by vitamin K from food or supplements has been observed. Doses of up to 25 g phylloquinone/kg bodyweight administered intraperitoneally or orally to mice, chickens and rats did not demonstrate any side effects. From the few studies there have been no reports of mutagenic or carcinogenic risk from vitamin K_1 (FNB, 2002; Food Standards Agency, 2001). All the same, injections of vitamin K_1 from day 6 to day 11 of gestation in mice did trigger teratogenic effects (Roche, 2001a).

Relatively few toxic reactions to vitamin K_1 have been observed in human beings. In rare cases allergic skin reactions may occur as well as convulsive pains, tachycardia, dysrhythmia and cyanosis as side effects following the intravenous administration of large doses (BGA, 1989; Deutsch, 1966). This low number of cases can be attributed to the fact that vitamin K is not available in OTC products unless it is contained in multivitamin preparations. Generally speaking it is only used for specific indications. Furthermore, vitamin K was rarely administered over longer periods which means that no long-term observations in man are available (Food Standards Agency, 2001).

In one study a higher risk of cancer following the intramuscular administration of vitamin K was observed in children (Golding *et al.*, 1992). However, no higher risk was observed for children who were given oral doses of vitamin K which means that these findings are of limited relevance for the UL which is based on oral intake data. The suspicion voiced of a link between intramuscular but not oral vitamin K prophylaxis and cancer in children was not confirmed by other studies (Ansell *et al.*, 1996; Ekelund *et al.* 1993; Göbel and von Kries, 1997; Parker *et al.*, 1998; von Kries *et al.*, 1996).

It is not possible using the available data to undertake a quantitative risk assessment which meant that no **LOAEL** (Lowest observed adverse effect level) could be identified and no numeric **Tolerable Upper Intake Level (UL)** could be derived for vitamin K (FNB, 2002; SCF, 2003).

8.3.2 Deficiency, possible risk groups

Vitamin K deficiency conditions may be triggered either by a real vitamin K deficiency (e.g. alimentary or absorptive) or during the therapeutic use of specific medicinal products by blocking the vitamin K cycle (Bässler *et al.*, 2002; Elmadfa and Leitzmann, 1998):

- Inadequate intake
  (e.g. in patients with eating disorders like Bulimia nervosa and in the case of parenteral alimentation without adequate substitution);
- Deficient absorption and utilisation
  (e.g. in the case of malabsorption, as a consequence of gastrointestinal disease or reduced utilisation in the case of liver cirrhosis and cholestasis);
- Disrupted transport
  (e.g. through lymph drainage disorders or not enough carrier protein (VLDL));
- Blockade of the vitamin K cycle
  - Inhibition of vitamin K epoxide and quinone reductase by anticoagulants
  - Inhibition of vitamin K carboxylase and epoxide reductase by antibiotics
  - Inhibition of reductase by high salicylate doses
  - Inhibition of carboxylase by high doses of vitamins A and E.

The detection of non-efficacious acarboxy precursors of the coagulation factors (PIVKA) and a prolongation of prothrombin time and, in severe cases, the onset of bleeding are considered to be clinical signs of a vitamin K deficiency.

Healthy adults who eat a mixed diet are not likely to suffer from a vitamin K undersupply or deficiency. A marginal vitamin K deficiency could be produced in adults in experiments through dietary restriction (<10 µg phylloquinone/day). This led to a significant increase in prothrombin time which was still in the normal range. Based on these data a requirement of 1 µg vitamin K_1/kg bodyweight and day was derived (Suttie *et al.*, 1988; Udall, 1965).

Since vitamin K is to be found in many foods and is also made available by intestinal bacteria, a vitamin K deficiency can generally be attributed in a secondary manner to chronic gastrointestinal diseases (e.g. Crohn's disease) or to interactions with medicinal products, particularly long-term antibiotic medication (e.g. ampicillin, cephalosporins or tetracyclines) or in conjunction with thrombosis or heart attack prophylaxis as a consequence of overdose of anticoagulants (cumarin derivatives like, for instance, marcumar) (Bechthold and Andrassy, 1988; Elmadfa and Leitzmann, 1998). In the case of foetal warfarin syndrome in children, whose mother's were treated with cumarin derivatives in early pregnancy, there are cases of skeletal deformities and hearing defects in the children as a consequence of disruptions of ossification through inhibition of osteocalcin formation. Furthermore, cerebral haemorrhages may occur in newborn babies (Hall *et al.*, 1980).

The risk of a vitamin K deficiency is particularly high in newborn and breastfed infants (Greer, 1995; Shearer, 1992; von Kries *et al.*, 1985). The causes are the low vitamin K transfer through the placenta and the initially insignificant bacterial vitamin K synthesis in the intestines ("sterile gut"). Furthermore, the immature liver is not yet capable of synthesising sufficient amounts of coagulation factors. The low vitamin K content in human milk (approx. 0.5 µg/100 ml) promotes a vitamin K deficiency, particularly if not enough fluids are taken in the first days. Newborn babies fed industrially manufactured infant formula (vitamin K content according to the Ordinance on foods for special dietary uses (DiätVO) of at least 4 µg/100 kcal) are also at risk if they are not fed from the first day. Bleeding may already occur in the first days (classic Morbus haemorrhagicus neonatorum). Late bleedings after 1-3 months are problematic particularly when severe cerebral haemorrhages occur. Newborn infants whose mother's took medication for epilepsy or blood thinning agents during pregnancy are particularly at risk. The low vitamin K content of human milk also promotes the onset of bleeding (Sutor *et al.*, 1983; von Kries *et al.*, 1985;). Therefore, oral administration of 2 mg vitamin K is recommended immediately after birth and once during the first two months of life. Administration is normally undertaken during the statutorily recommended U1 and U3 examinations. Sufficient vitamin K prophylaxis for the prevention of later vitamin K deficiency

bleeding is still necessary (Ernährungskommission, 1995). More recent studies show that vitamin K_1 supplementation of mothers during lactation is advantageous for the infant since this raised the levels of phylloquinone and MK-4 in human milk (Greer *et al.*, 1997; Thijssen *et al.*, 2002).

With the exception of anticoagulants from the cumarin group, interactions with medicinal products also occurred after the administration of high doses of salicylates (Elmadfa and Leitzmann, 1998). An overly high dose of vitamin K (500 µg and higher) can switch off the blood coagulation inhibiting effect of cumarins (Marcumar, Warfarin) and thus increase the risk of thrombosis (Geil, 1954; Shetty *et al.*, 1993). At a vitamin K intake of more than 250 µg/day above normal intake, higher doses of cumarins (warfarin) are necessary to adjust the INR (International Normalized Ratio) / Quick value in order to counteract a higher risk of thrombosis (Lubetsky *et al.*, 1999). However, there are also case reports of sensitive patients with a low vitamin K supply status in whom far lower amounts of 25 µg/day in the form of multivitamin tablets already lead to interaction (Kurnik *et al.*, 2003). The intra-individual variability is probably attributable in part to the polymorphism of the cytochrome P450 CYP2C9 as this enzyme is responsible for the degradation of warfarin (Khan *et al.*, 2004). In systematic dose-response relationship studies involving 12 healthy test persons, who had been adjusted to an INR of 2.0 with the cumarin derivative acenocoumarol and then given daily increasing doses of 50, 100, 150, 200, 250, 300 and 500 µg synthetic vitamin K_1 (as tablets) over a period of 7 weeks, a dose of 150 µg/day was established as the threshold value at which a statistically detectable lowering of the INR value could still be detected. A level of 100 µg/day is considered to be safe with regard to the adverse health interactions to this blood coagulation inhibiting medication (Schurgers *et al.*, 2004). Patients undergoing anticoagulant treatment do not, however, require a special diet or a low vitamin K diet. Instead a change in eating habits, for instance a sudden switch from a "normal" diet to a very low fat diet or a diet rich in leafy vegetables should, if possible, be avoided and should only be undertaken in conjunction with close monitoring of the coagulation parameters. Under certain circumstances, the taking of food supplements, e.g. vitamin K-containing multivitamin tablets should be avoided or the taking of these supplements should be discussed with the attending physician (BfArM, 1995; Booth and Centurelli, 1999; Großklaus, 1989; Roche, 2001b). On the other hand, it is also recommended that these patients should follow a diet with a steady vitamin K content or supplements on a scale of one- to two-fold the requirements (Booth *et al.*, 1997; Khan *et al.*, 2004). It is estimated that up to 1 million people in Germany take coagulation-inhibiting medication (DGE, 2001).

Excessive amounts of vitamins A and E trigger an antagonistic effect by inhibiting carboxylase which means that the bleeding time is prolonged by the relative vitamin K deficiency (Booth *et al.*, 2004; Olson, 1984).

For the Federal Republic of Germany there are no indications of an inadequate vitamin K status with the exception of newborn babies and breastfed infants. However, representative consumption data and validated biomarkers are not available (supply category 2).

8.3.3 Excessive intake, possible risk groups

Up to now, no cases of hypervitaminosis K have been described. The vitamins K_1 (phytomenadione) and K_2 (menaquinone) are practically non-toxic even at high doses. In rare cases allergic skin reactions may occur. The shock-like events described in isolated cases in conjunction with high-dose intravenous application cannot be attributed to vitamin K itself but to the pharmaceutical auxiliary used. Products with new galenics do not contain this substance which has led to a decisive improvement in intravenous tolerance (Bässler *et al.*, 2002; BGA, 1989).

8.4 Tolerable upper intake level for vitamin K

Up to now, a UL for an upper daily intake level for vitamin K could not be derived because of the lack of long-term studies (FNB, 2002; SCF, 2003). Naturally occurring vitamin K seems to be remarkably free of toxic side effects even when taken orally in milligram amounts. What, however, are missing are well designed, comparative studies in human beings with a sufficient number of test persons, of sufficient length and with various doses in order to make statements about long-term tolerance. This applies in particular to the recording of side effects in conjunction with the long-term use of high doses of vitamin K particularly since the latitude for the risk-benefit assessment of medicinal products is greater than for foods (Großklaus, 2000). For these reasons, it makes sense to orient this towards nutritional-physiological aspects. For adults and older children an amount of 1 to 2 µg/kg bodyweight and day seems to be safe and adequate, for infants around 10 to 20 µg.

The Expert Group on Vitamins and Minerals (EVM) of the United Kingdom observed in its draft report that because of the inadequate data from studies in human beings and animals no safe upper level (SUL) can be established for vitamin K. All the same, the EVM experts believe an intake level of 1 mg/day to be safe (so-called guidance level) at which in their opinion it is unlikely that side effects would occur. Because of the inadequate volume of data available, it was not, however, possible to draw up a guidance value for total intake (Food Standards Agency, 2003). The critical comment should be made that this so-called guidance level is far less reliable than a SUL because of the inadequate data situation. It should, therefore, be considered at best as a guidance value when setting maximum levels.

8.4.1 Derivation of a maximum level for vitamin K in food supplements

Since a UL could not be derived up to now, the proposed formula for a defined maximum level for vitamin K in food supplements cannot be used. Given the existing gaps in knowledge, the measures taken to establish uniform maximum levels should draw on the precautionary principle and be re-examined after the submission of new data.

8.4.1.1 Possible management options

a) Continuation of existing practice
i.e. the so-called "three-fold rule" which means that the three-fold recommended daily dose (240 µg) should not be exceeded in food supplements (BgVV, 1998).

Advantages: We do not know of any side effects from practice so far. The upper level is oriented towards nutritional-physiological requirements. A benefit of higher doses has not been proven so far in healthy persons.

Disadvantages: The proposed maximum level cannot be justified scientifically. It cannot be ruled out that disadvantageous interactions with anticoagulants may occur at this lower level (Schurgers *et al.*, 2004).

b) Setting the recommended maximum safe level at 1 mg in line with the EVM proposal

Advantages: The latitude for manufacturers is larger. The proposed value corresponds to the guidance level suggested in the United Kingdom (Food Standards Agency, 2003).

Disadvantages: This so-called guidance level only takes the *total* additional intake from food supplements into account. With this maximum level, patients undergoing anticoagulant treatment are at considerable risk when taking vitamin K-containing food supplements. At doses of more than 500 µg/day a warning would definitely have to be

prescribed, e.g. "contains higher amounts of vitamin K. Patients undergoing anticoagulant treatment should first consult their doctor!"

We, however, are of the opinion that doses of 1 mg and higher serve medicinal purposes and should not, therefore, be taken into account when setting maximum levels in food supplements.

c) Setting a recommended maximum level of 250 µg in line with the EVM proposal taking into account the suggested calculation.

Since no UL could be established for vitamin K because of the inadequate data available, the so-called guidance level for the determination of the tolerable upper intake level (**TL_{FS}**) of vitamin K in a single dietary supplement should be used with a certain degree of caution in the proposed formula. This leads to the following value:

$$\frac{1000\ \mu g\ ^{*}\ [GL] - 0\ \mu g\ ^{**}\ [DINF]}{4\ [MEF]} = 250\ \mu g\ [TL_{FS}]$$

* EVM, 2002

** The value zero is to be used here because the guidance level only applies to targeted additional intake and not to intake from all sources.

Legend:

UL	=	Tolerable Upper Intake Level (SCF) usually referring to the daily total intake
DINF	=	Dietary Intake by Normal Food (95 or 97.5 percentile)
MEF	=	Estimated Number of Consumed Products
TL	=	Tolerable Level in a single dietary supplement

Advantages: The maximum level derived applies for addition to supplements (individual products) and also takes into account dietary intake from products of the same kind. It is on the same scale of magnitude as the value in Option a).

Disadvantages: Because of the inadequate data available, the derived maximum level is to be treated with caution. A risk of disadvantageous interactions with anticoagulants cannot be ruled out which means that patients should consult their doctor before taking vitamin K-containing food supplements (Schurgers *et al.*, 2004).

d) Setting the admissible maximum level of 80 µg, referred to the daily dose indicated by the manufacturer

The maximum level was derived on the basis of the results of the dose-response studies to determine a safe dose of supplemented vitamin K_1 at which no significant interaction is to be expected with the onset of the blood-thinning effect of anticoagulants (Schurgers *et al.*, 2004). The value obtained in this way of 100 µg/day was divided by an uncertainty factor (UC) of 1.2 in order to take into account the low number of test persons (n=12) and/or inter-individual variability.

Advantages: This maximum level corresponds to the one-fold amount of the estimated value for adequate intake of vitamin K. A warning for specific risk groups is not necessary.

Disadvantages: None.

8.4.2 Derivation of a maximum level for vitamin K in fortified foods

A defined maximum level for vitamin K in fortified foods cannot be derived at the present time using the proposed formula. Non-differentiated, high fortification of conventional foods is not possible because of interaction with specific medicinal products, particularly if this leads to a sudden change in diet or vitamin K intake. According to EU law maximum levels have only been set up to now for foods for special medical purposes (20 µg/100 kcal).

8.4.2.1 Possible management options

a) Continuation of existing practice,
i.e. of the so-called "three-fold rule" which means that in the case of fortification the three-fold recommended daily dose (240 µg) in the recommended daily portion of the fortified food should not be exceeded (ALS, 1998).

Advantages: Experience is already available with this upper level at least for specific foods for special dietary purposes. One constraint is that no data are available about the extent to which these maximum levels are fully used up by the manufacturer.

Disadvantages: As a rule, fortified foods are consumed in an uncontrolled manner which means that there may be a considerable health risk for patients undergoing anticoagulant treatment. Hence, an indication of possible interactions with specific medicines is necessary for fortified foods of this kind which are rich in vitamin K.

b) Alternatively, for reasons of preventive health protection, an appropriate vitamin supplementation in the recommended daily portion should not exceed the one-fold amount of the estimated value for adequate vitamin K intake (corresponding to 80 µg).

Advantages: In the case of these vitamin K amounts oriented towards nutritional-physiological requirements, no health risks for consumers are to be expected. A warning for specific risk groups is not needed.

Disadvantages: The proposed maximum level is more restrictive than practice up to now.

c) Restricting fortification to specific food groups

Advantages: The voluntary fortification of specific groups of foods serves the purposes of preventive health consumer protection. From the nutritional angle, it should perhaps be restricted to foods for special dietary purposes since healthy people do not suffer from a food-related vitamin K deficiency.

Disadvantages: **F**or reasons of equal treatment, distortion of competition cannot be ruled out whereby consumer health protection has priority.

According to BfR, there is in general a low risk of adverse effects for vitamin K when used in food supplements or for the purposes of food fortification.

For reasons of preventive health protection, BfR recommends a maximum level of 80 µg, referred to the daily dose recommended by the manufacturer (Option d) for food supplements and a maximum level of 80 µg in the recommended daily portion (Option b) for fortified foods. Where appropriate, fortification should be restricted to specific food groups (Option c).

8.5 Gaps in knowledge

- There are no data on the chronic toxicity of vitamin K_1 in animals or any long-term observations in humans at doses which go beyond the adequate intake range in order to permit statements about long-term tolerance.
- There are no representative data on vitamin K intake from food or food supplements in Germany. What is also required is more information about the distribution and bioavailability of vitamin K in foods.
- There is a need for research on the extent to which a sub-clinical vitamin K deficiency, which has not as yet manifested itself in the coagulation system, may be involved in the development of osteoporosis. What are needed, more particularly, are validated biomarkers of bone metabolism. Clinical intervention trials in North America and Europe will help to clarify these open questions over the course of the next few years (FNB, 2002).

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9 Risk Assessment of Vitamin B_1

9.1 Summary

The data available for the Federal Republic of Germany on vitamin B_1 intake indicate that around one-third of women do not achieve the recommended intake. Less than optimal supply situations down to deficiencies may occur more particularly in conjunction with alcohol consumption. Based on the validated biomarkers a sub-optimum nutritional status was, however, only observed in a small proportion of the population whereas, generally speaking, the German population has a sufficient supply of vitamin B_1 (supply category 3).

The toxicity of vitamin B_1 is relatively low nor are there any reports about adverse effects from the excessive intake of vitamin B_1 from food or supplements. Up to now, a UL could not be derived because there are no systematic dose-response studies for vitamin B_1 in humans.

BfR considers the health risk from the use of thiamine in foods and fortified foods to be low (cf. Table 2).

BfR recommends that an upper level of 4 mg per daily portion not be exceeded when using vitamin B_1 in food supplements. This amount can be defended from the nutritional-physiological angle and adverse effects are not known or to be expected from this amount. it can, moreover, be justified by the fact that generally speaking the German population has an adequate supply of vitamin B_1, that a relative fall in bioavailability is reported in conjunction with the oral application of higher thiamine amounts and, moreover, no additional benefit has been shown from the intake of larger amounts.

For the fortification of foods for normal consumption with vitamin B_1, we recommend that the one-fold amount of recommended intake not be exceeded in the expected daily portion of a food. This recommendation also takes into account possible nutrient cumulation through the consumption of various fortified foods as well as the fact that vitamin intake beyond requirements does not offer any additional nutritional-physiological benefits.

Recommended intake	1.3 mg/day	
Intake [mg/day] (NFCS, 1994)	**m**	**f**
Median	1.36	1.1
P 2.5	0.69	0.54
P 97.5	2.63	2.25
Tolerable upper intake level	not defined database insufficient no known risk in normal doses	
Proposal for **maximum levels** in:		
food supplements	4.0 mg/daily dose	
fortified foods	1.3 mg/daily portion	

9.2 Nutrient description

9.2.1 Characterisation and identification

Thiamine (CAS No. 59-43-8) consists, in chemical terms, of a pyrimidine ring which is linked to a thiazole ring by a methylene group. The synonyms used are vitamin B_1, aneurine and antiberiberi factor. Thiamine is readily water soluble, sensitive to heat and oxidation, particularly in neutral and alkaline media.

Thiamine hydrochloride (CAS No. 67-03-8) and thiamine nitrate (CAS No. 532-43-4) are generally approved as vitamin compounds in foods (Additives Marketing Ordinance - ZVerkV, Annex 2, List 11, Ordinance on Vitaminised Foods - VitaminV).

9.2.2 Metabolism, function, requirements

Metabolism: Absorption of the naturally occurring water soluble vitamin is done by active, carrier-mediated transport which is subject to a saturation mechanism and is, therefore, dose-dependent. The intestinal threshold concentration is given as 2-3 µmol (µM) per litre (Bitsch, 1997; D-A-CH, 2000; Sauberlich *et al.*, 1979). The active process can be inhibited by alcohol. At higher oral doses a lower proportion is also absorbed by passive diffusion. The absorption co-efficient then, however, falls rapidly and is only 5-10% (D-A-CH, 2000) at oral doses of 5 mg and more per day. When administered orally, thiamine has a bioavailability of approximately 5.3% at a dose of 50 mg in healthy test persons. Altogether, a maximum of 8-15 mg can be ingested daily (BfArM, 1999; Food Standards Agency, 2000). Following intestinal uptake thiamine is normally converted in the liver to thiamine diphosphate (TDP). Uptake in the mucosa cell and from plasma to the cells and mitochondria of the various organs is done by a high affinity thiamine transporter which shows a higher concentration in the case of a deficiency condition (Singleton and Martin, 2001; Song and Singleton, 2002). High thiamine concentrations are found in the liver, kidneys and brain in addition to skeletal and heart muscle. Overall the storage capacity of the organism for thiamine is restricted to 25-30 mg. The biological half-life is stated as between around 10 and 20 days. Hence, relatively regular thiamine intake is necessary. Around 50% is eliminated unchanged or with thiamine-esterised sulphate. The rest normally consists of thiaminic acid, methylthiazole acetic acid and pyramine. High oral thiamine doses are quickly eliminated in urine after tissue saturation. The higher the thiamine intake, the lower the metabolisation and the higher the elimination of unchanged thiamine (Bässler *et al.*, 2002; D-A-CH, 2000; FNB, 1998). In the case of higher alcohol consumption intestinal absorption of thiamine falls whereas elimination of the vitamin increases (SCF, 2001). Furthermore, alcohol inhibits the activation of the free thiamine to the coenzyme thiamine diphosphate (McCormick, 1988).

Interactions: Thiamine is inactivated by the medicinal substance 5-flourouracil as 5-flourouracil competitively inhibits the phosphorylation of thiamine to thiamine pyrophosphate. Antacids and black tea prevent the absorption of thiamine (BfArM, 1999).

Function: Thiamine mainly functions in the form of thiamine diphosphate (TDP) as a coenzyme in important group transmission reactions in energy metabolism, e.g. when converting pyruvate to acetyl-CoA, when converting alpha-ketoglutarate to succinyl-CoA in the citric acid cycle and during transketolase in the pentose phosphate cycle. Furthermore, thiamine has an antagonistic effect on acetyl choline (Baumgartner, 1991).

Requirements: The thiamine requirements must be seen in relationship to the respective energy turnover (D-A-CH, 2000). A high protein and carbohydrate diet increase, a high lipid diet reduce thiamine requirements (Buddecke, 1980). In controlled clinical trials, a minimum thiamine requirement of 0.08 mg/MJ (0.33 mg/1000 kcal) was determined for adults (Sauberlich *et al.*, 1979; Wood *et al.*, 1980). In other studies an amount of 0.12 mg/MJ (0.5 mg/1000 kcal) was deemed necessary for tissue saturation and sufficient activity of the thiamine-dependent enzyme in adults. A lower energy requirement should not lead to a lower thiamine intake than 1.0 mg per day (D-A-CH, 2000; Nichols and Basu, 1994).

Table 10: Recommended thiamine intake

Age	Male persons mg/day	Female persons mg/day
Infants		
0 up to under 4 months (estimated value)	0.2	0.2
4 up to under 12 months	0.4	0.4
Children		
1 up to under 4 years	0.6	0.6
4 up to under 7 years	0.8	0.8
7 up to under 10 years	1.0	1.0
10 up to under 13 years	1.2	1.0
13 up to under 15 years	1.4	1.1
Adolescents and adults		
15 up to under 19 years	1.3	1.0
19 up to under 25 years	1.3	1.0
25 up to under 51 years	1.2	1.0
51 up to under 65 years	1.1	1.0
65 years and over	1.0	1.0
Pregnant women (from 4th month)		1.2
Lactating women		1.4

(D-A-CH, 2000)

As a consequence of the changing metabolite situation and the requirements of the foetus, an additional allowance of 0.2 mg/day thiamine is necessary during pregnancy. Bearing in mind the increased energy requirements and excretion in human milk, an additional allowance of 0.4 mg thiamine per day is calculated for lactating women (D-A-CH, 2000; Nail *et al.*, 1980).

In the case of chronic alcohol abuse, this requirement is far higher since the absorption and metabolism of thiamine are disrupted (Bitsch, 1997; D-A-CH, 2000).

In accordance with SCF the PRI (Population Reference Intake) is 100 µg thiamine per MJ energy intake which leads to an average recommended intake level of approximately 1.0-1.2 mg per day. For people with a lower energy intake than 8 MJ daily, the daily thiamine supply should not, however, be lower than 0.8 mg (Sauberlich *et al.*, 1979).

In the USA the RDA (Recommended Daily Allowance) was set as follows: children 0.2-0.9 mg, adolescents and adults 0.9-1.2 mg, pregnant women 1.4 mg, lactating women 1.4 mg (D-A-CH, 2000; Jellin *et al.*, 2002; Yates *et al.*, 1998).

9.2.3 Exposure (dietary and other sources, nutritional status)

Sources:

Foods: Good sources of thiamine are muscle meat, in particular pork (0.9 mg/100 g), liver (0.3 mg/100 g), some types of fish (plaice, tuna), cereals and cereal products depending on the degree of fineness (0.1-0.6 mg/100 g), pulses and potatoes (0.1-0.3 mg/100 g) (D-A-CH, 2000; Souci *et al.*, 2000). The mean value for preparation losses in gently prepared traditional foods is around 30% (Bognàr, 1995; D-A-CH, 2000).

Fortified foods: The main foods fortified with thiamine in Germany are beverages, cereals, sweets and dairy products. As a rule, at least 15% up to maximum 100% of recommended daily intake is added as thiamine (Kersting *et al.*, 1995).

Food supplements: In Germany 25% of women and 18% of men take food supplements more than once a week, usually in the form of multivitamin products. The daily intake of thiamine from food supplements, measured against recommended intake of the Deutsche

Gesellschaft für Ernährung (German Nutrition Society), is at least 100% (Beitz *et al.*, 2002; Mensink and Ströbel, 1999; Schellhorn *et al.*, 1998).

Medicinal products: In the case of the prophylactic treatment of thiamine deficiency conditions, the recommended oral dose is 5 mg thiamine chloride/thiamine hydrochloride per day to the extent that diet-related measures are not deemed to be sufficient. In the case of an existing mild thiamine deficit in adults, the oral dose is 5-30 mg per day for the duration of one month (Jellin *et al.*, 2002). For the treatment of clinically manifest thiamine deficiency conditions, the recommended oral dose is initially 300 mg thiamine chloride/thiamine hydrochloride per day followed by 50-200 mg per day, broken down into several doses over the day (BfArM, 1999; Jellin *et al.*, 2002).

Nutritional status: Representative data on thiamine supply in Germany were recorded in the VERA Study carried out in the 1980s whereby the median intake in men was 1.5 mg and in women 1.1 mg.

Based on biochemical parameters (reduced transketolase activity in the erythrocytes [α-ETK] and reduced thiamine elimination in urine), a sub-optimal supply status was only found in a small proportion of the Federal German population (4-6%). Measured thiamine values (urine) below the limit value were observed in more than 10% of underweight women. Regular alcohol and cigarette consumption is clearly a risk factor for inadequate thiamine supply (Heseker *et al.*, 1992).

Table 11: Daily thiamine intake in Germany

Age (years)	Male persons		Female persons	
	Median (mg)	Percentile 2.5-97.5 (mg)	Median (mg)	Percentile 2.5-97.5 (mg)
4 - 6	0.93	0.45 - 1.83	0.85	0.44 - 1.79
7 - 9	1.11	0.60 - 2.11	1.04	0.56 - 2.17
10 - 12	1.24	0.63 - 2.35	1.12	0.61 - 2.13
13 - 14	1.42	0.68 - 2.47	1.22	0.58 - 2.30
15 - 18	1.52	0.79 - 2.93	1.09	0.55 - 2.33
19 - 24	1.45	0.73 - 2.85	1.09	0.51 - 2.08
25 - 50	1.40	0.71 - 2.63	1.10	0.51 - 2.11
51 - 64	1.38	0.74 - 2.44	1.12	0.59 - 2.00
>64	1.28	0.64 - 2.26	1.09	0.57 - 2.04

(according to Adolf *et al.*, 1995)

According to the results of the 1998 Nutrition Survey, the median (and the 95% confidence interval) of vitamin B_1 intake (mg/day) for men and women, who regularly took vitamin supplements, was 1.50 (1.45; 1.56) and 1.13 (1.09; 1.18) respectively. In this context, around one-third of women did not meet the recommendation for vitamin B_1. This does not, however, mean that there was a supply deficiency as the DGE recommendations (1.0 mg/day) have a certain safety margin (Beitz *et al.*, 2002; Mensink *et al.*, 1999).

Studies on thiamine intake from food in the European Union showed a mean average intake of 1.2 mg thiamine (women); the average value of 1.0 mg was the lowest in the Netherlands and of 1.8 mg per day the highest in Portugal. In this respect SCF comes to the conclusion that the daily intake of thiamine can generally be deemed to be adequate in relationship to physiological needs. If one also takes into account additional intake from supplements, then the highest mean and 97.5 percentile of vitamin B_1 intake in the European Union was 2.28 and 6.35 mg/day (SCF, 1993).

9.3 Risk characterisation

9.3.1 Hazard characterisation (NOAEL, LOAEL)

Thiamine has relatively low toxicity. There are no reports about adverse reactions caused by excessive intake of thiamine from food or supplements (FNB, 1998; SCOGS, 1978). In the case of professional cyclists with a calculated oral intake of 30 mg per day and 10 mg i.m. over years, no adverse reactions were reported (Sauberlich *et al.*, 1979). In the case of oral intake of up to 500 mg thiamine per day for the duration of 1 month there was no visible toxicity in man (SCF, 1993). Adverse effects like headache, stupor, sweating, tachycardia, skin reactions with itchiness and urticaria may occur in human beings after longer oral intake of higher doses (50 mg/kg bodyweight or more than 3 g/day) (Bässler *et al.*, 2002; Food Standards Agency, 2000). In the case of parenteral administration of thiamine in doses between 100 and 300 mg, there were very rare cases of adverse effects, which were more frequent at parenteral doses up to 500 mg per day (Sauberlich *et al.*, 1979; Wrenn *et al.*, 1989). In individual cases anaphylactic reactions were documented after repeated intravenous administration of thiamine (Tetreault and Beck, 1956).

Since there are no systematic oral dose-response studies involving thiamine and the lowest toxicity of this vitamin, no **LOAEL** (Lowest observed adverse effect level) or **NOAEL** (No adverse effect level) can be set (SCF, 2001).

9.3.2 Deficiency, possible risk groups

At a thiamine intake below 0.2 mg/1000 kcal (4.2 MJ) there may already be symptoms of thiamine deficiency after 4-10 days. In isolated cases deficiency symptoms were observed at a thiamine intake of less than 0.05 mg/MJ (0.2 mg/1000 kcal) already after 9 days (D-A-CH, 2000).

Thiamine deficiency causes in particular disruptions of carbohydrate metabolism. A block of the transketolase reaction (pentose phosphate cycle) leads to an accumulation of pentose phosphates at three times the norm in the erythrocytes. Biochemical detection can be done by determining transketolase activity in erythrocytes (normal values 1.00-1.15, marginal 1.15-1.25, severe deficiency >1.25). An increase in the pyruvate and lactate levels in the blood as a consequence of reduced pyruvate decarboxylation, (secondary) acidosis and reduced thiamine elimination in urine (normal >66 µg/24 hours, marginal 27-65, severe deficiency <27) also provide diagnostic information (Buddecke, 1980; Food Standards Agency, 2000). In clinical terms the acute form of clear thiamine deficiency leads to metabolic acidosis linked, in some cases, with cardiac failure. Severe ongoing thiamine deficiency leads to the clinical picture of beriberi which, depending on its course and the involvement of other nutrients, is characterised by losses in neurological function, skeletal muscular dystrophy, myocardial insufficiency and oedemas. This disease, which is considered to be a classical avitaminosis, is broken down into the neurological form ("dry beriberi") with mainly neuritic disorders and polyneuropathies and the oedematous form ("wet beriberi") in which the focus, in clinical terms, is on cardiac insufficiency and the formation of oedemas (Bates, 2001). Infantile beriberi occurs in infants breastfed by women with a thiamine deficiency and manifests itself through a weak drinking ability, vomiting, apathy or unrest, in acute cases also with life-threatening cardiac insufficiency (D-A-CH, 2000).

Some of the causes of deficient thiamine supply are:

- Malnutrition and bad dietary habits, as a consequence of a zero calorie diet, slimming diet, one-sided eating habits of older people
- Malabsorption for instance in the case of Crohn's disease, sprue
- Chronic alcoholism

- Increased requirements, e.g. during pregnancy and lactation, chronic haemodialysis
- Diabetic acidosis
- Severe acute liver disorders
- Genetic defects in thiamine metabolism

Alcoholics are particularly at risk of thiamine deficiency (Bitsch, 1997; Bitsch and Hötzel, 1981; D-A-CH, 2000). In the case of chronic alcoholism a thiamine deficiency can lead to cardiomyopathy with dilatation of the right ventricle, polyneurophathy, Wernicke's disease (encephalopathy) and Korsakoff's (anamnestic) syndrome (BfArM, 1999).

High doses of thiamine (10-250 mg oral or 500 mg/day intramuscular) are necessary in the case of rare genetic defects in order to compensate for the reduced bonding affinity of the coenzymes (Ames *et al.*, 2002).

The data available for the Federal Republic of Germany on vitamin B1 intake indicate that around one-third of women do not achieve the recommended intake levels. A sub-optimal supply status down to deficiencies may occur particularly in conjunction with alcohol consumption. Based on the validated biomarkers, however, a sub-optimum supply status was only determined in a small portion of the population whereas in general the German population has a sufficient thiamine supply (supply category 3).

9.3.3 Excessive intake, possible risk groups

Thiamine has low toxicity. There are no known cases of hypervitaminosis attributable to excessive uptake from food including food supplements.

9.4 Tolerable upper intake level for thiamine

Based on the available data no **UL (Tolerable Upper Intake Level)** can be set at present for thiamine (FNB, 1998; SCF, 2001). No adverse effects were observed in the case of the 100-fold exceeding of the recommended intake level through supplements (Nordic Council, 2001). However, no systematic studies are available for this dose range. For that reason, this intake should be oriented towards nutritional-physiological aspects.

9.4.1 Derivation of a maximum level for thiamine in food supplements

Since, up to now, no tolerable upper intake level could be derived for total daily intake, the proposed formula for the derivation of a defined maximum level for thiamine in food supplements cannot be used. Given the existing gaps in knowledge, the measures to be taken to set uniform maximum levels should be based on the precautionary principle and revised on submission of new data.

9.4.1.1 Possible management options

a) Continuation of existing practice

In the case of food supplements the three-fold recommended daily dose of thiamine (3 x 1.0-1.3 mg corresponding to approximately up to 4 mg) per daily portion should not be exceeded (BgVV, 1998; D-A-CH, 2000).

Advantages: Good experience is available for this range. This upper level is oriented towards requirements – also taking into account the relatively dwindling bioavailability described above in conjunction with the oral application of higher thiamine amounts. It also takes sufficient account of inter-individual differences (concept of requirement orientation with an adequate additional allowance). Up to now, no adverse effects have

been reported for this range and no health risks are to be expected for consumers. It is indeed the case, as outlined above, that larger amounts of thiamine can indeed be considered as tolerable when administered orally, however there is no proof of an (additional) benefit for healthy persons from higher daily portions. Warnings are not necessary. Since, within the framework of medication-based treatment for mild thiamine deficiency in adults, the oral dose is 5-30 mg per day (Jellin *et al.*, 2002), the required "margin" would be maintained between food supplements and medicinal products.

Disadvantages: This maximum level ("three-fold rule") seems to have been laid down in an arbitrary manner. The criticism of an assumed inadequately differentiated assessment of B vitamins would not be dispelled.

b) No indication of upper levels for individual products or no maximum levels

Advantages: There are no identifiable advantages for the consumer.

Disadvantages: In the case of healthy people there are no signs of a benefit. More particularly, sufficient account would not be taken of the precautionary principle or of proper consumer health protection since the sparse data situation on the basis of which SCF or other bodies were unable to set a **UL**, does not indicate that higher amounts might not be linked to a health risk.

c) Upper levels far in excess of requirements based on individual efficacy or tolerance trials in patients, e.g. 100 mg thiamine daily as proposed by EVM UK, 2003, as a guidance level

Advantages: There are no identifiable advantages for the consumer. In the 1996 Gokhale clinical trial cited by EVM UK, a daily dose of 100 mg thiamine against placebo was tested over 60 and 90 days in 556 dysmenorrhoea patients aged between 12 and 21 years in India without there being any reports of adverse effects. This means that this upper level could be said to have a (narrow) scientific basis (EVM, 2003; Gokhale, 1996).

Disadvantages: An upper level of, for instance, 100 mg thiamine based on maximum tolerance studies is very far removed from nutritional-physiological aspects and the requirement-oriented approach. The relevance of individual studies like Gokhale, 1996, must be deemed to be questionable in the given context which is based on lifelong consumption of a substance by healthy consumers.

d) One-fold rule

The one-fold recommended daily dose of thiamine (= 1.0-1.3 mg) should not be exceeded per daily portion in the case of food supplements (BgVV, 1998; D-A-CH, 2000).

Advantages: This upper level is strictly oriented towards actual requirements and makes nutritional-physiological sense. For this range health risks for the consumer can be ruled out.

Disadvantages: There are no identifiable health disadvantages.

9.4.2 Derivation of a maximum level for thiamine in fortified foods

Since, up to now, no tolerable upper intake level could be derived for total daily intake, the proposed formula for the derivation of a defined maximum level for thiamine in fortified foods cannot be used. Given the existing gaps in knowledge, the measures to be taken to set uniform maximum levels should be based on the precautionary principle and revised on submission of new data.

9.4.2.1 Possible management options

a) Continuation of existing practice

In accordance with the Ordinance on Vitaminised Foods, fortification of foods with thiamine is permitted without there being any explicit mention of upper levels there. BgVV accepted thiamine additions up to three-fold the requirements referred to the expected daily portion.

Advantages: No bad experience has been reported on this. It should, however, also be mentioned that there are not sufficient data concerning the maximum level of fortification used by food manufacturers in individual cases. For this range no adverse effects have been reported up to now. Nor are any health risks to be expected for consumers. In the case of vitaminised foods intended to cover general requirements, the Working Group of Food Chemistry Experts of the *Länder* and BgVV is of the opinion that a major increase above the three-fold level of recommended daily vitamin intake does not offer any additional nutritional-physiological benefits (ALS, 1998).

Disadvantages: Since (fortified) foods are eaten in an uncontrolled manner without fixed daily portions, specific requirement-oriented maximum levels could scarcely be complied with. Depending on the consumption of various foods, large and excessive amounts of vitamins would be ingested under certain circumstances.

b) "One-fold rule" in line with the recommended daily dose of thiamine of 1.0-1.3 mg

Advantages: The one-fold recommended daily dose makes sense and is oriented towards nutritional-physiological aspects and preventive health protection since this, rather than higher fortification, takes account of the fact that foods as a rule are consumed in an uncontrolled manner without fixed daily portions.

Disadvantages: There are no identifiable health risks.

In the opinion of BfR, the risk of adverse effects from the use of thiamine in food supplements and fortified foods is low. After weighing up the above-mentioned management options, BfR recommends Option 9.4.1.1.a) for food supplements (up to 4 mg thiamine/daily dose) and Option 9.4.2.1.b) for fortified foods (up to 1.3 mg thiamine/daily intake). For risk management it is recommended that, for reasons of preventive health protection, the setting of maximum levels should be oriented towards nutritional-physiological aspects.

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10 Risk Assessment of Vitamin B_2

10.1 Summary

The data available for the Federal Republic of Germany indicate that around one-quarter of adult women do not reach the recommended intake for vitamin B_2. A sub-optimum nutritional status can, however, only be detected in underweight women and men and in conjunction with the consumption of cigarettes and alcohol. Vitamin B_2 intake is in the recommended intake range for the majority of the population (supply category 3).

Up to now, no UL could be derived for the total daily intake of riboflavin which means that the proposed formula cannot be used to calculate a maximum level for the addition of this vitamin to food supplements.

BfR, therefore, considers the health risk from the use of riboflavin in food supplements and fortified foods to be low (cf. Table 2).

BfR recommends that the upper level of 4.5 mg per daily portion of a food supplement not be exceeded when using vitamin B_2. This level is acceptable from the nutritional-physiological angle and adverse effects are neither reported nor expected at this level. Moreover, it is justified by the fact that in general the German population has a sufficient supply of vitamin B_2 and, furthermore, no additional benefit has been proven for higher intake levels.

Against this backdrop BfR recommends that, for the fortification of conventional foods with vitamin B_2, one-fold the recommended intake not be exceeded in the expected daily portion of a food. This recommendation takes account of possible nutrient cumulation through the consumption of various fortified foods and also of the fact that vitamin intake beyond requirements does not bring any additional nutritional-physiological benefits.

Recommended intake	1.5 mg/day	
Intake [mg/day] (NFCS, 1994)	**m**	**f**
Median	1.61	1.34
P 2.5	0.86	0.61
P 97.5	3.23	2.83
Tolerable upper intake level	not defined database not sufficient no known risk from customary doses	
Proposal for **maximum levels** in:		
food supplements	4.5 mg/daily dose	
fortified foods	1.5 mg/daily portion	

10.2 Nutrient description

10.2.1 Characterisation and identification

Riboflavin is chemically specified as an isoalloxazine ring bound to ribite, a pentahydric alcohol. Its chemical name is 7,8-dimethyl-10-(1‘-D-ribityl)isoalloxazine. Two synonyms are vitamin B_2 and lactoflavin.

Riboflavin (CAS No. 83-88-5) and riboflavin-5‘-phosphate-sodium (CAS No. 130-40-5) are generally authorised as vitamin compounds in foods (Additives Marketing Ordinance - ZVerkV, Annex 2, List 11; Ordinance on Vitaminised Foods - VitaminV). The substances are authorised food dyes, EC-No. E 101, which may be used for foods in general with the

exception of certain foods up to quantum satis (Additives Marketing Authorisation Ordinance - Zusatzstoff-ZulassungsVO, Annex 1, Part A).

10.2.2 Metabolism, function, requirements

Metabolism: Riboflavin, flavin-adenine dinucleotide (FAD) and flavin mononucleotide (FMN = riboflavin phosphate) are taken up from food. Both coenzymes are cleaved in the proximal small intestine. At low concentrations free riboflavin is actively absorbed by means of saturation kinetics and, at higher concentrations, by passive diffusion (D-A-CH, 2000).

In the dose range 2-25 mg oral riboflavin, the absorption rate is 50-60% (Elmadfa and Leitzmann, 1988).

In almost all tissues, particularly in the liver, riboflavin and FMN are converted into FAD. The tissue stores take the form of the enzyme-bound riboflavin. Excess riboflavin, not bound to plasma proteins, is mainly excreted via tubular secretion in urine.

Function: Riboflavin is a component of the coenzymes, flavin-adenine nucleotide (FAD) and flavin mononucleotide (FMN = riboflavin phosphate). As components of dehydrogenase and oxidase they play a central role in oxidative metabolism. It is, therefore, important for many stages in metabolism, including biosynthesis and the degradation of amino acids, fatty acids and carbohydrates.

Requirements: The dependence of riboflavin requirements on energy turnover is understandable from the position of the flavin enzyme in the oxidative metabolism (D-A-CH, 2000; Soares *et al.*, 1993; Van der Beek *et al.*, 1994).

Studies into FAD stimulation of erythrocyte glutathione reductase (EGR) showed that, at a riboflavin intake below 0.11 mg/MJ (0.5 mg/1000 kcal), elevated stimulation in most test persons resulted from inadequate supply. At intakes of 0.14 mg/MJ (0.6 mg/1000 kcal), by contrast, FAD stimulation was in the reference range (Bamji, 1969; Bitsch, 1997; D-A-CH, 2000).

Table 12: Recommended riboflavin intake

Age	Male persons (mg/day)	Female persons (mg/day)
Infants		
0 up to under 4 months (estimated value)	0.3	0.3
4 up to under 12 months	0.4	0.4
Children		
1 up to under 4 years	0.7	0.7
4 up to under 7 years	0.9	0
7 up to under 10 years	1.1	1.1
10 up to under 13 years	1.4	1.2
13 up to under 15 years	1.6	1.3
Adolescents and adults		
15 up to under 19 years	1.5	1.2
19 up to under 25 years	1.5	1.2
25 up to under 51 years	1.4	1.2
65 years and older	1.2	1.2
Pregnant women (from 4th month)		1.5
Lactating women		1.5

(D-A-CH, 2000)

SCF recommended the following daily intake levels: male children 0.8-1.6 mg/day, female children 0.8-1.3 mg/day, male adults 1.3 mg/day, female adults 1.1 mg/day (SCF, 1993).

In the USA the DRI (Dietary Reference Intake) was set as follows: children 0.5-0.9 mg/day, male adults 1.0-1.1 mg/day, pregnant women 1.4 mg/day, lactating women1.6 mg/day (FNB, 1998; Jellin *et al.*, 2002).

Reduced energy requirements should not lead to riboflavin intake below 1.2 mg/day (Boisvert *et al.*, 1993; D-A-CH, 2000).

During pregnancy an additional allowance of 0.3 mg vitamin B_2/day is recommended since requirements are clearly higher (reduced excretion of vitamin B_2 in urine, elevated EGR) (D-A-CH, 2000; FNB, 1998).

Human milk contains on average 38 µg vitamin B_2/100 ml. The additional allowance for lactating women (0.4 mg/day) is derived from the vitamin B_2 of 750 ml human milk and a vitamin B_2 turnover rate of 70% (D-A-CH, 2000; Souci *et al.*, 2000; WHO, 1965).

Riboflavin requirements increase during physical activity, severe disease, after operations and traumas, in conjunction with absorption disorders, chronic alcohol abuse and through interaction with various medicinal products (e.g. certain antidepressive agents) (Boisvert *et al.*, 1993; D-A-CH, 2000; Greb *et al.*, 1993; Soares *et al.*, 1993).

10.2.3 Exposure (dietary and other sources, nutritional status)

Sources:
Food: Foods rich in riboflavin are milk and dairy products (0.2-0.6 mg/100 g), meat (pork, beef, chicken: 0.2 mg/100 g), liver (pork, beef: 3.0 mg/100 g) and fish (e.g. sardines, mackerel, herring: 0.2-0.4 mg/100 g). Other major sources of riboflavin are fruit and vegetables (e.g. kale, peas, broccoli, yellow peppers, avocado: 0.2-0.3 mg/100 g) as well as cereals and cereal products (depending on the degree of fineness, e.g. rye wholemeal, oats: 0.2 mg/100 g) (Bässler *et al.*, 2002). Cow milk has a four times higher riboflavin content than human milk (D-A-CH, 2000).

Riboflavin is largely heat stable but is inactivated by light. In the case of correct storage and gentle preparation of food, vitamin losses of riboflavin are to be expected on a scale of 20% (Bognàr, 1995; D-A-CH, 2000).

Fortified foods: The main products fortified with riboflavin in Germany are beverages, e.g. mixed milk beverages, cereals and sweets. In the case of fortification of this kind, 15% to maximum 100% of recommended daily intake are normally added as riboflavin (Kersting *et al.*, 1995).

Food supplements: Studies indicate that around 25% of women and 18% of men take vitamin-containing food supplements at least once a week that frequently also contain riboflavin (Beitz *et al.*, 2002). The daily uptake from food supplements of this kind normally amounts to at least 100% measured against the intake recommendations of the Deutsche Gesellschaft für Ernährung (German Nutrition Society).

Medicinal products: Therapeutic doses for rapid saturation in the case of reduced tissue stores for the treatment of a riboflavin deficiency with medicinal products are daily doses of 5-25 mg and 5-30 mg riboflavin, distributed over several single doses (Jellin *et al.*, 2002; McKevoy, 1998).

Nutritional status: To assess the supply status the excretion of riboflavin in 24-hour urine can be used (D-A-CH, 2000; Horwitt *et al.*, 1950). A healthy adult excretes 120 µg riboflavin or more in urine in 24 hours. Less than 40 µg (Horwitt *et al.*, 1950) and 27 µg/g creatinine (Sauberlich, 1999) per 24 hours can be seen as an indication of a riboflavin deficiency.

The activation of erythrocyte glutathione reductase (EGR) through the addition of FAD can also be used to identify supply status (Coopermann and Lopez, 1984: D-A-CH, 2000). However, this method can supply misleading results when the concentration of the apoenzyme is changed, for instance in the case of a protein deficiency (D-A-CH, 2000; Horwitt, 1986).

According to the VERA Study (Adolf *et al.*, 1995) conducted in the 1980s, riboflavin supply in Germany was as follows:

Table 13: Daily riboflavin intake in Germany

Age (years)	Male persons		Female persons	
	Median (mg)	Percentile 2.5-97.5 (mg)	Median (mg)	Percentile 2.5-97.5 (mg)
4 - 6	1.24	0.62 - 2.54	1.15	0.62 - 2.76
7 - 9	1.41	0.68 - 2.65	1.32	0.65 - 2.95
10 - 12	1.54	0.74 - 3.11	1.32	0.69 - 2.58
13 - 14	1.59	0.81 - 3.09	1.32	0.62 - 2.79
15 - 18	1.64	0.76 - 3.76	1.29	0.60 - 2.80
19 - 24	1.62	0.78 - 3.54	1.26	0.61 - 2.79
25 - 50	1.54	0.80 - 3.16	1.27	0.61 - 2.65
51 - 64	1.55	0.81 - 2.97	1.31	0.71 - 2.50
>64	1.51	0.75 - 2.84	1.31	0.62 - 2.73

(according to Adolf *et al.*,1995)

Riboflavin supply from foods has been the subject of surveys in various European countries (SCF, 2000).

In the Netherlands the mean daily uptake of riboflavin is 1.54 mg (97.5 percentile: 2.87 mg/day) (Hulshof *et al.*, 1997-1998). In Italy data indicate a mean riboflavin intake of 1.6 mg/day (97.5 percentile: 2.7 mg/day) (Turrini, 1994-1996). Data from Austria indicate a mean intake of 1.49 mg/day (97.5 percentile: 3.29 mg/day) (Elmadfa *et al.*, 1998). In the United Kingdom the mean intake from all sources for men and women is 2.3 mg/day and 1.8 mg/day respectively (EVM, 2000). In Ireland the data point to a mean intake from all sources for adults (18-64 year-olds) of on average 2.1 mg/day (97.5 percentile: 4.6 mg/day) (IUNA, 2000).

10.3 Risk characterisation

10.3.1 Hazard characterisation (NOAEL, LOAEL)

Up to now, there have been no reports of adverse effects in human beings as a consequence of high or excessive riboflavin uptake from foods or supplements. In 49 patients no side effects were observed after taking 400 mg/day (Schoenen *et al.*, 1994). However, the available data are not sufficient in order to derive a **UL** (tolerable upper intake level) (FNB, 1998; SCF, 2000). However, the limited consumption data for riboflavin available up to now indicate that this substance does not constitute a risk to human health in conjunction with the assumed daily intake levels from all sources. This does not mean that riboflavin does not have any potentially adverse effects when higher amounts are consumed.

10.3.2 Deficiency, possible risk groups

It may be difficult to diagnose a riboflavin deficiency since, on the one hand, the symptoms in the case of hypovitaminosis are not very characteristic and, on the other, this is usually combined with a deficiency of other vitamins, too. An exclusively riboflavin deficiency is extremely rare and depends on the ability to maintain the riboflavin bound in the tissue during a deficiency state. On release of the vitamin following protein cleavage, it is reused for the synthesis of the new enzymes. Only the relative small part bound to the enzyme cannot be reused. The reserve capacity for riboflavin is 2-6 weeks. Risk groups for sub-optimum supply are young women, pregnant women and older people.

The VERA Study conducted in Germany showed that underweight men and women have a comparatively high prevalence for low measured values of various vitamins including riboflavin. Smokers compared to non-smokers, in some cases depending on the average number of cigarettes smoked, have significantly lower measured values of various vitamins, including riboflavin. The same applies to people with high alcohol consumption (Heseker *et al.*, 1992).

Studies on the supply situation in Germany indicate that around one-quarter of adult women do not reach the recommended intake levels of 1.2 mg per day for riboflavin (Beitz *et al.*, 2002).

Riboflavin deficiency can lead to growth disorders, seborrhoiec dermatitis around the nasal labial crease, infections in the oral mucosa and tongue, conjunctivitis and angular cheilosis. In severe cases there may be vascularisation and inflammation of the cornea, cataract and also normocytic anaemia. Severe riboflavin deficiency also impairs the metabolism of pyridoxine and niacin, folic acid and vitamin K (D-A-CH, 2000). During pregnancy a riboflavin deficiency can lead to skeletal anomalies in the rat foetus (Buddecke, 1980).

A riboflavin deficiency can occur, for instance in conjunction with:

- severe malnutrition and bad dietary habits, e.g. false choice of food by older people
- alcoholism
- long-term parenteral diet without riboflavin substitution
- malabsorption, e.g. Crohn's disease, sprue, chronic enteritis
- increased requirements, e.g. pregnant, lactating women
- diabetes mellitus as a consequence of losses in urine
- chronic haemodialysis
- phototherapy for jaundice of the newborn hyperbilirubin anaemia (Icterus neonatorum)
- medicinal product interactions, e.g. through barbiturates, chlorpromazine, hormonal contraceptives.

The data available for the Federal Republic of Germany on vitamin B_2 indicate that around one-quarter of adult women do not reach the recommended intake levels for this vitamin. However, sub-optimum supply status can only be identified in underweight women and men and in conjunction with cigarette and alcohol consumption. For the majority of the population riboflavin intake is in the recommended intake range (supply category 3).

10.3.3 Excessive intake, possible risk groups

The highest mean uptake of riboflavin from foods and supplements for men aged between 31-50 was given as 6.9 mg daily. The highest intake (95 percentile) was 11 mg riboflavin per day for women over 70 years of age (FNB, 1998)

No negative effects of high doses of riboflavin from food or from supplements (e.g. 400 mg per day over 3 months) were observed (Schoenen *et al.*, 1994). Minor adverse effects were only reported in two cases at this high dose, for instance gastrointestinal diarrhoea and polyuria (Schoenen *et al.*, 1998).

Riboflavin can lead to a yellow-orange colouring of urine (Micromedex).

10.4 Tolerable upper intake level for riboflavin

Based on the existing data and with regard to the overall low toxicity of this vitamin, it is not possible to set a **UL (tolerable upper intake level)** for riboflavin (SCF, 2000).

JECFA has set a group ADI of 0-0.5 mg/kg bodyweight for riboflavin and riboflavin-5-phosphotase as a dye (JECFA, 1969; 1981). This value was confirmed by a 13-week feed study in Wistar rats in whom the dose of 50 mg per kg bodyweight did not trigger any relevant toxicological effects (SCF, 1998).

However, no long-term experience, more particularly with high doses in humans is available. For these reasons intake should be oriented towards nutritional-physiological aspects. The requirements of children and adults are covered with 0.8-1.0 mg/day whereby the 97.5 percentile of intake not exceed 3.8 mg (Adolf *et al.*, 1995).

10.4.1 Derivation of a maximum level for riboflavin in food supplements

As no tolerable upper intake level for total daily intake could be derived up to now, the proposed formula for the derivation of a defined maximum level for riboflavin in food supplements cannot be used. Given the existing gaps in knowledge the measures to be taken to set uniform maximum levels should be based on the precautionary principle and revised on submission of new data.

10.4.1.1 Possible management options

a) Continuation of existing practice

For adolescents and adults the three-fold recommended daily dose of riboflavin (3 x 1.2-1.5 mg corresponding approximately to up to 4.5 mg) should not be exceeded per daily portion in food supplements (ALS, 1998; Bässler *et al.*, 2002; D-A-CH, 2000).

Advantages: No negative experience is available for this range. This upper level is oriented towards requirements and takes sufficient account of inter-individual differences (concept of requirement orientation with adequate allowance). For this range no side effects have been reported up to now nor are any health risks expected for the consumer. It is indeed the case, as outlined above, that larger amounts of riboflavin administered orally can indeed be considered tolerable. However, there is no evidence of an (additional) benefit of higher daily portions for healthy individuals. Warnings are not necessary. Since, within the framework of medication-based therapy for rapid resaturation in the event of reduced tissue stores during the treatment of a riboflavin deficiency, the oral dose was 5-25 mg or 5-30 mg per day (Jellin *et al.*, 2002; McKevoy, 1998), the required "margin" would be maintained between food supplements and medicinal products.

Disadvantages: This maximum level ("three-fold rule") seems to have been set in an arbitrary manner. The criticism of a supposedly inadequately differentiated assessment of the B vitamins would not have been dispelled.

b) No indication of upper levels for individual products or no maximum levels

Advantages: There are no identifiable advantages for the consumer.

Disadvantages: There are no signs of a benefit in healthy individuals. More particularly the precautionary principle and, by extension, proper consumer health protection would not be sufficiently taken into consideration since the inadequate data which resulted in the SCF and other bodies not being able to set a **UL**, does not mean that higher intakes may not be linked to a health risk. (SCF made it quite clear in this respect for riboflavin, "This does not mean that there is no potential for adverse effects from high intakes" (SCF, 1993; 2000). FNB specified in this context, "Since data on the adverse effects of riboflavin intake are limited, caution may be warranted." (FNB, 1998)).

c) Upper levels set considerably higher than requirements on the basis of individual efficacy or tolerance trials in patients, e.g. 40 mg riboflavin daily as proposed by EVM UK, 2003 as a guidance level

Advantages: There are no identifiable advantages for the consumer. In the clinical trial by EVM UK Schoenen *et al.* (1998), a daily dose of 400 mg riboflavin was tested for at least 90 days in 55 migraine patients whereby only two minor and non-specific adverse effects were reported (Schoenen *et al.*, 1998). Based on this value EVM extrapolates a so-called guidance level for riboflavin of 40 mg per day which means that this upper level could be attributed a (narrow) scientific basis (EVM, 2003).

Disadvantages: An upper level of, for instance, 40 mg riboflavin based on maximum tolerance studies is very far removed from nutritional-physiological aspects and the requirement-oriented approach. The relevance of individual studies is, therefore, to be considered questionable in this context which is based on lifelong consumption of a substance by healthy consumers.

d) One-fold rule

The one-fold daily recommended dose of riboflavin (= 1.2-1.5 mg) should not be exceeded per daily portion of supplements by adolescents or adults (ALS, 1998; Bässler *et al.*, 2002; D-A-CH, 2000).

Advantages: This upper level is strictly oriented towards actual requirements and makes nutritional-physiological sense. For this range no health risks are expected for consumers.

Disadvantages: There are no identifiable health disadvantages.

10.4.2 Derivation of a maximum level for riboflavin in fortified foods

Since, up to now, no tolerable upper intake level could be derived for total daily intake, the proposed formula for the derivation of a defined maximum level for riboflavin cannot be used in fortified foods. Given the existing gaps in knowledge, the measures to be taken to set uniform maximum levels should be based on the precautionary principle and revised on submission of new data.

10.4.2.1 Possible management options

a) Continuation of existing practice

In accordance with the Ordinance on Vitaminised Foods, fortification of foods with riboflavin is permitted without it making any explicit reference to upper levels. BgVV accepted riboflavin additions up to three times requirements referred to the expected daily portion.

Advantages: No bad experience is available in this respect. By way of constraint, it should be mentioned that no sufficient data are available concerning the maximum level of fortification undertaken in individual cases by food manufacturers. No side effects have been reported for this range up to now nor are any health risks expected for the consumer. In the case of vitaminised foods, which serve to cover general needs, the Working Group Food Chemistry Experts of the *Länder* and *BgVV* is of the opinion that a major increase beyond three-fold the recommended daily vitamin intake does not offer any additional nutritional-physiological benefits (ALS, 1998).

Disadvantages: Since (fortified) foods are normally consumed in an uncontrolled manner without any fixed daily portions, certain requirement-oriented maximum levels could scarcely be complied with. Depending on the consumption of various foods, considerable and excessive amounts of vitamins could be consumed under certain circumstances.

b) "One-fold rule" in line with the recommended daily dose of 1.2-1.5 mg riboflavin

Advantages: The one-fold recommended daily dose makes sense and is oriented towards nutritional-physiological aspects and to preventive health protection since this, rather than higher fortification, takes into account the fact that foods are consumed as a rule in an uncontrolled manner without any fixed daily portions. In the case of vitaminised foods which serve general needs, a major increase in recommended daily vitamin intake does not offer any additional nutritional-physiological benefits (ALS, 1998).

Disadvantages: There are no identifiable health disadvantages.

The risk of adverse effects in conjunction with the use of riboflavin in food supplements and fortified foods is considered to be low by BfR. After weighing up the above-mentioned management options, BfR recommends Option 10.4.2.1b) (up to 1.5 mg riboflavin/daily intake) for fortified foods and Option 10.4.1.1a) (up to 4.5 mg riboflavin/daily dose) for food supplements. For the purposes of risk management it is recommended that, for reasons of preventive health protection, the setting of maximum levels should be oriented towards nutritional-physiological aspects.

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11 Risk Assessment of Niacin

11.1 Summary

The surveys available for the Federal Republic on niacin intake indicate that the average intake is far higher than the amount needed to cover requirements. The biochemical studies undertaken to estimate niacin supply do not provide any evidence of deficiency (supply category 3/4).

Given the different hazard potential of nicotinic acid and nicotinamide, various bodies have derived different ULs for these two compounds. Whereas the intake of high nicotinamide concentrations only rarely leads to side effects and no adverse effects are known or to be expected from dietary uptake, nicotinic acid may already have a vasodilating effect and increase the fibrinolytic activity of the blood at doses over 30 mg. According to the Federal Institute of Risk Assessment (BfR), the use of niacin in the form of nicotinamide in food supplements and fortified foods poses a low health risk. By contrast, the health risk linked to the use of nicotinic acid is considered to be high (cf. Table 2).

If one takes the UL for nicotinic acid as the basis for the derivation of the maximum level, this leaves no scope for the use of niacin in food supplements as this UL is already achieved through average intake from normal food. BfR recommends that the use of niacin in food supplements be oriented towards nutritional requirements and that the maximum level of 17 mg not be exceeded in a daily dose.

In the case of fortified foods a major increase in recommended daily vitamin intake does not offer any additional nutritional-physiological benefits. Therefore, we recommend that, for appropriate vitamin addition, the one-fold amount (17 mg) of the recommended daily vitamin intake not be exceeded in the expected daily portion of a food.

Recommended intake	17 mg/day	
Intake [mg/day] (NFCS, 1994)	**m**	**f**
Median	15.1	11.5
P 2.5	6.78	4.98
P 97.5	29.1	21.4
Tolerable upper intake level	Nicotinic acid 10 mg/day Nicotinamide 900 mg/day	
Proposal for **maximum levels** in:		
food supplements	17 mg/daily dose	
fortified foods	17 mg/daily portion	
Nicotinic acid should definitely not be used.		

11.2 Nutrient description

11.2.1 Characterisation and identification

Niacin is a collective term for the vitamins nicotinic acid and nicotine acid amide as well as the co-enzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). They can be interconverted in the organism. Nicotinic acid and nicotinamide are included amongst vitamins although they are not really vitamins since they can by synthesised from tryptophan in the organism. Only when there is a tryptophan deficiency (e.g. diet mainly consisting of maize) is niacin intake necessary.

Nicotinic acid (pyridine-3-carboxylic acid, CAS No. 59-67-6) and nicotinic acid amide (nicotinamide, pyridine-3-carboxylic acid, CAS No. 98-92-0) are both substances acting as vitamins. In the human organism they serve to synthesise the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) (Bässler *et al.*, 2002). In Germany nicotinic acid and nicotinic acid amide are equated with additives (ZVerkV) and also permitted as additives in foods for special dietary purposes (Ordinance on foods for special dietary purposes - DiätVO, Annex 2, No. 4.8). Both compounds are also listed in EU Directive 2001/15/EC on substances that may be added for specific nutritional purposes in foods for particular nutrition uses and in Directive 2002/46/EU on food supplements.

11.2.2 Metabolism, function, requirements

Metabolism: Nicotinamide mostly occurs in food in the form of the two coenzymes, NAD and NADP. It is already absorbed in the stomach, mostly however in the upper small intestine after being cleaved into free nicotinic acid. At a low concentration, absorption is sodium-dependent or carrier-mediated, at higher doses (3-4 g) it involves passive diffusion (Bässler *et al.*, 2002). Nicotinic acid is also rapidly and almost fully absorbed in the entire small intestine.

Niacin has a high first-pass metabolism which means that in the low dose range nicotinamide and nicotinic acid only reach systemic circulation in the form of the coenzymes, NAD and NADP which are stored in erythrocytes and tissue. End products of niacin metabolism are primarily excreted in urine as N_1-methyl nicotinamide and N_1-methyl-2-pyridone-5-carboxylic acid amide (Gaßmann, 1997; Knip *et al.*, 2000).

Function: in the human organism niacin, in the form of the two coenzymes NAD and NADP, in conjunction with specific enzymes, is involved in a number of oxidation and reduction reactions. They play an important role in glycolysis, lipid synthesis and energy generation. Their function is the reversible uptake and release of reduction equivalents (hydrogen) (Bässler *et al.*, 2002).

Requirements: Since the organism in the liver and kidneys can directly convert some of the essential amino acid tryptophan taken up with nutrient proteins to NAD and NADP, the entire supply of niacin consists of an exogenous portion from food and an endogenous portion from tryptophan metabolism. That is why niacin requirements or the desirable level of intake is given in "niacin equivalents" whereby one niacin equivalent corresponds to 1 mg nicotinic acid or 60 mg tryptophan since in theory 1 mg niacin can be formed in metabolism from 60 mg tryptophan. Because of the endogenous synthesis route, daily niacin requirements can only be estimated (Bässler *et al.*, 2002).

In the SCF expert opinion concerning the derivation of a UL for niacin dated 17 April 2002, it is observed that, because of endogenous niacin synthesis from tryptophan, niacin requirements are sufficiently met under normal balanced diet conditions. Hence, the additional intake of preformed niacin in the diet and the daily intake of 9-8 mg niacin equivalents recommended by SCF in 1993 is not necessarily needed (SCF, 2002).

Given the dependence on energy consumption WHO and FAO have proposed a calculation basis of 1.6 mg NE (niacin equivalents)/MJ (= 6.7 mg/1000 kcal) for recommended intake whereby adults should not ingest less than 13 mg NE per day (Gaßmann, 1997). This recommendation was also taken on board by D-A-CH. This leads to the following intake recommendations for children and adults (DGE/ÖGE/SGE/SVE, 2000):

Table 14: Recommended niacin intake

Age	Recommendation	
	male	female
Children		
1 up to under 4 years	7 mg NE/day	
4 up to under 7 years	10 mg NE/day	
7 up to under 10 years	12 mg NE/day	
10 up to under 13 years	15 mg NE/day	13 mg NE/day
13 up to under 15 years	18 mg NE/day	15 mg NE/day
Adolescents and adults		
15 up to under 25 years	17 mg NE/day	13 mg NE/day
25 up to under 51 years	16 mg NE/day	13 mg NE/day
51 up to under 65 years	15 mg NE/day	13 mg NE/day
>65 years	16 mg NE/day	13 mg NE/day

Since there is increased conversion of tryptophan into niacin during pregnancy, the exogenous niacin requirements do not increase. Because of the increased energy need (+ 1.1 MJ/day and 255 kcal/day), an additional niacin intake of 2 mg/day is, however, recommended for pregnant women (DGE/ÖGE/SGE/SVE, 2000); from the 4th month onwards pregnant women should consequently take 15 mg NE per day.

Up to 1.3 mg preformed niacin from food (and theoretically 2.8 mg NE from tryptophan, where the conversion rate is unknown) in secreted in 750 ml human milk. Since breastfed infants do not show any sign of deficiency, an estimated value of rounded up 2 mg/day preformed niacin is the recommended intake for young (non-breastfed) infants and for breastfeeding women an additional intake is recommended, too (DGE/ÖGE/SGE/SVE, 2000; Fankhänel and Gaßmann, 1998).

This leads to the following intake recommendation:

	Recommendation
Breastfeeding women	17 mg NE/day
Breastfed infants	
Non-breastfed infants	
0 up to under 4 months	2 mg niacin/day
4 up to under 12 months	5 mg NE/day

11.2.3 Exposure (dietary and other sources, nutritional status)

Sources: Niacin is contained in different concentrations in almost all foods. In Germany the main niacin sources are meat, fish, bread, cake and pastries, beer, potatoes, milk and dairy products. In foods of animal origin niacin occurs in a non-bound manner. Bioavailability from foods of animal origin is almost 100%. In foods of plant origin niacin is mainly bound to macromolecules and only bioavailable to 30% (Mensink *et al.*, 2002; Sebrell and Butler, 1938). The content of free nicotinic acid is particularly high in roasted coffee. This has to do with demethylation of trigonelline during the roasting process (BGA, 1990)

Nutritional status: Since far in excess of half of niacin is synthesised by the organism from tryptophan and the bioavailability of niacin from foods varies considerably, it is scarcely possible to assess niacin supply by means of an exact intake calculation. In the VERA Study (Volume IV), excretion of the metabolite NMNA = N-methyl nicotinamide was, therefore, measured in 24-h urine in order to assess the niacin supply status (Heseker *et al.*, 1992). The NMNA values show a very large scatter. In 5.3% of the test persons values were measured below the reference intake. The VERA Study (Volume III) reaches the conclusion that if one includes all groups of people in the German population a median intake of 12.9 mg preformed niacin from food (without niacin equivalents) per day is reached (Heseker *et al.*,

1994), whereby the highest niacin intake in the 97.5 percentile of 35-44 year-old men is 32.3 mg.

The DONALD study, which describes the diet of a cohort of children and adolescents aged between 1 and 18 in a longitudinal manner, examined the influence of fortified foods on niacin intake in addition to niacin uptake from normal food. The total intake of niacin equivalents increases with age and in all age groups is 20-50% above recommended intake which means that nutrient fortification is not necessary (Kersting *et al.*, 2000). According to the DONALD study existing fortified foods cover, at present, about 20% of niacin supply in the age groups of children and adolescents in Germany. The recommendations are met through intake levels of up to 160% (Kersting *et al.*, 1995; Sichert-Hellert and Kersting, 2001).

According to the results of the Nutrition Survey conducted within the framework of the Federal Health Survey in 1998, there was a median intake of 38 mg NE for men and 29 mg NE for women. In the case of regular users of vitamin supplements median niacin intake was 42.5 mg (25-75 percentile: 36.6-52.5 mg) (Mensink *et al.*, 2002). This means that in the Federal Republic of Germany the niacin intake of the population is far higher than the daily intake recommended by D-A-CH; only 0.6% of the population do not reach these levels (Mensink *et al.*, 2002). According to SCF the consumption data of other European countries also show that the average intake is around 200% of the recommended intake of niacin equivalents (SCF, 2002). The high protein and, by extension, tryptophan intake means that a sufficient niacin supply of the population is guaranteed.

Medicinal products: Nicotinamide is used as a medicinal product for treatment and prophylaxis in conjunction with niacin deficiency conditions as a consequence of malnutrition or bad dietary habits, increased requirements or malabsorption linked to gastrointestinal diseases (BGA, 1989). For the purposes of prophylaxis, oral doses of 8-10 mg/day are recommended, for treatment 40-250 mg/day.

Besides the treatment and prophylaxis of nicotinic acid deficiency conditions as a consequence of malnutrition or bad dietary habits, increased requirements or deficiency conditions caused by gastrointestinal diseases, nicotinic acid is also used as a medicinal product to treat primary and secondary hyperlipoproteinaemias (BGA, 1990). For prophylaxis 15-30 mg/day are recommended, for treatment several daily doses of 50-100 mg. For the treatment of hyperlipidaemias a daily dose of 3-6 g is suggested.

11.3 Risk characterisation

11.3.1 Hazard characterisation (NOAEL, LOAEL)

Risk assessment is done on the basis of studies with niacin-containing supplements and medicinal products. There are no indications of adverse effects through niacin uptake from food.

Because of their differing hazard potential, a separate assessment of nicotinic acid and nicotinamide is necessary.

Nicotinic acid: At doses above 30 mg, nicotinic acid has a vasodilating effect and can increase the fibrinolytic activity of blood. Furthermore, at this dose flushing, pruritus, heat discomfort and, in some cases, urticaria are to be expected. The administration of 300-1500 mg nicotinic acid can lead to nausea and vomiting. At high doses over a longer period there may be incidences of heartburn, loss of appetite, bloatedness, hyperacidity, nausea, vomiting, diarrhoea and signs of liver damage (increase in transaminases and/or alkaline phosphatase) (Gray *et al.*, 1994; Rader *et al.*, 1992). Furthermore, doses upwards of 3 g can

lead to a reduction of glucose tolerance, an increase in the uric acid level and, in isolated cases, to toxic macular changes (Bässler *et al.*, 2002; BGA, 1990; Gaßmann, 1997).

The occurrence of flushing constitutes a limiting side effect for nicotinic acid. From a dose of 50 mg/day upwards, this adverse effect was observed in various clinical trials (SCF, 2002; Sebrell and Butler, 1938; Spies *et al.*, 1938). This dose is considered to be the LOAEL by the American Food and Nutrition Board (FNB) whereas SCF has defined a LOAEL of 30 mg based on the study by Sebrell, in which 2 out of 6 patients, who took nicotinic acid orally over 92 days, already reacted with occasional flushing at a dose of 30 mg (FNB, 1998; SCF, 2002; Sebrell and Butler, 1938; Spies *et al.*, 1938).

Nicotinamide: The uptake of high nicotinamide concentrations only rarely causes side effects. In very rare cases gastrointestinal disorders and, in isolated cases, hepatotoxic reactions were described at high dose nicotinamide treatments of up to 3 g/day (Bässler *et al.*, 2002; Knip *et al.*, 2000; Rader *et al.*, 1992). Based on clinical trials in patients with a high risk of diabetes mellitus, who took higher doses of nicotinamide for up to 3 years without major side effects, the Scientific Committee on Food (SCF) of the European Commission defined for **nicotinamide** a dose of **25 mg/kg/day** as the **NOAEL**, a **LOAEL** was not defined (Knip *et al.*, 2000; SCF, 2002). There were no cases of flushing or pruritus in clinical trials (Bässler *et al.*, 2002; Knip *et al.*, 2000).

Even at high doses nicotinamide only leads to minor side effects and is, therefore, frequently given priority over nicotinic acid (Gaßmann, 1997; Heseker *et al.*, 1994).

11.3.2 Deficiency, possible risk groups

The signs of niacin deficiency are characterised by the 3-D symptoms: dermatitis – diarrhoea – dementia. Niacin deficiency has a non-characteristic prodromal stage with loss of appetite and weight, indisposition, sleeplessness, attacks of vertigo and finally pellagra, a characteristic, sharply delimitated, oedematous dermatitis. Partial atrophy of the intestinal mucosa is the cause of diarrhoea. A serotonin deficiency in the brain is considered to be the main cause of the neuropsychiatric changes (Bässler *et al.*, 2002; Hanck, 1986). In developing countries protein deficiency and a one-sided diet with maize or sorghum are considered to be the most frequent causes. In these types of cereals niacin is largely bound to the peptide niacytin and is not absorbed. In industrial countries with a balanced food offering pellagra symptoms (pellagroid) are normally only observed in conjunction with chronic alcoholism, cirrhosis of the liver and chronic diarrhoea (Crohn's disease, Colitis ulcerosa). A niacin deficiency may also occur in the case of non-treated autosomal-recessive hereditary Hartnup disease as a consequence of intestinal and tubular absorption disruptions of neutral amino acids (BGA, 1989; 1990; Manske *et al.*, 1999) or in conjunction with elevated consumption of tryptophan for serotonin synthesis in the case of a carcinoid syndrome (Hanck, 1986). The nicotinic acid metabolism can also be disrupted by various medicinal products at different points and trigger a niacin deficiency, e.g. through certain analgesics, anti-diabetic products, psychiatric drugs, anti-epileptics, tuberculostatic agents, immunosuppressants and cytostatic agents (Hanck, 1986).

The surveys available for the Federal Republic on niacin indicate that on average far more is taken in than is deemed necessary to cover requirements. The biochemical studies undertaken to estimate niacin supply do not provide any indication of deficiency conditions (supply category 3/4).

11.3.3 Excessive intake, possible risk groups

Niacin has a low toxicity. Up to now, no cases of hypervitaminosis have been described in conjunction with excessive uptake from food and supplements.

Nicotinic acid: At very high intake levels of nicotinic acid per day, occasional adverse effects may occur like heartburn, loss of appetite, bloatedness, hyperacidity, nausea, vomiting, diarrhoea and signs of liver damage (increase in transaminases and/or alkaline phosphatase). In addition, reductions in glucose tolerance, an increase in uric acid levels and isolated toxic macular changes have been described. Following intake over several months of 1.5-3 g nicotinic acid per day, hepatotoxic effects occurred like jaundice, abnormal liver values (e.g. elevated serum glutamate-oxalacetate transaminase and alkaline phosphatase levels) whereby these symptoms partly occurred in an isolated manner or already after administration of 0.5 g/day over two months (Bässler *et al.*, 2002; BGA, 1990).

Nicotinamide: The taking of high doses of nicotinamide rarely causes side effects (Bässler *et al.*, 2002; Knip *et al.*, 2000).

11.4 Tolerable upper intake level for niacin

Whereas SCF lays down separate maximum levels in its assessment for nicotinic acid and nicotinamide because of their different hazard potential, the American FNB defines a maximum level for niacin taking into account the different toxicities. Based on a LOAEL of 50 mg, the FNB derives the following ULs for daily **niacin** intake for individual groups of people (FNB, 1998):

Age	UL
1 - 3 years	10 mg niacin/day
4 - 8 years	15 mg niacin/day
9 - 13 years	20 mg niacin/day
14 - 18 years	30 mg niacin/day
≥ 19 years	35 mg niacin/day
Pregnant and lactating women	35 mg niacin/day

Nicotinic acid: Based on erythema that occurs at doses of 30 mg upwards, SCF established a value of **10 mg/day for adults** as the **UL** (tolerable upper intake level) with regard to the daily intake of **nicotinic acid** in its expert report. In this context it explicitly points out that this level does not apply to pregnant or lactating women because of a lack of experience and clinical trials. When setting the UL for children, the average bodyweight of the respective age group was taken as the basis (SCF, 2002). Hence the following apply

Age	UL
1 - 3 years	2 mg nicotinic acid/day
4 - 6 years	3 mg nicotinic acid/day
7 - 10 years	4 mg nicotinic acid/day
11 - 14 years	6 mg nicotinic acid/day
15 - 17 years	8 mg nicotinic acid/day

The Expert Group on Vitamins and Minerals (EVM) in the United Kingdom described the data available in its draft report both for nicotinic acid and nicotinamide as inadequate for the derivation of a safe maximum level. Instead, it suggested a so-called guidance value for nicotinic acid of 17 mg/day based on a NOAEL of 50 mg for flushing which only refers to supplements (Food Standards Agency, 2003).

Nicotinamide: For nicotinamide SCF set a UL of 12.5 mg/kg bodyweight/day resp. **900 mg/day for adults** based on a NOAEL of 25 mg/kg bodyweight and an uncertainty factor of 2 since adults may eliminate nicotinamide more slowly than the study group which mainly consisted of children. This does not apply to pregnant or lactating women (SCF, 2002). The following values apply for children:

Age	UL
1 - 3 years	150 mg nicotinamide/day
4 - 6 years	220 mg nicotinamide/day
7 - 10 years	350 mg nicotinamide/day
11 - 14 years	500 mg nicotinamide/day
15 - 17 years	700 mg nicotinamide/day

EVM set a guidance value of 500 mg/day for nicotinamide based on a NOAEL of 25 mg/kg/d which only refers to supplements (Food Standards Agency, 2003).

According to SCF, niacin is mainly used in the form of nicotinamide in food supplements and for the fortification of foods. It has a far lower toxicity than nicotinic acid. Because of the adverse effects which already occur at low doses of nicotinic acid, we are of the opinion that it should not be added to food supplements or used for food fortification (SCF, 2002).

11.4.1 Derivation of a maximum level for niacin in food supplements

In other chapters of our report we had calculated maximum levels for micronutrients for use in food supplements with the help of the following formula:

$$TL = \frac{UL - DINF}{MEF}$$

Legend:

UL	=	Tolerable Upper Intake Level (SCF) usually referring to the daily total intake
DINF	=	Dietary Intake by Normal Food (95. or 97,5 percentile)
MEF	=	Estimated Number of Consumed Products
TL	=	Tolerable Level in a single dietary supplement or fortified food

This formula is only suited to a limited degree to the derivation of a maximum level for the use of niacin in food supplements because an assessment of niacin supply based on intake calculations is very difficult since more than half of niacin is synthesised from tryptophan and no distinction was made between nicotinic acid and nicotinamide when collecting consumption data.

Various options for the use of niacin in food supplements are presented below.

a) Continuation of existing practice

In its information brochure *"Questions and Answers about Food Supplements"*, published in 1998, BgVV suggested that no more than three-fold the daily niacin intake level in food supplements recommended by DGE at that time should be authorised whereby the age group with the highest requirements should be taken as the basis. Hence, **60 mg** (3 x 20 mg) niacin was set as the maximum dose (ALS, 1998; BgVV, 1998).

Advantages: There are no identifiable health advantages.
Disadvantages: From the angle of the risk assessment undertaken for nicotinic acid, the level of 60 mg/d niacin seems too high for the safe handling of food supplements.

b) Limiting the maximum level in food supplements to one-fold the recommended daily dose of niacin (= rounded up 17 mg)

Advantages: This upper level corresponds to the recommended intake and is oriented towards actual requirements. Health risks for the consumer can be ruled out.

Disadvantages: As the UL for nicotinic acid defined by SCF is 10 mg/d, the maximum level of 17 mg niacin in food supplements would also run the risk of exceeding the UL of nicotinic acid unless nicotinamide was solely authorised for use in food supplements.

c) Taking over the D-A-CH recommendation of using maximum 35 mg niacin in food supplements

Advantages: No identifiable advantage.

Disadvantages: see Option b)

11.4.2 Derivation of a maximum level for niacin in fortified foods

a) Continuation of existing practice of the unlimited potential addition of niacin to foods

Advantages: There are no identifiable health advantages.

Disadvantages: The Ordinance on Vitaminised Foods (VitaminV) was taken over in 1942 into food law (VitaminV, 1994). Since then the use of vitamins to fortify foods has increased. As a rule, fortified foods are eaten in uncontrolled manner without fixed daily portions which means that, depending on choice of food and eating habits, niacin intake could reach a scale at which adverse effects cannot be ruled out.

As nicotinic acid has a very low safety margin, there is definitely a need for regulation.

b) Fortification of foods with one-fold the recommended daily intake (17 mg) niacin

Advantages: The one-fold recommended daily intake is oriented towards nutritional-physiological aspects since this, rather than higher fortification, takes into account the fact that foods as a rule are eaten in an uncontrolled manner without fixed daily portions.

Disadvantages: As the UL for nicotinic acid defined by SCF is 10 mg/d, the maximum level of 17 mg niacin would also run the risk of exceeding the UL for nicotinic acid unless nicotinamide was expressly authorised for the fortification of foods.

It is recommended that nicotinic acid be deleted as a reliable niacin compound as the health risk in the case of overdose must be classified as very high. By contrast, the risk linked to the use of nicotinamide even at high doses is estimated to be low.

As niacin supply in the Federal Republic of Germany is more than sufficient, there is no reason to add niacin to food supplements. As long as nicotinic acid is included under niacin, it is recommended that the maximum level for the use of niacin in food supplements be restricted to the recommended intake of 17 mg per daily dose (Option b).
For the fortification of foods with niacin, BfR recommends that an upper level of 17 mg not be exceeded in the expected daily portion (Option b).

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12 Risk Assessment of Vitamin B_6

12.1 Summary

The data available for the Federal Republic of Germany on vitamin B_6 intake indicate that on average far more is ingested than is necessary to cover requirements (supply category 4). Biochemical studies undertaken to assess vitamin B_6 status confirm sub-optimum values for a small proportion of the federal German population only. These risk groups include underweight persons, older people with a low food intake and people with high chronic alcohol consumption or alcohol abuse.

In line with the risk classification of nutrients taken over by BfR concerning the 97.5 percentile of expected intake and a UL of 25 mg/day for adults (SCF) concerning any adverse health effects which may occur, vitamin B_6 is classified in the medium risk category ("moderate risk").

For food supplements BfR recommends, for reasons of precautionary health protection, a maximum level of vitamin B_6 per daily portion of 5.4 mg for adults (children and adolescents correspondingly less).

In the case of fortified foods, BfR suggests for reasons of precautionary health protection that the one-fold recommended daily dose of vitamin B_6 not be exceeded per expected daily portion corresponding to 1.2 mg and 1.6 mg for adults and adolescents and correspondingly less for children.

Recommended intake	1.2-1.6 mg/day	
Intake [mg/day] (NFCS, 1994)	**m**	**f**
Median	1.79	1.43
P 2.5	1.0	0.68
P 97.5	3.43	2.86
Tolerable upper intake level	Adults 25 mg/day Children/adolescents age-dependent 5-20 mg/day	
Proposal for **maximum levels** in: food supplements	5.4 mg/daily dose (adults)	
fortified foods	1.2-1.6 mg/daily portion	

12.2 Nutrient description

12.2.1 Characterisation and identification

According to a proposal of the IUPAC-IUB Commission (1973) vitamin B_6 is the official name for all 3-hydroxy-2-methyl pyridine derivatives with biological activity of pyridoxine (CAS No. 65-23-6). Furthermore, the most important biologically active derivatives include pyridoxal and pyridoxamine and the respective 5´-phosphoric acid esters which are coenzymes. Interconversion between all six derivatives is possible during metabolism and, therefore, they have the same vitamin activities. They form the metabolically active pyridoxal-5'-phosphate and pyridoxamine-5'-phosphate (Bässler *et al.*, 2002).

Pyridoxine (as alcohol: pyridoxol, as aldehyde: pyridoxal, as amine: pyridoxamine) and pyridoxine hydrochloride (CAS No. 58-56-0) are generally authorised as vitamin compounds for addition to foods (Additives Marketing Ordinance - ZVerkV, Annex 2, List 11, Ordinance on Vitaminised Foods - VitaminV). According to a Proposal for a Regulation of the European

Parliament and the Council of 10 November 2003 (COM (2003) 671 final) pyridoxine hydrochloride and pyridoxine-5'-phosphate (CAS No. 54-47-7) may be added to foods. Pyridoxine hydrochloride and pyridoxine-5'-phosphate are also included in the European list of substances which may be used for the manufacturing of food supplements (Directive 2002/46/EC, 10.06.2002).

Vitamin B_6 belongs to the group of water soluble vitamins. Pyridoxine hydrochloride is very stable in aqueous acid solutions but not, however, in neutral and alkaline solutions and is sensitive to daylight and UV light. Pyridoxine is relatively heat stable whereas pyridoxamine and, above all, pyridoxal are heat unstable. The heat instability of vitamin-B_6 is responsible for losses during the sterilisation and drying of milk. The exposure of milk to sunlight in clear glass bottles can destroy almost 50% of vitamin B_6 in the space of a few hours. Despite gentle preparation of the foods, losses of on average 20% of this vitamin must be included in the calculations. In the case of pyridoxal and pyridoxamine from foods of animal origin these losses may even be as high as 30-40% (Bässler *et al.*, 2002; D-A-CH, 2000).

12.2.2 Metabolism, function, requirements

Metabolism: Vitamin B_6 is mainly absorbed in the proximal jejunum, in the ileum by passive diffusion at higher doses and by an active, requirement-driven process at low doses after hydrolysis (Bässler, 2002). The bioavailability of glycosylated vitamin B_6 of plant origin varies considerably (0-80%) (Gregory, 1997) whereas the free vitamin B_6 forms can be absorbed rapidly and effectively. Its bioavailability from a normal mixed diet in Germany can be given at around 75%. The main portion of the absorbed vitamin B_6 is taken up and phosphorylated by the liver cells. Large portions of the vitamin B_6 retained in the body are stored in the liver or bound in the muscle as pyridoxal-5'-phosphate by means of glycogen phosphorylase. Only 0.1% is to be found in the blood stream. Enzyme-bound pyridoxal-5'-phosphate is, therefore, the most important storage form of vitamin B_6. Pyridoxine has a half-life of 15-20 days (EVM, 2003). In the case of adequate supply the total body level of vitamin B_6 is approximately 100 mg of which on average 2 mg are excreted every day (Heseker, 1997). Other authors assume a total body level of 6 to 27 mg (EVM, 2003). In the liver and, to a lower degree, in the kidneys non-enzyme-bound pyridoxal-5'-phosphate is dephosphorylated into pyridoxal and oxidised to biologically inactive 4-pyridoxin acid, the most important degradation product in the metabolism of this vitamin. 4-pyridoxin acid is eliminated by the kidneys and excreted in urine (Heseker, 1997).

Function: In its coenzyme forms pyridoxal phosphate (PLP) and pyridoxamine phosphate (PMP), vitamin B_6 is involved in numerous enzymatic conversions, mainly in the amino acid metabolism, e.g. within the framework of homocysteine metabolism and in nucleotide synthesis. The vitamin is involved in many group transfer reactions, above all transaminations and decarboxylations. In addition, functions of the nervous system, immune system and haemoglobin synthesis are influenced by vitamin-B_6 (D-A-CH, 2000; Reynolds, Leklem, 1988; Zempleni, 1997). Aside from its coenzyme function PLP also seems to modulate the effect of steroid hormones and to play a role in gene expression (Heseker, 1997).

Requirements: The reserve or storage capacity of an adult human being for vitamin B_6 is sufficient for a period of 2-6 weeks (D-A-CH, 2000). Healthy adults remain free from biochemical deficiencies at a daily intake of 1.2 mg up to 2 mg within the framework of a normal mixed diet (Sauberlich, 1964; Selhub *et al.*, 1993). The need for vitamin B_6 is not a steady parameter but depends on protein turnover because of its central role in amino acid metabolism (Hansen *et al.*, 1997; Miller *et al.*, 1986; Sauberlich, 1964). For the derivation of recommendations a factor of around 0.02 mg (D-A-CH, 2000) or 0.015 mg (SCF, 2000) vitamin B_6 per gram recommended protein intake is suggested and taken as the basis for intake recommendations. In the case of higher protein intake than the recommended level,

the recommended intake for vitamin B_6 also increases in line with the above factor. Human milk contains on average 14 µg vitamin B_6 per 100 ml (Souci *et al.*, 2000). Given the lack of direct measurements of vitamin B_6 requirements, an estimated value from intake from human milk is given for fully breastfed infants. The current reference intakes are around 20% below the DGE recommendations from 1991 which can be attributed to the lower protein recommendation in the current D-A-CH reference intakes (2000).

Table 15: Recommended vitamin B_6 intake

Age	Male persons (mg/day)	Female persons (mg/day)
Infants		
0 up to under 4 months (estimated value)	0.1	0.1
4 up to under 12 months	0.3	0.3
Children		
1 up to under 4 years	0.4	0.4
4 up to under 7 years	0.5	0.5
7 up to under 10 years	0.7	0.7
10 up to under 13 years	1.0	1.0
13 up to under 15 years	1.4	1.4
Adolescents and adults		
15 up to under 19 years	1.6	1.2
19 up to under 65 years	1.5	1.2
65 years and over	1.4	1.2
Pregnant women (from 4th month) and **lactating women**	-	1.9

(in accordance with D-A-CH, 2000)

It can be assumed that there is an increased need for vitamin B_6 during pregnancy and lactation as well as, more particularly, amongst patients with specific kidney diseases like, for instance, haemodialysis patients, and in conjunction with chronic uraemia and renal insufficiency (Bässler, 2002).

Interactions: Various medicinal products like hydralazines, specific hydrazide-containing tuberculostatic agents, phenytoin, D-penicillamin, L-dopa may increase the need for vitamin B_6 (Bässler, 2002).

12.2.3 Exposure (dietary and other sources, nutritional status)

Sources:
Foods: Pyridoxal and pyridoxamine are mainly to be found in foods of animal origin (liver, kidneys, muscle meat, eggs, milk and dairy products) whereas pyridoxine is contained in cereals, potatoes and leafy vegetables. The main sources include potatoes (15%), meat, sausage products, bread (each around 10%), dairy products (8-10%), fruit and vegetables (8-10%) as well as juices and poultry (<5%). Fats, oils and sugar do not contain almost any vitamin B_6 (Mensink *et al.*, 2002; Bognàr, 1995; Bässler, 2002).

Table 16: Vitamin B_6 levels in foods

Food	Vitamin B_6 content (mg/100 g)
Salmon	0.98
Walnuts	0.87
Beef liver	0.70
Avocado	0.53
Chicken	0.50
Herring	0.45
Pork, low fat	0.39
Potatoes	0.19
Tomatoes	0.10
Apples	0.10
Gouda cheese	0.07
Yoghurt	0.05
Dairy milk, 3.5% fat	0.04

(Heseker, 1997)

Fortified foods: The main foods fortified with vitamin B_6 in Germany are beverages, cereals, sweets and dairy products. As a rule, at least 15% and at the most 100% of daily recommendations of vitamin B_6 are added. Children's needs for vitamin B_6 are covered up to 80% by fortified foods (Sichert *et al.*, 2001; Kersting *et al.*, 1995).

Food supplements: In Germany 25% of women and 18% of men take a food supplement more than once a week, mostly in the form of multivitamin products. The daily intake of vitamin B_6 from food supplements, measured against the recommended intake of the Deutsche Gesellschaft für Ernährung (German Nutrition Society) is at least 100% (Beitz *et al.*, 2002; Mensink and Ströbel, 1999; Schellhorn *et al.*, 1998). Within the framework of co-ordinated management practice, food supplements are accepted in Germany which do not contain more than the three-fold recommended intake of vitamin B_6 per daily portion (BgVV, 2001).

Medicinal products: In the case of the prophylactic treatment of clinical vitamin B_6 deficiency conditions, the recommended oral dose is 1.5-25 mg pyridoxine hydrochloride per day if nutritional measures are not considered to be sufficient. For the treatment of severe vitamin B_6 deficiency conditions, oral doses of 20-300 mg per day are administered (BGA, 1988). In the drug samples for *water soluble and fat soluble vitamins in fixed combinations,* 1-6 mg per day is recommended as a prophylactic dose and 6-20 mg per day as a therapeutic dose (BfArM, 1995).

Nutritional status:
Intake: Representative data on vitamin B_6 supply (not including supplements or fortified foods) were collected in Germany in the National Food Consumption Study (NFCS) carried out in 1980 whereby the median (97.5 percentile) of daily intake was 1.74 (3.43) for 19-24 year-old men and 1.30 (2.70) mg for women (Table 17).

The data in the NFCS/VERA Study show that a sub-optimum supply status was only found in a small proportion of the federal German population (4.2%). Far more significantly unfavourable α-EAST and 4-PA values were observed in the case of underweight women than for normal or overweight persons. 9% of the underweight persons examined were found to have high α-EAST values. Smokers have lower 4-PA excretion values and lower EAST enzyme activities than non-smokers. In women increasing cigarette consumption is linked with increasing shares of critical α-EAST and 4-PA values (Heseker *et al.*, 1992).

Table 17: Daily vitamin B_6 intake in Germany

Age (years)	Male persons		Female persons	
	Median (mg)	Percentile 2.5-97.5 (mg)	Median (mg)	Percentile 2.5-97.5 (mg)
4 - 6	1.10	0.54 - 2.40	0.98	0.48 - 2.54
7 - 9	1.27	0.67 - 2.67	1.19	0.62 - 2.96
10 - 12	1.42	0.70 - 2.92	1.29	0.68 - 2.49
13 - 14	1.58	0.87 - 2.84	1.31	0.63 - 2.40
15 - 18	1.67	0.92 - 3.50	1.27	0.66 - 2.67
19 - 24	1.74	0.86 - 3.43	1.30	0.62 - 2.70
25 - 50	1.79	0.93 - 3.30	1.38	0.66 - 2.64
51 - 64	1.81	0.96 - 2.99	1.44	0.80 - 2.55
≥ 65	1.71	0.89 - 2.93	1.41	0.72 - 2.65

(according to Adolf *et al.*, 1995)

Based on the data from the random income and consumption sample (EVS), the median daily intake of pyridoxine by male and female persons is on average 1.7 mg and 1.5 mg respectively, expressed as a percentage of the reference intakes of 132 and 136% (DGE, 2000).

The average vitamin B_6 intake is far higher than the recommended intake for men of 1.6 mg and for women of 1.2 mg according to more recent data from the Federal Health Survey. Only a small percentage of the population does not reach the reference intake (Mensink *et al.*, 2002).

Biomarkers: Determinations of vitamin B_6 in plasma and in erythrocytes are the easiest way of recording vitamin status. One biochemical parameter for inadequate supply is reduced 4-pyridoxine acid excretion in urine. For a more exact assessment of the vitamin B_6 supply status, the level of the activation coefficient of the erythrocytic aspartate aminotransferase (α-EAST) is used. A reference intake for the satisfactory covering of requirements is an α-EAST <2.5 (Hansen *et al.*, 1997; D-A-CH, 2000). Whereas the α-EAST value and the concentration of pyridoxal-5'-phosphate in plasma reflect medium-term supply, the excretion of 4-pyridoxine acid describes immediately prior vitamin B_6 intake (Heseker, 1997).

The data available for the Federal Republic on vitamin B_6 intake indicate that on average far more is taken in than is considered necessary to cover requirements (supply category 4). The biochemical studies undertaken to estimate vitamin B_6 supply confirm sub-optimum values only for a small proportion of the German population. The risk groups include underweight persons, older people with low food intake and people with chronically high alcohol consumption or alcohol abuse.

12.3 Risk characterisation

12.3.1 Hazard characterisation (NOAEL, LOAEL)

Vitamin B_6 has a low acute toxicity. Up to now, no adverse effects in human beings have been observed as a consequence of larger uptake levels of vitamin B_6 occurring naturally in foods. The isolated administration of high doses of this vitamin was linked more particularly to neurotoxic effects which were already described in 1940 in animal experiments and for the first time in 1983 in human studies. Since overall the data were not uniform or consistent it was not possible to identify a systematic dose-response relationship for vitamin B_6. There are grounds, both in human studies and in animal experiments, for assuming a causal relationship between higher vitamin B_6 intake and the onset of neuropathies or neurotoxic effects. In addition to the dose level, the period of administration also seems to be of particular importance (EVM, 2003; FNB, 1999; SCF, 2000). On the basis of the available data SCF (2000) was not able to establish a clear **NOAEL** (no adverse effect level).

12.3.2 Deficiency, possible risk groups

A pure vitamin B_6 deficiency only occurs extremely rarely in healthy people since the vitamin is to be found in many foods and the body has sufficient reserves. Inadequate supply can – together with deficits of other water soluble vitamins – be observed for instance in conjunction with chronic alcohol abuse (Heseker, 1997) and bad dietary habits or malnutrition. Severe vitamin B_6 manifests itself in the form of seborrhoiec dermatitis around the nose and eyes with glossitis and cheilosis, a hypochromic, microcytic, iron refractory anaemia and neurological disorders like peripheral neuritis with sensitivity disorders, states of confusion, cerebral convulsions in infants, disruptions of the neurotransmitter system, disorders of the cerebral metabolism, growth disorders (Bässler, 2002; D-A-CH, 2000; SCF, 2000). Elevated renal excretion of oxalic acid with an inclination to nephrolithiasis was described as another consequence of a vitamin B_6 deficiency (Harrison *et al.*, 1981).

12.3.3 Excessive intake, possible risk groups

In conjunction with the long-term intake of high dose supplements (50-500 mg pyridoxine and more per day), toxic effects were observed in sensitive and motor neurons (SCF, 2000; FNB, 1999; EVM, 2003; Bässler *et al.*, 1998; Dalton and Dalton, 1987; Dalton, 1985; Parry and Bredersen, 1985; Schaumburg *et al.*, 1983). This led to ataxia and severe, peripheral sensitive neuropathies with loss of reflex and disruptions, amongst other things, of touch and temperature sensations which were, in some cases, irreversible. Another adverse effect described in humans was the onset of photosensitivity in a 35 year-old man who had taken 200 mg pyridoxine in the form of a multivitamin product (Morrimoto *et al.*, 1996). Erythematous skin damage following exposure to sunlight was also observed in specific patients who had taken high dose pyridoxine (35 mg/kg bodyweight and day) over 4 years (Coleman *et al.*, 1985). Doses of 100 or 500 mg/day over 10 days were taken by 58 students; at the higher dose they led to a significant decrease ($p<0.002$) in memory performance whereas a reduction of the learning effect in the lower dose group was not significant ($p<0.07$) compared to the placebo group (Molimard *et al.*, 1980). A special vulnerability of specific groups in the population when taking elevated vitamin B_6 could not be identified (EVM, 2003; SCF, 2000).

12.4 Tolerable upper intake level for vitamin B_6

When taking 500 mg vitamin B_6 or more per day, a relevant toxicological potential for adults is clearly identifiable whereas for the dose of 100 mg vitamin B_6 per day in conjunction with long-term administration, adverse effects of this kind cannot be ruled out with sufficient certainty (SCF, 2000). In an earlier SCF expert opinion, a dose per day of more than 50 mg in adults was considered to be potentially harmful (SCF, 1993). Based on the data from Dalton and Dalton (1987) SCF established a **UL (tolerable upper intake level)** for adults of 25 mg/day. For the age groups of children and adolescents, lower ULs were derived based on bodyweight (SCF, 2000):

Table 18: Tolerable Upper Intake Level (UL) of vitamin B_6

Age (years)	UL [mg/day]
1 - 3	5
4 - 6	7
7 - 10	10
11 - 14	15
15 - 17	20
Adults	25

(according to SCF, 2000)

By contrast, the American Food and Nutrition Board derived a UL of 100 mg/day for adults based on a NOAEL of 200 mg/day and an uncertainty factor of 2 (FNB, 1999). It did, however, stress the weaknesses of the data from Dalton and Dalton (1987), who had retrospectively examined 172 women who had been given doses of 50-500 mg pyridoxine/day for the treatment of premenstrual syndrome. Of them 103 reported neurological symptoms and 69 women had no side effects.

Since the available human data are insufficient, the Expert Group on Vitamins and Minerals (EVM) of the United Kingdom derived a safe upper level on the basis of animal experiments (Food Standards Agency, 2000; 2001; 2003). After doses of 50 mg/kg bodyweight/day a loss of myelin was observed in animal experiments with dogs (Phillips *et al.*, 1978). Based on this dose, taking into account an uncertainty factor (UF) of 3 for the extrapolation to the NOAEL and a UF of 10 for the transfer of animal experiment data to human beings and a further UF of 10 for the inter-individual differences, a safe upper level of 50/300 = 0.17 mg/kg bodyweight/day was derived for *supplements* corresponding to an intake of 10 mg/day for an adult weighing 60 kg. EVM points out, more particularly, that only inadequate tolerance data for human beings are available for the dose range between 10 mg and 200 mg vitamin B_6 per day in conjunction with long-term administration.

12.4.1 Derivation of a maximum level for vitamin B_6 in food supplements

The comments show that different recommendations and estimates by various scientific bodies are available concerning the upper safe intake level of vitamin B_6. Furthermore, it can be noted that there are still considerable gaps in knowledge about the toxicity of vitamin B_6 in conjunction with the long-term administration of higher doses in human beings. The data available for the Federal Republic of Germany on vitamin B_6 intake indicate that on average far more is taken in than is considered necessary to meet requirements. On the basis of the existing data, sub-optimum values of vitamin B_6 intake are only to be expected for a small proportion of the federal German population, under certain circumstances in underweight individuals, older people with a low food intake and people with chronically high alcohol consumption or alcohol abuse.

BfR takes account of the precautionary principle and it uses as the basis for the derivation of maximum levels for vitamin B_6 in foods the lower tolerable upper intake level (UL), i.e. the one which was derived by SCF i.e. 25 mg/day vitamin B_6 upper intake for adults (SCF, 2000). This is also because the safe upper level of 10 mg per day proposed by EVM for adults is based on animal experiment data and refers to the dose for supplements but not to total daily intake.

The data available for Germany on vitamin B_6 supply show that the mean vitamin B_6 intake (median) for adults and adolescents is below the UL of 25 mg per day, in males depending on age between 1.67 mg and 1.81 mg and in females depending on age between 1.27 mg and 1.44 mg. The 97.5 percentile for males is between 2.93 mg and 3.50 mg and for females between 2.55 mg and 2.7 mg per day (Adolf *et al.*, 1995).

In the introduction to this report the following formula was presented for the derivation of the vitamin and mineral amount available for additional intake from food supplements and fortified foods:

$$R = UL - DINF$$

Using a **UL** of 25 mg/day and the 97.5 percentile of vitamin B_6 intake for adults (see above: supply status), this leads to a residual amount R which is available for additional intake:

$$R = 25\ mg/day - 3.43\ mg/day$$

$$R = 21.57\ mg/day$$

For the additional intake of vitamin B_6 it seems appropriate to allocate 50% of the residual amount R to food supplements and 50% to fortified foods. This then leads to:

R = Total intake level FS + Total intake level FF
R = 10.785 mg/day FS + 10.785 mg/day FF

When considering the residual amount of additional intake of vitamin B_6 from food supplements taking into account a factor of 2 for multiple exposure (consumption of up to 2 food supplements per day containing vitamin B_6), then, again using the formula presented in the introduction to this report, this leads to the following tolerable level TL for vitamin B_6 in food supplements:

$$TL_{FS} = \frac{\text{Total intake level FS}}{\text{MEF}}$$

$$TL_{FS} = \frac{10.785\ mg/day}{2}$$

$$TL_{FS} = 5.4\ mg/day \text{ for adults}$$

Legend:

UL	=	Tolerable Upper Intake Level (SCF) usually referring to the daily total intake
DINF	=	Dietary Intake by Normal Food (95. or 97.5. percentile)
MEF	=	Estimated Number of Consumed Products
TL	=	Tolerable Level in a single dietary supplement or fortified food
R	=	Residual amount of intake available for uptake from food supplements and fortified foods

12.4.1.1 Possible management options

a) Maximum level for vitamin B_6 in food supplements per daily portion for adults of 5.4 mg in accordance with the above derivation.

Advantages: This maximum level results, as outlined above, from appropriate consideration of the UL and the expected vitamin B_6 intake including the expected multiple exposure from various products. Furthermore, it is more or less in the range of the previously accepted maximum dose whereby the daily portion of vitamin B_6 should not exceed more than the three-fold amount in food supplements measured against recommended intake on the basis of co-ordinated administrative practice. No negative experience has been reported for this range. This upper level is oriented towards requirements and takes sufficient account of inter-individual differences (requirement orientation with adequate allowance). So far no adverse effects have been reported for this range. With regard to the known data situation on vitamin B_6 it can be assumed that no health risks are to be expected for the consumer. Warnings are not necessary. Taking into account the age-dependent UL (see above: upper safe daily intake level) and the corresponding 97.5 percentile of intake (see above: supply status), this would lead to a maximum level of 4.1 mg/day vitamin B_6 in food supplements for adolescents. For children correspondingly less.

Disadvantages: There are no identifiable health disadvantages.

b) "One-fold rule": In the case of food supplements the one-fold recommended daily dose of vitamin B_6 should not be exceeded per daily portion corresponding to 1.2 mg-1.6 mg for adolescents and to 1.2 mg-1.5 mg for adults.

Advantages: This upper level is strictly oriented towards actual requirements and makes nutritional-physiological sense. At this range health risks for consumers can be ruled out.

Disadvantages: There are no identifiable health disadvantages.

12.4.2 Derivation of a maximum level for vitamin B_6 in fortified foods

12.4.2.1 Possible management options

a) In accordance with the derivation outlined above, this would mean a maximum level of 10.8 mg vitamin B_6 for adults in fortified foods per daily portion which would have to be correspondingly divided up over various fortified foods ("multiple exposure"), for children and adolescents correspondingly less. In accordance with the Ordinance on Vitaminised Foods, the fortification of foods with vitamin B_6 is permitted without it making any explicit mention of upper levels. Up to now BgVV/BfR has accepted additions up to three-fold the requirements referred to the expected daily portion within the framework of co-ordinated administrative practice and this is roughly in the range of the derived maximum level described above.

Advantages: There are no reports of bad experience with this. One constraint is that insufficient data are available concerning the maximum level of fortification undertaken by food manufacturers in isolated cases. Normally, manufacturers adopt a requirement-oriented approach to vitamin B_6 fortification. So far, there are no known adverse effects for this range. With regard to the known data situation on vitamin B_6 it can be assumed that no health risks for consumers are to be expected from this range.

Disadvantages: Since (fortified) foods are normally consumed in an uncontrolled manner without any fixed daily portions, specific requirement-oriented maximum levels could scarcely be complied with. Under certain circumstances a related assumed multiple exposure of for instance 2 or 3 different fortified foods may still be too low. If various fortified foods are consumed, this may lead to considerably elevated vitamin levels.

b) "One-fold rule": In the case of fortified foods the one-fold recommended daily dose of vitamin B_6 should not be exceeded per expected daily portion which corresponds to 1.2 mg-1.6 mg for adults and correspondingly less for children (see above: recommended intake).

Advantages: This upper level is strictly oriented towards actual requirements, preventive health protection and nutritional-physiological aspects. This, rather than a higher level of fortification, takes into account the fact that foods as a rule are consumed in an uncontrolled manner without any fixed daily portions. For this range health risks for the consumer can be ruled out.

Disadvantages: There are no identifiable health disadvantages.

In line with the risk classification of nutrients taken over by BfR, vitamin B_6 is to be classified in the medium risk category ("moderate risk") in respect of the 97.5 percentile of expected intake and the level of the UL of 25 mg/day for adults (SCF) with regard to any adverse health effects.

For reasons of precautionary health protection, BfR recommends for food supplements per daily portion a maximum level of vitamin B_6 of 5.4 mg for adults (children and adolescents correspondingly less) in line with the derivation outlined above (Option a.).

In the case of fortified foods BfR proposes, for reasons of precautionary health protection, that the one-fold recommended daily dose of vitamin B_6 not be exceeded per expected daily portion, which means for adults and adolescents 1.2 mg up to 1.6 mg, children correspondingly less (Option b.).

12.5 Gaps in knowledge

Particularly in the case of the dose range between 10 mg and 200 mg vitamin B_6 per day in conjunction with long-term administration, the available tolerance data for human beings are largely inadequate; there is a need for research in this area.

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13 Risk Assessment of Folic Acid

13.1 Summary

The Nutrition Surveys conducted in Germany indicate that 80-90% of the population (in all age groups) do not reach the recommended intakes for folate equivalents through the consumption of normal, unfortified foods. With the fortified foods already on the market, in theory around 50% of the adult population and 75% of children and adolescents could reach the recommended intakes for this vitamin. There is, however, uncertainty about the contribution which fortified foods can make to covering the requirements of adults. Furthermore, there are no representative data about folate concentrations in serum or in erythrocytes (supply category 1/2).

A risk classification for the use of folic acid in food supplements and fortified foods is not possible using the criteria laid down by BfR since the UL derived for this vitamin only applies to synthetic folic acid. In the opinion of BfR the risk of adverse health effects in conjunction with the use of synthetic folic acid in foods must be described as moderate.

For the general population BfR believes that additional intake of 200 µg folic acid (=400 µg folate equivalents) from supplements is adequate. Since, however, additional daily folic acid intake of 400 µg (=800 µg folate equivalents) is recommended to women of childbearing age and the use of this dose in the past has not led to adverse effects, a maximum level of 400 µg folic acid per daily intake of food supplements should be used for the entire population.

Assuming that only one food supplement of 400 µg is taken daily, other foods can be fortified with folic acid as well. Since salt fortified with folic acid in normal household use can lead to intakes of 100-200 µg folic acid per day, BfR is of the opinion that a maximum of 200 µg folic acid per portion should be used to fortify other conventional foods.

If, in future, flour were also to be fortified with folic acid in addition to table salt, depending on the chosen flour fortification levels, the maximum levels for the addition of folic acid to conventional foods must be redefined or it must be examined whether and if so, which foods can be fortified with folic acid besides salt and flour.

Recommended intake	400 µg/day (folate equivalents)	
Intake [µg/day] (NFCS, 1994)	**m**	**f**
Median	198	165
P 2.5	99.5	72.9
P 97.5	450	385
Tolerable upper intake level	Adults: 1 mg/day (only applies to folic acid)	
Proposal for **maximum levels** in: food supplements	400 µg folic acid/daily dose	
fortified foods	200 µg folic acid/portion	

13.2 Nutrient description

13.2.1 Characterisation and identification

Folate is the generic term for a water soluble vitamin. The name comes from the Latin term "folium" – the leaf – since the vitamin was first detected in leafy green vegetables. A distinction must be made between *folates* which occur naturally in foods and synthetic *folic*

acid used for therapeutic purposes and for supplementation. Folates consist of a pteridine and a para-aminobenzoic acid ring, at whose carboxy end up to 8 glutaminic acid residues are bound (pteroyl polyglutamates). Folic acid, by contrast, is a fully oxidised pteroyl polyglutamate with only one glutaminic acid residue.

Up to now, the addition of folic acid to conventional foods has not been explicitly regulated in Germany. The vitamin could be added to foods without requiring authorisation. In the Annex of the European Directive 2002/46/EC folic acid is envisaged in the form of pteroyl-monoglutamic acid for use in food supplements. Furthermore, the compound is listed in the Annex to the Proposal for a *Regulation on the Addition of Vitamins and Minerals and of certain other Substances to Foods* (COM(2003) 671 final of 10 November 2003). Accordingly, it may, in future, be added to conventional foods.

13.2.2 Metabolism, function, requirements

Metabolism: In food folates are normally present as pteroyl polyglutamates. They are mainly absorbed in the proximal part of the small intestine by an active absorption mechanism which is supported at higher folate doses by a passive transport mechanism. Absorption is stimulated by glucose and sodium; the optimum pH is 6.0 (Bässler *et al.*, 2002). Pteroyl polyglutamates must be hydrolysed with the help of a zinc-dependant carboxy peptidase to monoglutamates prior to absorption in the brush border of the mucosa cells whereas the vitamin ingested as folic acid will be reduced prior to resorption to tetrahydrofolate (THF) and partially methylated or formylated (Selhub *et al.*, 1983 in: Brouwer *et al.*, 2001). After transport to the liver full methylation takes place there and the resulting 5-methyl tetrahydrofolic acid is then bound to albumin and macroglobulin and transported to the cells where it is demethylated and converted into polyglutamate derivatives. The polyglutamate form is the storage form of the vitamin. Folic acid and its derivatives are distributed to all tissues; the distribution pattern of the various forms of folate shows dependency on the cell proliferation rate of the tissue. The total body store of human beings is estimated at 5-10 mg of which the liver contains around half. The biological half-life of this amount is approximately 100 days (IOM, 2000).

The amounts of 10-90 µg folic acid excreted daily with bile are regulated by enterohepatic circulation and almost fully reabsorbed. In the case of normal folic acid intake around 1-12 µg are excreted by the kidneys daily in the form folate compounds like 5-methyl-THF and 10-methyl-THF, as well as inactive metabolites like pteridine. It is not possible to assess the importance of faecal excretion since endogenously formed folates from microbia folate biosynthesis which takes place in the intestines are always excreted with the faeces. Folic acid migrates to human milk where it reaches concentrations of around 50 µg/L (Bässler *et al.*, 2002; IOM, 2000).

Function: The main task of folates in the human organism is the transfer of 1-carbon units. In this context folate/folic acid is not efficacious as such but in reduced form as 5,6,7,8-tetrahydrofolate (THF), which can be bound to at least six different C1 groups like hydroxymethyl and formyl groups. The conversion of serine to glycine is the main source of 1-carbon units for the organism (Bässler *et al.*, 2002). The C1 residues are needed for purine synthesis (C8 and C2 of the purine ring) and for the methylation of homocysteine into methionine whereby vitamin B_{12} is necessary as a co-factor. S-adenosyl methionine, which is created through the reaction between methionine and ATP, supplies a methyl group for further methylations like, for instance, ethanolamine to choline, noradrenaline to adrenaline or phosphatidyl ethanolamine to lecithin. In the case of SAM-dependent methylations homocysteine is created as an intermediate product. The following diagram gives an overview of the metabolism of the vitamin and the close relationship in metabolism between folic acid and homocysteine. Given its role in DNA, RNA and protein metabolism, folate/folic

acid is of primary importance for adequate cell growth, normal cell proliferation and optimum cell differentiation.
Nutrient interactions: *folate and vitamin B_{12}*: The function of the folates is closely linked to that of vitamin B_{12}. Both vitamins are involved in the conversion of homocysteine to methionine, an irreversible metabolism route, through which 5-methyl-THF is transferred to a methyl group on homocysteine. The reaction is catalysed by methylene-THF-reductase and methionine synthase whereby the co-factor vitamin B_{12} is required. The reaction is blocked in the case of a vitamin B_{12} deficiency which means that the amount of reactive THF is reduced and is not sufficiently available for the formation of 5,10-methylene-THF or for DNA synthesis (so-called methyl trap).

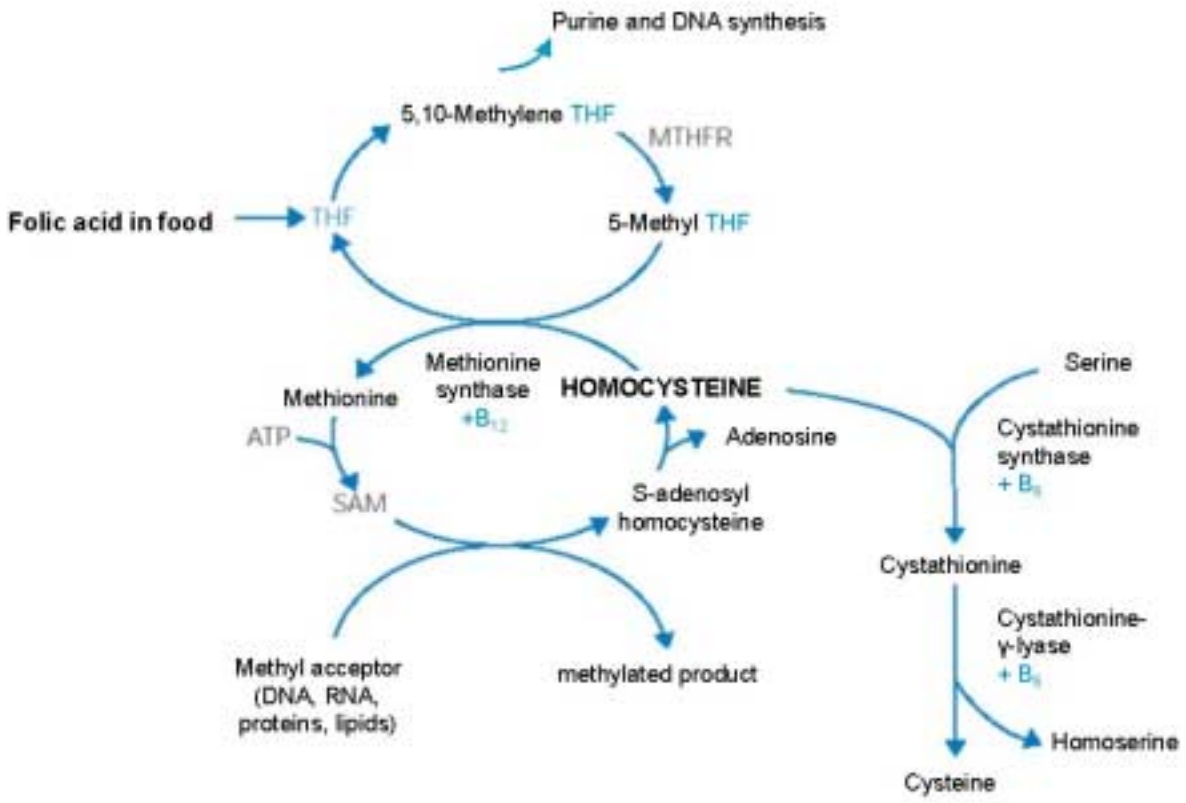
Folate and vitamin B_6: Homocysteine can either be remethylated to methionine or converted via cystathionine to cysteine. The reactions are catalysed through a vitamin B_6-dependent cystathionine-β-synthase and cystathionase (Bailey *et al.*, 2001; Bässler *et al.*, 2002). This shows that there is also a close relationship between the metabolism routes of folate and vitamin B_6.

Bioavailability: The bioavailability of food folates can be influenced by the relationship between monoglutamates and polyglutamates which occur in food, the release of folates from the cell structure, the type of food matrix and the presence of other nutrient components like organic acids, folate-binding proteins and reducing substances (Bässler *et al.*, 2002; Brouwer *et al.*, 2001; Molloy, 2002; Sanderson *et al.*, 2003). Dietary folates (polyglutamate) must be hydrolysed prior to absorption whereas synthetic folic acids can be absorbed without prior hydrolysis as monoglutamate (Krishnaswamy and Nair, 2001; Sanderson *et al.*, 2003). Overall a 1.7 to 2-fold better bioavailability of folic acid compared to food folate is assumed (DGE/ÖGE/SGE/SVE, 2000; IOM, 2000; Molloy, 2002).

The availability of folic acid from bread, which was baked using fortified flour, seems to differ considerably between manufacturing processes. The data fluctuate between 18 and 68% (Colman, 1982 in: de Bree *et al.*, 1997). Pfeiffer *et al.* (1997) did not find any difference in the availability of folic acid from fortified white bread, wheat germ bread, rice, pasta or water. Vahteristo *et al.* (2002) also showed that the intake of the same folate amounts from ordinary foods (rye products, orange juice) compared with that from bread fortified with folic acid, led to similar increases in plasma and erythrocyte folate concentrations.

Requirements: The level of homocysteine concentration in blood serves as an early indicator of inadequate folate supply. Several studies have shown that through the daily intake of 50-100 µg folic acid (as a supplement) haematological deficiency symptoms can be prevented. All the same, a maximum reduction of the homocysteine concentration is only achieved following regular daily intake of 400 µg folate equivalents (Holmes and Gates, 2003; Riddell *et al.*, 2000; Ubbink *et al.*, 1994; van Oort *et al.*, 2003). Any folate/folic acid intake beyond that only influences the homocysteine level to a minor degree.

Figure 4: Folate metabolism[4]



(according to: Koch *et al.*, 1998)

On this basis recommended intakes were derived for adolescents and adults and children's' requirements were calculated depending on their lower bodyweight (DGE/ÖGE/SGE/SVE, 2000):

Due to genetic polymorphisms, which go hand in hand with altered properties of 5,10-methylene-tetrahydrofolate reductase (MTHFR), the need for folate/folic acid increases (Girelli *et al.*, 2003; Guinotte *et al.*, 2003; Molloy, 2002). Inadequate intake of the vitamin can have negative health consequences for individuals who are homozygotic for these polymorphisms. Around 10% of the population show a thermolabile variant of methylene-tetrahydrofolate reductase for instance because of a homozygosity for the C677T mutation which leads to elevated homocysteine concentrations in conjunction with inadequate folate and/or vitamin B_{12} supply. These individuals need higher levels of folate to normalise the metabolism disorder triggered by the polymorphism (Bailey *et al.*, 2001).

[4] THF = Tetrahydrofolate
SAM = S-adenosyl methionine
MTHFR = Methylene tetrahydrofolate reductase

Table 19: Recommended folic acid intake

Age	Recommendation (µg folate equivalent* per day)
Infants**	
0 - <4 months	60
4 - <12 months	80
Children	
1 - 3 years	200
4 - 6 years	300
7 - 9 years	300
10 - 12 years	400
13 - 14 years	400
Adolescents and adults***	
15 - 18 years	400
>19 years	400
Pregnant women***	600
Lactating women	600

* Calculated according to the sum of active folate compounds in normal food
➔ 1 µg folate equivalent = 1 µg food folate = 0.6 µg folic acid (taken together in a meal) = 0.5 µg folic acid (taken on an empty stomach)

** Estimated values

*** Women who are pregnant or wish to become pregnant are advised to take 400 µg/day folic acid in the form of supplements beyond the recommended intake in order to prevent neural tube defects. This additional intake should begin at the latest 4 weeks after the commencement of pregnancy and continue up to the end of the first trimester of pregnancy because the closure of the neural tube normally takes place 4 weeks after conception (between the 22nd and 28th day of pregnancy) or around 6 weeks after the first day of the last menstruation. If a woman already has a child with NTD, an additional intake of 4 mg synthetic folic acid is recommended per day (Koletzko and von Kries, 1995).

13.2.3 Exposure (dietary and other sources, nutritional status)

Sources:
Good sources of **folate** are leafy vegetables like spinach and lettuce but also tomatoes, potatoes, some types of cabbage, fruit and cereals as well as bread, cake and pastries made of wholemeal flour. Wheat germ and soya beans are especially rich in folate. Amongst the foods of animal origin liver contains the highest concentrations of this vitamin whereas other types of meat and fish have relatively low levels of folates (DGE/ÖGE/SGE/SVE, 2000).

As folates are water soluble, light sensitive and heat sensitive, their content in processed foods depends on the type of preparation. Losses of between 50 and 90% can occur during cooking (McKillop *et al.*, 2002). Since more than 60% of ingested folates come from food, which was consumed without further preparation, the mean value of preparation losses is given as around 35% (DGE/ÖGE/SGE/SVE, 2000).

Food supplements: For reasons of preventive health protection BgVV accepted up to now a maximum level of 900 µg per daily intake for the addition of folic acid to food supplements (BgVV, 1998). There is no overview about the food supplements containing folic acid or their doses on the market at the present time.

According to a market survey by the Forschungsinstitut für Kinderernährung (Research Institute for Children's Nutrition), Dortmund, 25% of the food supplements on sale on the German market specifically for children contain folic acid (Kersting and Alexy, 2000).

Fortified foods: In Germany the addition of folic acid to conventional foods was permitted up to now without authorisation. Since many manufacturers have used this opportunity in recent years a wide range of foods with folic acid are now on the market.

According to a market survey by the Gesellschaft für Konsumforschung (GfK) (Society for Consumption Research) 45.5% of cereal products purchased by consumers in Germany between April 2001 and March 2002, 1.5% of dairy products and 11% of soft drinks, including juices, contained folic acid. The folic acid levels for cereals were between 30 and 340 µg per 100 g product. For dairy products the folic acid levels were between 20 and 80 µg per 100 g. In the case of soft drinks, products with 30, 50 and 100 µg folic acid per 100 ml were by far the most frequently purchased products.

The database (LEBTAB database) set up and regularly updated by the Forschungsinstitut für Kinderernährung, Dortmund (Research Institute for Children's Nutrition) has records of the foods fortified with folic acid which appeared in the dietary protocols of the study group from 1990 to 2001. The data base containing 10 mg soft drinks, dairy products and infant formula with ≤100 µg folic acid per 100 g, cereals, juices and food supplements with ≤200 µg folic acid per daily dose and drink powder and sweets with 150-650 µg and 200-1300 µg per 100 g. It should, however, be borne in mind that drink powder, cereals and sweets are normally consumed in smaller amounts than, for instance, soft drinks or juices.

Since 2002 some table salt fortified with iodine and fluoride in Germany has also been fortified with 1 mg folic acid per 100 g. According to information from the company Südsalz the table salt was purchased by 10% of consumers in Germany during the last quarter of 2003 (around one year after its launch on the market). It is on sale throughout Germany (is available on average in one in three shops) and it is to be made increasingly well known by means of comprehensive advertising (30% product familiarity at the end of 2003). In theory, regular use of this table salt would cover folate intake requirements: at an assumed additional salt level in the household of 2 g/day and a folic acid content of 10 mg/100 g salt, additional folic acid intake of around 200 µg/day (~400 µg folate equivalent/day) would be reached whereby preparation losses are not taken into account.

There are now also bread baking mixtures on the market with 125 µg folic acid per 100 g flour and a series of other foods like, for instance, instant soups, ready-to-eat dishes and margarine which contain folic acid of between 40 and 400 µg folic acid per portion.

Nutritional status:
Dietary intake: The nutrition surveys conducted in Germany, which did not take account of the consumption of fortified foods and food supplements, indicate that the recommended intakes for folate equivalents are not reached via normal food in general (Gonzalez-Gross *et al.*, 2002):

According to the National Food Consumption Survey (NFCS) women (>18 years) in the 1980s reached a mean folate intake of 227 µg/day [P2,5=100 µg/day; P97,5=523 µg/day]. Men took in on average 261 µg/day [P2.5=125; P97.5=600 µg/day] (Adolf *et al.*, 1995). The EPIC study conducted in 2000, also showed that men and women only reach on average 50-70% of the recommended intake of this vitamin (Schulze *et al.*, 2001). In the nutrition survey conducted in 1998 within the framework of the Federal Health Survey, a mean dietary folate intake for men was observed of 271 µg/day [P25=221 µg/day; P75=330 µg/day]. Women ingested on average 226 µg/day [P25=188 µg/day; P75=273 µg/day] (Mensink *et al.*, 2002).

For the first time, the contribution of food supplements to folate supply was also recorded in the Nutrition Survey. The study groups were split into "non-consumers" and "regular consumers" of food supplements. Men, who regularly took food supplements, had a mean intake of folate equivalents (FE) of 338 µg/day [P25=267 µg/day; P75=492 µg/day]. Women who regularly took food supplements achieved a mean FE intake of 290 µg/day [P25=220 µg/day; P75=431 µg/day] (Mensink *et al.*, 2002).

In a recent re-evaluation of the German Nutrition Survey it was calculated which contribution foods fortified with folic acid can make to help adults aged between ≤19 up to ≥65 reach recommended intake. It was assumed that the study population consumed cereal products, soft drinks and dairy products fortified with folic acid – either with a low (a) or with a high (b) level of folic acid instead of the non-fortified options. The uptake of folic acid from fortified table salt could not be taken into account since no information about frequency or level of use of table salt was recorded in the Nutrition Survey. The main results are:

(a) The proportion of men, who do not reach the recommended intake of 400 µg folate equivalents when consuming products fortified with a low level of folic acid in addition to their normal diet would fall from 84 to 54%. Intake would increase most amongst men aged between 25 and <51 and least in the age group ≥65. The proportion of women who do not reach the recommendations would fall from 90 to 63% whereby the increase would be the most effective in young women. 0.1% of men and 0.3% of women (age group 25-<51 years) would ingest more than 1000 µg folic acid under these conditions and thus exceed the UL.

(b) The proportion of men who do not reach the recommendations when consuming products fortified with a high level of folic acid would fall to 43% and the proportion of women to 51%. The UL would be exceeded by 1.3% of men, particularly between the ages of 25 and <51, and by 0.9% of women.

➔ *The model calculations reveal that for around 50% of adults adequate intake would be possible through the foods enriched with folic acid already on the market as long as these products are regularly consumed. There is, however, some uncertainty about the actual contribution which foods fortified with folic acid make to folate supply in the group of adults.*

In **children and adolescents** (4-18 years) the mean folate equivalent intake was between 200 and 250 µg/day according to the National Food Consumption Survey. For male adolescents (15-18 years) it was up to 285 µg/day. The 2.5 percentile was between 90 and 115 µg/day up to maximum 130 µg/day in the case of male adolescents. At the 97.5 percentile intakes were determined between 450 and 600 µg/day (and >700 µg/day for male adolescents) (Adolf *et al.*, 1995). The results of the DONALD study, in which folate intakes by children and adolescents aged between 1 and 18 were recorded longitudinally, scarcely differed from those of the NFCS for these age groups (Kersting *et al.*, 2000).

The DONALD study was also re-assessed with a view to the consumption of foods fortified with folic acid. In contrast to the data from the Nutrition Survey, this study has continuously asked about the consumption of fortified foods since 1990 which means that the results are not only based on model calculations and assumptions but on the actual consumption by various age groups in the study population. Folic acid intake via fortified table salt was not taken into account in the DONALD study either.

If one divides the study group into those who eat fortified foods and those who don't, then the mean value for folate intake increases amongst those who do not take any fortified foods or food supplements from 70 µg/day (66 µg/day) amongst the male (female) infants to 187 µg/day (155 µg/day) amongst 15-18 year-old boys (girls). The recommended intakes are not reached by any of the age groups apart from the infants, not even in the 90 percentile. In all age groups folate intake is around 50% lower than in those who consume fortified products. Amongst those who consume products of this kind, the mean value for folate intake increases from 123 µg/day (120 µg/day) amongst male (female) infants to 429 µg/day (350 µg/day) amongst the 15-18 year-old boys (girls). Independently of age and gender, foods fortified with folic acid account for 50% and food supplements for 8% (only amongst 15-18 year-olds) of folate intake from all sources.

➔ *The re-evaluation of the DONALD study shows that 75% of the children and adolescents examined reached the recommended intake for folate equivalents if the consumption of fortified foods and food supplements is taken into account.*

Intake by women of childbearing age: A sub-group (n=1244) of the study population in the nutrition survey was included in a special survey about folic acid intake of women of childbearing age (18-40 years). Mean folate intake by these women was 228.5 µg/day [P25=191 µg/day; P75=282 µg/day] (Thamm *et al.*, 1999; Thamm, 2001).

A few years after the publication of the recommendation that women who are pregnant or who would like to become pregnant should take an additional 400 µg folic acid per day periconceptually, studies showed that very few women in Germany comply with this recommendation. In a survey of 253 women in childbed in Munich, 4.3% indicated that they had taken folic acid supplements in early pregnancy (Genzel-Boroviczény *et al.*, 1997). Similar figures are confirmed by other authors (Gärtner *et al.*, 1997; Egen and Hasford, 2003; Rösch *et al.*, 1999).

When assessing intake data it must be borne in mind that folate levels stated in current food databases are a subject of controversy. Some authors assume that folate levels are underestimated by around 20-30% because of unreliable analytical methods (Tamura, 1998). By contrast, a Dutch study showed that folate levels in foods, which were not analysed with microbiological methods but using HPLC, were found to have values which were on average 25% below the values listed in the nutrient tables (Konings *et al.*, 2001).

Biomarker: Conclusions about the current intake situation can be drawn from the determination of serum folate concentrations. A folate level below 7 nmol/L (3 µg/L) is defined as inadequate (IOM, 2000). To determine folate status over a longer period (2-3 months retrospectively), the folate concentration in the erythrocyte is considered to be a good indicator and a reliable source of information on tissue store. A fall in this concentration below 317 nmol/L (140 µg/L) points to a folate deficiency (Pietrzik and Prinz-Langenohl, 1998).

In pregnant women Daly *et al.* (1995) showed for Ireland that an erythrocyte folate concentration <150 µg/L compared with >400 µg/L correlated with an eight-fold risk of neural tube defects (NTD). At concentrations between 150 and 200 µg/L the risk was still four times, between 200 and 300 µg/L three and between 300 and 400 µg/L two times higher.

In the folic acid study mentioned already, which was carried out within the framework of the Nutrition Survey specifically amongst women of childbearing age, folic acid concentrations in the serum and in erythrocytes were determined in addition to consumption data (n=1266): the median serum concentration was 7.6 µg/L [P5= 4.2 µg/L; P95=12.9 µg/L]. In the erythrocytes the median was 266 µg/L [P5=161.5 µg/L; P95=498 µg/L] (Thamm, 2001). Although according to the Nutrition Survey not one woman in the group reached the recommended intake, less than 5% of the study population would have to be classified as undersupplied taking into account the biomarkers. Assuming, however, that the relationship between marginal folate status and the risk of NRD[5] noted by Daly *et al.* (1995) was applicable to Germany, the risk of NTD would be eight-fold amongst around 3% of the study group examined, four-fold however for around 14%, three-fold for 48% and double for almost 22% (Thamm, 2001).

[5] Since there is no national malformation register in Germany in which prematurely terminated pregnancies are also recorded, there is no exact information about the number of neural tube defects in this country. Regional surveys about malformations, regularly conducted in Mainz using the register of births and drawing on malformation monitoring since 1980 in the Land Saxony-Anhalt, indicate frequencies of 1.84 (CI 95%: 1.44-2.33) and 1.17 (CI 95%: 0.86-1.1) per 1000 births for the period 1980-99 (EUROCAT, 2002). In 2002 the frequency of NTD in the Land Saxony-Anhalt was 0.84 per 1000 live births and had, therefore, fallen slightly compared with the previous year (Fehlbildungsmonitoring Sachsen-Anhalt, 2003).

The nutrition surveys carried out in Germany indicate that 80-90% of the population (in all age groups) do not reach the recommended intake for folate equivalents through the consumption of normal unfortified foods. Theoretically around 50% of the adult population and 75% of children and adolescents could reach the recommended intake for this vitamin from the fortified foods already on the market. However, there is uncertainty about the contribution which fortified foods actually make to meeting the requirements of adults. Furthermore, there are no representative data about folate concentrations in serum or erythrocytes (supply category 1/2).

13.3 Risk characterisation

13.3.1 Hazard characterisation (NOAEL, LOAEL)

So far, there is no evidence of a risk from a high folate intake from natural sources which means that no NOAEL or LOAEL could be established for natural folate (SCF, 2000; EVM, 2003; IOM, 2000). Since the population in Germany reaches on average only 50-70% of the recommended dietary folate intake, there is no danger in any case of excessive intake of this vitamin from normal food.

No systematic toxicological studies have been conducted either for synthetic folic acid (PGA) or for synthetically reduced folate compounds. There are signs from animal studies that 60-90 mg/kg synthetic folic acid administered intravenously may have a neurotoxic or epileptogenic effect. The studies are, however, contradictory and, in the opinion of SCF, cannot be used for the derivation of a NOAEL or a LOAEL. From human studies there are no signs of neurotoxicity from taking folic acid (SCF, 2000).

The administration of more than 1 mg folic acid can trigger convulsions amongst epileptics and can also weaken the effect of antiepileptic drugs since folic acid induces an elevated hepatic metabolism of individual anti-epileptic drugs (barbiturates, phenytoin). Sometimes, therefore, higher doses of these drugs are needed. On the other hand, anti-epileptic drugs inhibit the uptake of folic acid (Staub and Gallmann, 1996). Interactions between folate absorption in conjunction with the parallel taking of medicinal products to treat cancer or rheumatism are possible. At present, there is no clear evidence that elevated folate or folic acid intake has a negative effect on the efficacy of these medicinal products. The intake of 1 mg folic acid per day does not seem to impair the therapeutic effect of low-dose methotrexate and at higher doses folic acid may possibly contribute to reducing the side effects of this medicinal product (Campbell, 1996).

Various authors have observed that intake levels of more than 250 µg folic acid (as a single dose) can no longer be converted fully to 5-methyl tetrahydrofolate. As a consequence, some of this substance reaches the plasma as non-metabolised folic acid. So far it is not known what effects the vitamin in this form can have on the organism (Bailey *et al.*, 2001 in: Quinlivian and Gregory III, 2003; IOM, 2000; Kelly, 1997).

From an intake of 5 mg folic acid upwards, a parallel vitamin B_{12} deficiency can be "masked", i.e. the identical haematologic symptoms in the case of a vitamin B_{12} and folate deficiency are improved through the folic acid intake whereas neurological symptoms, which go hand in hand with a vitamin B_{12} deficiency, are not prevented and may even be exacerbated (Drazkowski *et al.*, 2002; IOM, 2000; SCF, 2000). Because of this relationship FNB has established a LOAEL of 5 mg. A NOAEL could not be determined since no data are available about the onset of masking when taking folic acid between 1 and 5 mg (IOM, 2000). SCF followed the FNB evaluation (SCF, 2000).

13.3.2 Deficiency, possible risk groups

Megaloblastic anaemia may occur as a consequence of chronic clinical folate deficiency. Furthermore, because of the importance of folates for DNA synthesis, there may be disruptions in cell proliferation that have a particularly negative impact on the rapidly proliferating cells in bone marrow and in the intestinal tract and go hand in hand with a reduction in the number of white cells (neutrophiles, lymphocytes, monocytes, eosinophiles and basophiles) and thrombocytes (Bailey *et al.*, 2001; Molloy, 2002).

Inadequate folate supply during pregnancy is linked with a higher risk of premature births, low birth weight and foetal growth retardation (Scholl and Johnson, 2000). Furthermore, various studies have shown that an elevated periconceptual intake of folic acid, in combination with multivitamin products or on its own, reduces the risk of neural tube defects and other congenital malformations (Czeizel and Dudas 1992; Czeizel 1995; 2000; Moore *et al.*, 2003; Tönz *et al.*, 1996). The genesis of neural tube defects (NTD) is, however, conditioned by various factors. Up to now it was not possible to determine by means of which mechanism folic acid is involved in the closure of the neural tube (Fleming, 2001).

A link between sub-optimum folate supply and the onset of cardiovascular diseases is also under discussion. This is due to the fact that homocysteine cannot be remethylated to methionine in the case of inadequate folate supply and accumulates in the organism or reacts with another homocysteine molecule to homocystine. This damages the endothelium and may lead to vascular occlusion (Ubbink *et al.*, 1996). The results from large intervention studies will provide information on whether these observations have a causal link.

Furthermore, a series of epidemiological studies indicates that there is a link between low folate intake and low plasma folate levels and an elevated risk of the onset of colorectal cancer. Smaller intervention studies, conducted up to now, in order to confirm the suspected protective effect in man, did not, however, provide any unequivocal evidence and the results from large intervention studies are not yet available (Kim, 2003).

13.3.3 Excessive intake, possible risk groups

Up to now, no adverse effects have been observed as a consequence of folate intake from common food.

As mentioned above, intake of >5 mg folic acid per day can "mask" a parallel vitamin B_{12} deficiency. Risk groups for the masking of a vitamin B_{12} deficiency are older people (>60 years), who suffer from a vitamin B_{12} but also a folate deficiency more frequently than the average population (Clarke *et al.*, 2003). It can be assumed that the most frequent cause of a vitamin B_{12} deficiency is an absorption disorder for food-bound vitamin B_{12}. No data are available for the Federal Republic of Germany about the incidence of vitamin B_{12} malabsorption. Despite adequate intake in all age groups, the VERA Study reports a high prevalence of low vitamin B_{12} plasma levels amongst men aged 65 and over whereby plasma concentrations below the reference intake were only measured in 4.3% of the total random sample (Heseker *et al.*, 1992). We know from other studies that between 10 and 15% of all people aged 60 and above suffer from a vitamin B_{12} deficiency whereas pernicious anaemia, the final stage of an autoimmune disorder with loss of the gastric mucosa cells that form the intrinsic factor, only occurs in around 2% of all persons aged 60 and above. In the Framingham study a 15% prevalence of undiagnosed cobalamine deficiency was observed amongst the over 60s which can probably be attributed to malabsorption (Lindenbaum *et al.*, 1994 in: Andrès *et al.*, 2002). According to Baik and Russell (1999) 10-15% of this age group seem to be affected by vitamin B_{12} deficiency.

Sleep disorders, agitation, hyperactivity, nausea, flatulence, a disturbed sense of taste and allergic reactions like erythema, pruritus and urticaria have been reported in conjunction with higher levels of folic acid intake (~15 mg), (Bässler *et al.*, 2002).

In animal experiments folic acid intake far in excess of requirements led, in the presence of pre-malignant lesions or neoplastic foci, to a progression of the lesions (Kim, 2003). Given the relatively high prevalence of colorectal adenoma amongst the western population (USA: ~25% amongst the population >50 years), these results must be taken seriously and further examined in the interests of public health. Furthermore, the observation that people with a polymorphism of thymidylate synthase [TSER 2rpt/2rpt] at a dietary folate intake of more than 440 µg/day ran a 1.5 times higher risk of developing colonrectal cancer than those whose folate intake was below 440 µg/day, shows that there are still gaps in knowledge about the link between folate metabolism and the onset or prevention of cancer. It cannot be accepted as proven that folic acid supplementation is always advantageous for every genotype (Ulrich *et al.*, 2002).

13.4 Tolerable upper intake level for folates and folic acid

Since there is no known risk from folate intake from natural sources, neither SCF nor any other scientific body has set a **UL** for natural folates (EVM, 2003; IOM, 2000; SCF, 2000).

Based on the LOAEL defined by FNB of 5 mg/day and taking into account an uncertainty factor of 5 (given that no NOAEL could be established), an **upper intake level for the intake of synthetic folic acid of 1 mg/day** was derived (IOM, 2000). SCF agreed with this and added that there is no evidence that groups in the population must be especially protected against high intake and that this UL can, therefore, be applied to pregnant and lactating women as well.

No data are available about the long-term effects of high-dose folic acid intake amongst infants. For that reason it is recommended that the folate needs of this group should be exclusively covered from normal food. Lower ULs are derived for all age groups of children and adolescents in line with their bodyweight (SCF, 2000):

Age (years)	UL [µg RE/day]
1 - 3	200
4 - 6	300
7 - 10	400
11 - 14	600
15 - 17	800

Since the masking of symptoms of an undiagnosed vitamin B_{12} deficiency is probably less relevant amongst children and adolescents than amongst adults, the ULs are not very well suited to assessing the risk of adverse effects for those age groups. On the other hand, it cannot be ignored that an estimation of the long-term effect of folic acid ingestion by children is rendered more difficult by the fact that scarcely any experience is available about the taking of folic acid supplements by these groups in the population (Molly, 2003).

13.4.1 Derivation of a maximum level for folic acid in food supplements and fortified foods

The application of the formula used in other chapters of this report leads to the following:

R = UL – DINF
R = 1000 - 0 [µg]
R = 1000 µg

Legend:

UL	=	Tolerable Upper Intake Level (SCF) usually referring to the daily total intake
DINF	=	Dietary Intake by Normal Food (95. or 97.5 percentile)
MEF	=	Estimated Number of Consumed Products
TL	=	Tolerable Level in a single dietary supplement or fortified food
R	=	Residual or maximum amount for safe addition to foods including dietary supplements

The amount of folic acid (= "R") available for use in food supplements or fortified foods is 1000 µg as the UL only refers to folic acid and not to folate taken up from food.

With a view to the current fortification practice and the supply situation of the population, it makes sense in future to also divide the amount "R" of folic acid between food supplements and fortified foods. If we assume multiple consumption for each of the product groups and if food supplements and fortified foods are assigned equal portions then this leads to:

$$TL_{FS} = \frac{\text{Intake food supplement}}{2} \qquad TL_{FF} = \frac{\text{Intake fortified foods}}{2}$$

$$TL_{FS} = \frac{500\ \mu g}{2} \qquad TL_{FF} = \frac{500\ \mu g}{2}$$

$$TL_{FS} = \mathbf{250\ \mu g} \qquad TL_{FF} = \mathbf{250\ \mu g}$$

13.4.1.1 Possible management options for the use of folic acid in food supplements

Up to now, BgVV/BfR had accepted a maximum level of 900 µg per daily intake for the addition of folic acid to food supplements (BgVV, 1998).

a) Retention of the previously accepted maximum level of 900 µg folic acid (=1800 µg folate equivalents) per daily dose.

   Advantage: None identifiable

   Disadvantage: Children and adolescents who took one food supplement would already exceed the UL set for these age groups. Adults who consumed two food supplements would also exceed the UL. There is no scope left for the fortification of conventional foods.

b) Setting the maximum level at 250 µg folic acid (= 500 µg folate equivalents) per daily dose in line with the derivation using the formula.

   Advantage: By taking just one food supplement, one would reach the daily recommended intake of an adult. In this way, food supplements would make a major contribution to improving the supply situation amongst the population. The maximum level of 250 µg per daily dose would leave enough scope for the use of folic acid in fortified foods. It is unlikely that the UL would be exceeded by the adult population even if they took several food supplements.

   Disadvantage: The dose is lower than the 400 µg folic acid per day recommended for periconceptual supplementation which means that special recommendations would be required for women of childbearing age, e.g. information on the products that women of childbearing age should take two food supplements per day.

c) Setting the maximum level on the level of recommended intake, i.e. 200 µg folic acid (= 400 µg folate equivalents) per daily dose

Advantages and disadvantages: See b)

d) Setting the maximum level at 400 µg folic acid (=800 µg folate equivalents) per daily dose in line with the recommendation for periconceptual supplementation

Advantage: By regularly taking a food supplement with 400 µg folic acid per daily dose, women of childbearing age could ingest the amounts of folic acid recommended in a targeted manner for this population group to cover their specific requirements and to prevent NTD. Food supplements are already available on the market at this dose and so far no harmful effects have been observed through their ingestion.

Disadvantage: Up to now we do not know what (long-term) effects can be triggered by unmetabolised folic acid in serum (was observed at a single dose >250 µg folic acid). The remaining scope for the fortification of foods would be relatively small. Children up to the age of 10 would already reach or exceed the UL by taking one food supplement.

13.4.1.2 Possible management options for the use of folic acid in fortified foods

Some of the table salts on sale in Germany which have already been iodised and fluoridised have also been fortified with 100 µg/g folic acid. It is known that in November 2003 10% of German households already used this salt and that the intake of folic acid or folate equivalents (FE) by consumers of this salt is around 100-200 µg/d folic acid resp. 170-340 µg FE per day. Because of its widespread use within the population salt can help to raise folic acid intake in all age groups of the population (>1 year). The sole use of salt could contribute to reaching around 50% of the recommended intake. Furthermore, salt is known as a carrier food for nutrient fortification of iodine and fluoride and is generally accepted by the population.

Moreover, discussions are underway about whether in Germany, given the low folate intake of the population and the fact that only few women of childbearing age take on board the recommendations of periconceptual folic acid supplementation to reduce the NTD risk, a targeted folic acid fortification of selected basic foods (e.g. flour) would make sense.

The taking of one food supplement per day of 400 µg folic acid and the use of salt fortified with folic acid (100-200 µg folic acid/day) in the home would lead to folic acid intake from these two sources of 500-600 µg per day. This means that a total level of folic acid of 400-500 µg is still available for the fortification of conventional foods.

The following proposals for maximum levels for the fortification of conventional foods with folic acid are made subject to the assumption that no more than 400 µg folic acid per day is ingested from food supplements. If one assumed multiple consumption from food supplements (as in other chapters of the report), then this would reduce the margin to the UL to such an extent that a fortification of conventional foods beyond salt would not be acceptable.

a) No change to the current unregulated practice, i.e. fortification of different food (groups) without setting a maximum level

Advantage: The new assessments of the Nutrition Survey and the DONALD study have shown that a large share of the population (50% of adults and 75% of children and adolescents) reach or could reach the recommended intakes by consuming already fortified foods (100-200 µg up to maximum 400 µg folic acid per portion)

without, on the other hand, having to expect that a large part of the population would exceed the UL. Since the 18 to 25 age groups in particular consume foods from the groups of products which are frequently on sale on the market with folic acid supplements, the group of young women, i.e. a large proportion of those of childbearing age would automatically improve their folic acid intake.

Disadvantage: Given the major variations in amounts and frequencies of consumption of fortified foods, no even or predictable improvement in the supply situation of the population would be possible. Through the consumption of fortified foods coupled with an otherwise normal diet, very few women of childbearing age would reach the recommended intakes of 1200 or 1400 µg folate equivalents per day (in early pregnancy).

b) Setting a maximum level of 100 µg per portion for the use of folic acid in fortified foods

Advantage: The addition of this amount of folic acid to conventional foods can make a valuable contribution to improving folate supply. The margin to the UL is sufficiently large which means that several portions of foods fortified with 100 µg folic acid could be consumed and the likelihood that the UL would be exceeded is small.

Disadvantage: There is no certainty that the groups in the population, whose folate intake is low, actually eat fortified foods or that women of childbearing age could achieve requirement-oriented folate/folic acid intake through the consumption of fortified foods.

c) Setting a maximum level of 200 µg per portion for the use of folic acid in fortified foods

Advantage: A portion of a food, which has been fortified with this amount of folic acid, would already cover the daily requirements of an adult in respect of folate equivalents.

Disadvantage: See b). Individuals, who regularly use salt fortified with folic acid and take food supplements with folic acid, could exceed the UL for folic acid through the additional consumption of other fortified foods.

The risk classification for the use of folic acid in food supplements and fortified foods is not possible using the criteria laid down by BfR since the UL derived for this vitamin solely applies to synthetic folic acid. In the opinion of BfR the risk of adverse health effects in conjunction with the use of synthetic folic acid in foods must be described as moderate.

For the general population BfR considers an additional intake of 200 µg folic acid (=400 µg folate equivalents) from food supplements to be adequate. Since, however, it is recommended that women of childbearing age take an additional daily folic acid supplement of 400 µg (=800 µg folate equivalents) and the use of this dose in the past has not led to any adverse effects, the maximum level of 400 µg folic acid per daily dose (Option d) in food supplements should be recommended for the overall population.

Assuming that only one food supplement of 400 µg per day is taken, other foods can also be fortified with folic acid. Since salt fortified with folic acid as used in households can lead to intakes of 100-200 µg folic acid per day, BfR is of the opinion that maximum 200 µg folic acid per portion should be used for the fortification of other foods (option c).

To the extent that flour, in addition to table salt, will also be fortified with folic acid in future, depending on the selected flour fortification levels, the maximum levels for the addition of folic acid to conventional foods should be revised or it should be examined whether and, if so, which foods if any, in addition to salt and flour, could be fortified with folic acid.

13.5 Gaps in knowledge

- It is not known what contribution fortified foods and food supplements make in Germany to folate supply.
- It is not known by means of which mechanism folic acid has a preventive effect on the onset of neural tube defects.
- The folic acid fortification programmes in other countries have not been up and running for long enough or not enough findings are available about changes in folate supply or the reduction in NTDs since 2000 in order to be able to assess the impact of chronic folic acid intake from fortified foods.
- No data are available about the long-term effects of the ingestion of large amounts of synthetic folic acid.
- It is still to be clarified which (positive or negative) effects the vitamin can have on certain groups amongst the population taking into account the gene polymorphisms relevant for the folate metabolism.
- There are no representative figures about the prevalence of vitamin B12 deficiency and pernicious anaemia in Germany.
- So far it has not been proven unequivocally whether the link between high homocysteine levels and the onset of cardiovascular disease is a causal relationship and whether the long-term administration of folic acid can reduce not only the homocysteine level but also the risk of the onset of these diseases.

13.6 References

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14 Risk Assessment of Pantothenic Acid

14.1 Summary

For the Federal Republic of Germany there are no signs of inadequate intakes of pantothenic acid. However, representative consumption studies are lacking nor is there any precise information about requirements (supply category 2). The health risk linked to the use of the vitamin in food supplements and fortified foods is estimated to be low.

Because of the lack of systematic studies and the low toxicity of pantothenic acid, a tolerable upper intake level for total daily intake could not be derived. Hence, using the proposed formula no defined maximum levels can be derived at present for pantothenic acid in food supplements or fortified foods either. As an alternative, for reasons of preventive health protection and because of the gaps in knowledge, the three-fold level of the estimated value for adequate intake (18 mg) per day should not be exceeded in food supplements. It can be assumed that at this pantothenic acid level no health risks are to be expected for the consumer.

A substantial increase in daily vitamin intake does not offer any additional nutritional-physiological benefits. Hence, in the case of fortified foods an appropriate vitamin addition in the expected daily portion should not exceed one-fold the estimated value for adequate intake for pantothenic acid (6 mg).

Estimated values for adequate intake	6 mg/day
Intake [mg/day]	**m** **f**
Median	? * ? *
P 2.5	? * ? *
P 97.5	? * ? *
	* No representative intake data for the Federal Republic of Germany
Tolerable upper intake level	Not defined Low toxicity No systematic studies available
Proposal for **maximum levels** in:	
food supplements	18 mg/daily dose
fortified foods	6 mg/daily portion

14.2 Nutrient description

14.2.1 Characterisation and identification

The biologically active form of pantothenic acid (CAS No. 79-83-4) is bound to the dextrorotatary D configuration. It is designated chemically as (R)-N-(2,4-dihydroxy-3,3-dimethyl-1-oxobutyl)-β-alanine or as D(+)-N-(2,4-dihydroxy-3,3-dimethylbutyryl)-β-alanine ($C_9H_{17}NO_5$). Admissible pantothenic acid compounds or vitamin sources, which are added to foods for special dietary purposes and food supplements and which can be used or were proposed for the fortification of foods, include sodium-D-pantothenate (CAS No. 867-81-2), calcium-D-pantothenate (CAS No. 137-08-6) and D-panthenol (CAS No. 81-13-0) (Ordinance on Vitaminised Foods; Ordinance on Foods for Special Dietary Purposes; Ordinance on Food Supplements and amending the Ordinance on Vitaminised Foods; Commission of the European Communities, 2003). The synthetically produced alcohol, panthenol, can be readily oxidised in the organism to pantothenic acid.

14.2.2 Metabolism, function, requirements

In foods pantothenic acid is mainly found in bound form as a component of coenzyme A. For absorption the substance is released from the coenzyme and absorbed as pantothenic acid or pantetheine. Besides passive diffusion, it is thought that an active carrier-mediated Na^+-dependent transport with saturation kinetics is involved in absorption. Excretion takes place after release from coenzyme A in urine mainly in unchanged form as pantothenic acid or as 4-phosphopantothenate. Around 15% of the absorbed pantothenic acid is exhaled as CO_2 or appears in faeces (Bässler *et al.*, 2002; Miller *et al.*, 2001). Any excess pantothenic acid is mainly excreted by the kidneys. Excretion correlates to a high degree with dietary intake.

As a component of coenzyme A, a cofactor and acyl group carrier of enzymatic reactions and as a component of the fatty acid synthase complex, pantothenic acid is of central importance for the intermediary metabolism.

For pantothenic acid only estimated values for adequate intake can be given. In Germany an estimated value for adequate intake of 6 mg per day was derived for adolescents and adults, including pregnant and lactating women. For infants values of 2-3 mg/day are given, depending on their age and values of 4-5 mg are given for children (1-15 years), again depending on their age (D-A-CH, 2000). The Scientific Committee on Food (SCF) gave 3-12 mg/day as an acceptable intake range for adults (SCF, 1992).

14.2.3 Exposure (dietary and other sources, nutritional status)

Sources:
Pantothenic acid can be found in a number of foods. There are high levels in beef, veal and pork liver (7-8 mg/100 g) or herring (7.4 mg/100 g). In other foods of animal origin like beef or pork the levels are approximately 0.5-0.7 mg/100 g, in chicken eggs approximately 1.4 mg/100 g, in milk approximately 0.3 mg/100 ml. Among foods of plant origin, wholegrain wheat (1.2 mg/100 g), oats (1.1 mg/100 g) or tomatoes (1 mg/100 g) have higher levels of pantothenic acid (Bässler *et al.*, 2002).

Medicinal products: In the medicinal product monograph *Dexpanthenol/Panthenol/Pantothenic acid*, daily doses of up to 10 mg/day are indicated for the prophylactic treatment of pantothenic acid deficiency conditions, which cannot be remedied through diet and up to 100 mg/day are indicated for treatment of these deficiencies. The maximum daily dose should not exceed 500 mg – broken down into several single doses – according to the monograph (BGA, 1993). In the drug samples for *water and fat soluble vitamins in a fixed combination*, doses of 2-10 mg per day are indicated for prophylaxis and 10-50 mg for therapeutic treatment (BfArM, 1995).

Dietary status: There are some uncertainties surrounding the recording of actual intake levels of pantothenic acid (Bässler *et al.*, 2002; Gaßmann, 1999). For Germany daily intakes in adults of around 4-5 mg are mentioned (D-A-CH, 2000). The average intakes of 4-7 mg/day mentioned by SCF in 1992 are on a similar scale whereby some individuals took 3-12 mg (SCF, 1992). The SCF opinion on the derivation of a tolerable upper intake level (UL) of pantothenic acid from 2002 indicates similar intake amounts (SCF, 2002).

14.3 Risk characterisation

14.3.1 Hazard characterisation (NOAEL, LOAEL)

Pantothenic acid or panthenol apparently have a low toxicity.

In various animal studies on acute toxicity, toxicity of long-term administration and reproduction toxicity (Chung *et al.*, 1954; Everson *et al.*, 1954; FDA, 1974; Unna and Greslin, 1941; Weiss *et al.*, 1950) pantothenic acid and panthenol showed a low degree of toxicity. However, in studies by one working group in rats (50 mg/day, 70 days) elevated uterus weights, elevated weights of the vesiculae seminales (Fidanza *et al.*, 1959a) and increased excretion of 17-ketosteroids were reported (Fidanza *et al.*, 1959b). It should be added that all the above-mentioned animal studies are quite old and were not, therefore, conducted in line with the standards customary today.

With regard to data obtained from human beings, SCF maintains in its report for the derivation of a tolerable upper intake level for pantothenic acid that in a literature search from 1966 onwards no reports could be found about adverse effects following the oral intake of pantothenic acid or panthenol (SCF, 2002). In the meantime a single case report has been published about the occurrence of a life-threatening eosinophilic pleuropericarditis in conjunction with trimetazidine medication and additional intake of 300 mg pantothenic acid and 10 mg biotin per day (Debourdeau *et al.*, 2001). However, the interpretation of this individual case report is difficult because of the parallel administration of three substances.

Concerning the administration of higher pantothenic acid levels, another study is quoted in the SCF report according to which no side effects occurred in conjunction with the administration of 2 g pantothenic acid per day (General Practitioner Research Group, 1980). It is also pointed out that diarrhoea and water retention may occasionally occur in conjunction with daily intakes of 10-20 g (Harris and Lepkovsky, 1954; SCF, 2002).

In summary, it can be said that because of the lack of systematic studies and the low toxicity of pantothenic acid (calcium pantothenate or panthenol) no **LOAEL** (Lowest observed adverse effect level) or **NOAEL** (No observed adverse effect level) could be identified by SCF and no UL (tolerable upper intake level) could be derived (SCF, 2002). The American Food and Nutrition Board (FNB) (IOM, 2000) came to the same conclusion.

14.3.2 Deficiency, possible risk groups

Given the widespread occurrence of pantothenic acid in foods, an isolated pantothenic acid deficiency is rare. As a rule, in such cases there are also deficiencies of other vitamins. Specific deficiency symptoms have been described up to now following the administration of artificial pantothenic acid antagonists and/or the administration of pantothenic acid-free semi-synthetic diets or in the case of malnutrition amongst prisoners of war during the Second World War (burning feet syndrome) (Fry *et al.*, 1976; Glusman, 1947; Hodges *et al.*, 1958; Hodges *et al.*, 1959).

There is no risk of deficiency through intake of pantothenic acid on the level of the estimated value for adequate intake of 6 mg/day.

For the Federal Republic of Germany there is no indication of inadequate intakes of pantothenic acid. However, representative nutrition surveys are missing nor is there any precise information about requirements (supply category 2).

14.3.3 Excessive intake, possible risk groups

The available data indicate that pantothenic acid has a low toxicity. Hypervitaminosis through excessive intake from food including food supplements has not been described up to now. At very high intake levels of 10-20 g/day minor adverse effects like occasional diarrhoea and water retention can occur (SCF, 2002). However, no systematic studies on the effect of high intake levels are available.

14.4 Tolerable upper intake level for pantothenic acid

Given the lack of systematic studies and the low toxicity of pantothenic acid (calcium pantothenate or panthenol) no tolerable upper intake level for total daily intake could be derived by SCF or the American Food and Nutrition Board (IOM, 2000; SCF, 2002). Nor was the British Expert Group on Vitamins and Minerals (EVM) able to derive a safe upper level for pantothenic acid. With the limitation "for guidance purposes only" a value of 200 mg per day is given for additional intake (from supplements) (Food Standards Agency, 2003). The report of the Nordic Council of Ministers on the addition of vitamins and minerals to foods and the report of the French CSHPF do not give any upper levels for the intake of pantothenic acid (CSHPF, 1995; Nordic Council, 2001).

14.4.1 Derivation of a maximum level for pantothenic acid in food supplements

As no tolerable upper intake level for total daily intake could be derived up to now, the proposed formula for deriving a defined maximum level for pantothenic acid in food supplements cannot be used. Because of the existing gaps in knowledge, the measures to be taken to set maximum levels should be based on the precautionary principle and revised on submission of new data.

14.4.1.1 Possible management options

a) Continuation of existing practice

   At present, pantothenic acid additions of up to three-fold the estimated value for adequate intake (18 mg) are accepted in food supplements per recommended daily portion (ALS, 1998; D-A-CH, 2000).

   Advantages: Experience is already available with this upper level. According to this, it can be assumed that no health risks for consumers are to be expected at these pantothenic acid levels.

   Disadvantages: In the opinion of BfR no health disadvantages are identifiable.

b) One-fold rule

   Limiting the addition of pantothenic acid per recommended daily portion to one-fold the estimated value for adequate intake (6 mg).

   Advantages: The proposed maximum level is oriented towards actual requirements. It makes nutritional-physiological sense.

   Disadvantages: In the opinion of BfR no health disadvantages are identifiable.

c) No setting of maximum levels for individual food supplements.

   Advantages: There are no identifiable advantages.

Disadvantages: This would not do justice to the precautionary principle or proper consumer protection since the inadequate data situation that led to SCF and other bodies not being able to set a **UL**, does not mean that higher levels could not constitute a health risk.

d) Derivation of upper levels on the basis of the "guidance value" of the British EVM

The British EVM has derived a value of 200 mg per day for the additional intake of pantothenic acid (from supplements) "for guidance purposes only". It is not expected that this additional intake will lead to any adverse effects in the general population (Food Standards Agency, 2003).

Advantages: There is a numerical value which is derived by a recognised scientific body. Based on the procedure proposed in Chapter 3.2, an upper level could be derived for individual food supplements (50 mg pantothenic acid)[6].

Disadvantages: The guidance values are linked to considerable uncertainties since they may possibly not be applicable *to all age groups* or to *lifelong intake* (Chapter 5: General Principles for Assessing Micronutrients; Food Standards Agency, 2003).

14.4.2 Derivation of a maximum level for pantothenic acid in fortified foods

Since, up to now, no tolerable upper intake level could be derived for total daily intake, the proposed formula for the derivation of a defined maximum level for pantothenic acid in fortified foods cannot be used. Because of the existing gaps in knowledge, the measures to be taken to set maximum levels should be based on the precautionary principle and revised on submission of new data.

14.4.2.1 Possible management options

a) Continuation of existing practice

At present, pantothenic acid additions up to three-fold the estimated value for adequate intake (18 mg) are accepted in fortified foods per recommended daily portion or per expected daily portion (ALS, 1998) whereby no maximum levels for addition are indicated in the Ordinance on Vitaminised Foods.

Advantages: Experience is already available with this fortification practice. No negative effects have been described up to now.

Disadvantages: Sufficient data are not available about how high the levels of fortification actually are in individual cases. The level of fortification in many areas is down to the discretion of the manufacturer, since fortified foods are eaten according to needs and they need not carry any consumption recommendations and no agreement has been reached on the size of expected daily portions. Depending on the number of fortified foods consumed, high vitamin levels may be ingested under certain circumstances since – at least theoretically – a vitamin addition of up to 18 mg pantothenic acid would have to be assumed per portion.

b) Limiting the maximum level to the one-fold the estimated value for adequate intake with a maximum level of maximum 6 mg pantothenic acid per recommended or expected daily portion

[6] When broken down into equal total tolerable intakes for food supplements and fortified foods and a multiple exposure factor for food supplements of 2

Advantages: This proposal is oriented towards nutritional-physiological aspects and towards preventive health protection since this, rather than higher fortification, takes into account the fact that fortified foods are normally consumed in an uncontrolled manner without any fixed daily portions.

Disadvantages: In the opinion of BfR there are no identifiable health disadvantages.

The risk of adverse effects when using pantothenic acid in food supplements and fortified foods is considered to be low in the opinion of BfR. After weighing up the advantages and disadvantages of the options outlined above, BfR recommends Option a) for food supplements (18 mg per recommended daily dose) and Option b) for fortified foods (6 mg per daily portion).

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15 Risk Assessment of Biotin

15.1 Summary

The requirement for biotin is not known. It is assumed that the biotin intake of healthy adults covers their requirements in the same way as biotin intake from human milk covers the requirements of breastfed infants. Aside from patients who receive too little biotin because of special circumstances, the German population is sufficiently supplied with biotin. Pregnant women may be one possible risk group (supply category 2).

For human beings no symptoms of biotin overdose are described. BfR considers the health risk from the use of biotin in food supplements and the addition of biotin to fortified foods to be low. Because of the data situation, however, a risk cannot be ruled out with certainty. For reasons of preventive consumer protection, BfR is of the opinion that no more than 180 µg biotin per daily dose should be added to food supplements and a maximum of 60 µg per portion to fortified foods.

Estimated value for adequate intake	30-60 µg/day	
Intake [µg/day] (Mensink *et al*., 2002)	**m**	**f**
Median	42.9-61.9	37.3-43.6
P 10	27.6-35.9	24.3-28.7
P 90	69.7-101.3	55.2-74.8
Tolerable upper intake level	Not defined No systematic dose-response studies available	
Proposal for **maximum levels** in:		
food supplements	180 µg/daily dose	
fortified foods	60 µg/portion	

15.2 Nutrient description

15.2.1 Characterisation and identification

Biotin (CAS No. 58-85-5; cis-hexahydro-2-oxo-1H-thieno(3,4)imidazole-4-pentanoic acid) is a water soluble vitamin, whose synthesis is limited to most bacteria, several fungi and plants. Of the eight theoretically possible stereoisomers, only d-(+)-biotin (D-biotin) occurs in nature and is biologically active. Alternative (historic) designations like vitamin H or vitamin B_7 should be avoided.

15.2.2 Metabolism, function, requirements

Metabolism: Dietary biotin is mainly bound to protein. During digestion of the protein biotin-containing peptides are formed including biocytin (biotinyl-ε-lysine) for the cleavage of which the ubiquitous enzyme biotinidase is necessary. Opinions differ as to whether biotinyl peptides can be absorbed in the human intestine. Biotinidase deficiency leads to a biotin deficiency when the elevated biotin requirement is not met. In the case of low/normal intake biotin is absorbed with the help of an active, sodium-dependent transporter. At higher concentrations in the intestine this is mainly a transporter-independent process. Whereas in the past a very variable bioavailability of biotin of 24-58% was assumed, the bioavailability of higher doses of 20 mg free biotin seems to be 100% (Zempleni and Mock, 1999).

Absorbed biotin is released into blood by a transporter-dependent process where the largest portion is free (81%), 12% bound covalently to the serum biotinidase and 7% non-specifically bound to the plasma albumin and globulins (Mock and Malik, 1992). Biotin uptake into organ

cells is probably by means of specific energy-consuming, sodium dependent transport processes (Said *et al.*, 1998). Proliferating lymphocytes take up more biotin as a consequence of an increase in transporter protein (Zempleni and Mock, 2000a). A sodium-dependent multivitamin transporter mediates the transport of biotin (and of lipoic acid and pantothenic acid) against a concentration gradient through the placenta to the foetus. The biotin levels in foetal blood are three to seven times higher than in maternal blood during the 18^{th} to 24^{th} week of pregnancy. The expression of this and other transporters for biotin falls as the blood levels increase (Crisp *et al.*, 2004)

Biotin is mainly excreted in urine whereby biotin clearance corresponds to 0.4 times creatinine clearance. The half-life elimination of a 600 µg single dose of biotin from plasma was 110 minutes (Bitsch *et al.*, 1989). Normal adults excrete around 24 µg biotin plus biotin metabolites/day.

Biotin uptake in the intestine is inhibited by a protease resistant protein avidin which occurs in raw egg. Pro molecule it binds 4 molecules of biotin. Longer heating of avidin to 100°C leads to denaturation and releases biotin.

Biotin is degraded in the human body whereby the beta-oxidation of the valerate side chain leads to the formation of bisnorbiotin and bisnorbiotin methyl ketone whereas the oxidation of sulphur in the thiophene ring leads to biotin-D, L-sulphoxide and biotin sulphone which can be detected in the plasma and urine and do not have any vitamin activity. Biotin only accounts for around 50% of the avidin-binding substances in the plasma and urine (Mock *et al.*, 1993; Zempleni and Mock, 1999). In the case of biotin measurements with the help of the avidin-binding method, a prior separation of biotin metabolites by means of HPLC is, therefore, recommended (Mock, 1997).

Interactions: High lipoic acid intake and alcohol can competitively inhibit intestinal and cellular biotin uptake at least in rats (Zempleni *et al.*, 1997). Some anti-epileptic medicines (primidone and carbamazepine) inhibit intestinal biotin uptake and force biotin from its bond to biotinidase and can lead to elevated biotin requirements (Krause *et al.*, 1984; Mock and Dyken, 1997).

Function: In human beings biotin is an essential cofactor in four carboxylases which catalyse the binding of bicarbonate to organic acids: actetyl-CoA-carboxylase, pyruvate carboxylase, propionyl-CoA-carboxylase and 3-methylcrotonyl-CoA-carboxylase. These carboxylases play a decisive role in fatty acid synthesis, in the supply of metabolites for the citric acid cycle and the degradation of isoleucine, valine, methionine, threonine, of the cholesterol side chain, odd-numbered fatty acids and leucine.

The binding of biotin to the epsilon amino group of lysine of apocarboylases is brought about by an ATP-dependent holocarboxylase synthetase.

The proteolytic degradation of holocarboxylases releases biocytin which can be cleaved by the biotinidase which is present in almost all tissues. This releases biotin which again becomes available. Biotinidase has a further function as biotintransferase which biotinylates and debiotinylates the histones (alkaline DNA-binding proteins) and possibly influences chromatin structure, DNA repair and gene expression (Ballard *et al.*, 2002; Hymes and Wolf, 1996). Biotin influences the expression of genes of non-biotin-dependent enzymes as has been demonstrated in depletion-repletion studies in animals (glucokinase, ornithintranscarbamylase) (Chauhan and Dakshinamurti, 1991; Maeda *et al.*, 1996).

Stimulation of the proliferation of immunologically active circulating mononuclear cells by mitogens stimulated biotin uptake in these cells and 3-methylcrotonyl-CoA-carboxylase activity. Biotin supplementation of 750 µg/day over 14 days and of 2 mg/day over 21 days

led, in the cultivated and stimulated mononuclear blood cells of the healthy test persons, to the increased expression of genes for interleukin-1β and interferon-γ and to the reduced expression of the gene for interleukin-4. It influenced the release of various interleukins, i.e. biotin influences the immune response (Wiedmann *et al.*, 2003) without anything being known about the clinical importance of these effects.

Requirements: Biotin requirements are not known. It is assumed that the biotin intake of healthy adults covers their requirements and that biotin intake from human milk covers the requirements of breastfed infants.

The estimated values by DGE (DGE/ÖGE/SGE/SVE, 2000) for adequate biotin intake are presented in the following table:

Table 20: Estimated values for adequate biotin intake

Age	Estimated value [µg/day]
0 - <4 months	5
4 - <12 months	5 - 10
1 - <7 years	10 - 15
7 - <10 years	15 - 20
10 - <13 years	20 - 30
13 - <15 years	25 - 35
15 - >65 years	30 - 60
Pregnant women	30 - 60
Lactating women	30 - 60

15.2.3 Exposure (dietary and other sources, nutritional status)

Sources: Biotin is present at very different levels in most natural foods. Liver, kidneys, egg yolk, some vegetables like soya beans, nuts, spinach, mushrooms and lentils (20-100 µg/100 g edible portion) are particularly rich in biotin. Lean meat, fruit, cereals and bread contain 1-20 µg/100 g. In many foods of plant origin biotin is available in a water-extractable form whereas in foods of animal origin and yeast it is firmly bound in complexes. No reliable data are available about the bioavailability of biotin from various foods.

In human milk the biotin level varies within a 24-hour period. It increases five to thirty-fold from colostrum to mature milk (Mock *et al.*, 1992a; Salmenperä *et al.*, 1985) and is then twenty to fifty times higher than the plasma concentration of women (Mock *et al.*, 1992b). 30 to 40 days postpartum the biotin level was on average 7 µg/L, i.e. a fully breastfed infant would receive around 6 µg biotin with 800 ml human milk (Mock *et al.*, 1997a).

Food supplements (n=110), which are directly (42%) or indirectly (58%) marketed for children contain (in 23% of cases) biotin in levels up to double (300 µg) the reference intake in the Nutrient Labelling Ordinance. The median level contained in these products was 90 µg per daily portion (Kersting and Alexy, 2000). The contribution of food supplements to the biotin intake of 4030 adults in the Nutrition Survey 1998, 9% of whom took food supplements, was low (Mensink *et al.*, 1999).

No reliable data are available about the scale of **food fortification** with biotin for Germany.

Medicinal products contain up to 25 mg biotin per single dose for the prophylaxis and treatment of biotin deficiency conditions. In the Summary of Product Characteristics (SPC) it is pointed out that 200 µg biotin are sufficient for prophylaxis.

Nutritional status:

Dietary intake: In the 1998 Nutrition Survey biotin **intake** from foods by the 4030 participants aged between 18 and 79, who did not take any food supplements, was on average 52.9

µg/day (95% confidence interval 52.2-53.7) for men and 42.5 µg/day (95% confidence interval 42-43.1) for women. The biotin intake of those persons (9.4% of men, 9.5% of women), who regularly took multivitamin products more than once a week, was on average around 3 µg/day higher (Mensink *et al.*, 1999). From the random income and consumer sample in 1993, an average biotin intake of 45.1 and 40.3 µg/day for male and female participants was calculated for 38,924 participants aged between 4 and more than 65 years. Up to the age of 15 years the average biotin intake of the test persons was above 100% of the reference intakes DGE/ÖGE/SGE/SVE (2000). In the higher age groups it varied between 65 and 170% of the reference range of 30-60 µg biotin/day (DGE, 2000).

Biomarkers: Serum assays are not suitable for the assessment of the biotin **supply status**. The biotin concentrations in the serum of healthy adults are 60 ± 14.9 ng/L (range 34-89 ng/L). In addition, bisnorbiotin (46 ± 33 ng/L; range 5-145) and biotin sulphide (3.7 ± 8 ng/L; range 0-31) were detected as avidin-binding substances (Mock *et al.*, 1997b). Biotin excretion in urine was between 4.4 and 19.3 µg/day plus the same amount of biotin metabolites.

In only 5 out of 10 healthy adults, whose biotin deficiency condition had been triggered by giving them egg albumin over a period of 20 days containing enough avidin to bind more than seven times normal biotin intake, the biotin level was below the normal range in the serum after this period. The biotin and bisnorbiotin excretion in the urine fell significantly, by contrast, from the 3rd day of the test period and was below the lower normal limit after 14 days in 8 out of 10 test persons. The most sensitive parameter was a significant increase in 3-hyroxyisovalerate excretion from the 3rd day. On the 10th day it was above the normal range for all persons. The increase in 3-hydroxy-isovalerate excretion is a consequence of the reduced activity of 3-methycrotonyl-CoA-carboxylase (Mock *et al.*, 1997b). There were no clinical symptoms of biotin deficiency. The biotin depletion effect caused by egg albumin consumption on 3-hydroxy-isovalerate excretion can be further enhanced through a leucine load. A normal diet with or without a biotin supplement (80 µg/day) led within a week to normalisation of excretion (Mock *et al.*, 2002a). Also the determination of the activity of propionyl-CoA-carboxylase activity in blood lymphocytes and the amount of odd-numbered fatty acids in the plasma or erythrocyte lipids are suitable as parameters for biotin status (Mock *et al.*, 2002b).

15.3 Risk characterisation

15.3.1 Hazard characterisation (NOAEL, LOAEL)

Rats, which were given subcutaneous injections of biotin in single or repeat doses of a total of 50 to 100 mg/kg bodyweight, manifested disorders of the oestrus, absorption of foeti and placentae with reduction of uterus weight which showed a lower glycogen and protein level. This could not be confirmed in other studies in rats and mice. Neither SCF nor FNB believe these studies to be suitable for the derivation of a **UL** for man (IOM, 1998; SCF, 2001). For human beings no symptoms of a biotin overdose are described.

15.3.2 Deficiency, possible risk groups

Food-related biotin deficiency is rare. It was described in isolated cases in conjunction with parenteral alimentation, the chronic consumption of raw eggs and biotin-free diets.

The symptoms, which take months to years to develop, are alopecia and seborrhoic dermatitis with a susceptibility to mycotic infection and periorificial localisation. Adults may manifest signs of impairment of the nervous system like depression, lethargy, muscle pain, hyperesthasia and paraesthesia. In infants the symptoms develop more quickly and may be linked with convulsions, muscle hypotonia and retarded development.

A sub-clinical biotin deficiency characterised by low biotin and high 3-hydroxy-isovalerate excretion is observed in patients receiving anticonvulsive treatment (Mock and Dyken, 1997), under chronic haemodialysis (Yatzidis *et al.*, 1984), in alcoholics and in chronic gastrointestinal diseases.

During normal pregnancy biotin excretion in urine falls significantly in 50% of women and 3-hydroxy-isovalerate acid excretion increases although the serum levels of biotin are higher in early pregnancy than in non-pregnant controls (Mock *et al.*, 1997c). Supplementation with 300 µg biotin/day reduces 3-hydroxy-isovalerate excretion (Mock *et al.*, 2002c).

Sub-clinical biotin deficiency has proved to be teratogenic in various animals (cleft palate, micrognathia, microglossia and hypoplasia of the extremities; (chickens, turkeys, rats, mice) (Zempleni and Mock, 2000b; Mock *et al.*, 2003).

Genetic defects of biotinidase and of holocarboxylase synthetase lead to multiple carboxylase deficiency which goes hand in hand with a typical pattern of organic acids in the urine and serum. They manifest a wide spectrum of clinical symptoms (similar to those of food-related manifest biotin deficiency). If untreated it may prove fatal in newborn babies (Burri *et al.*, 1981; Wolf *et al.*, 1983). Recently, a third genetic defect was described with reduced biotin uptake in cells which also leads to multiple carboxylase deficiency with biotin dependency (Mardach *et al.*, 2000).

Aside from patients who receive too little biotin because of special circumstances, the German population is adequately supplied with biotin. Pregnant women may be a possible risk group (supply category 2).

15.3.3 Excessive intake, possible risk groups

Chronic biotin intakes for therapeutic purposes amounting to more than 200 times the reference intake, have not led to any identifiable negative effects.

The biotin intake determined for Germany from food and food supplements is far below these therapeutic doses.

15.4 Tolerable upper intake level for biotin

No systematic studies on biotin tolerance in humans are available. There are many isolated observations of patients with food-related biotin deficiency and biotin deficiency caused by metabolism defects who received high doses of biotin: 10 mg/day orally over 7 weeks in the case of a one-year-old child (Mock *et al.*, 1985), over 2 weeks in the case of five infants and young children (Velázquez *et al.*, 1995), during the 2nd half of pregnancy with one child with multiple carboxylase deficiency (Packman *et al.*, 1982; Roth *et al.*, 1982), between 10 and 100 mg/day over many years in patients with biotinidase and holocarboxylase synthetase deficiency (Zempleni, 2001). In no case was an adverse effect observed which could have been linked to taking biotin. A LOAEL and a NOAEL could not be identified; nor could a UL be derived.

Based on a study in 20 diabetics, some of whom took 9 mg biotin/day over four years without any adverse effects and using an uncertainty factor of ten, the British Expert Group on Vitamins and Minerals (EVM, 2003) has, derived a guidance value of 0.97 mg biotin/day from all sources, which can probably be tolerated without any side effects.

15.4.1 Derivation of a maximum level for biotin in food supplements

Since a tolerable upper intake level for total daily intake of biotin cannot be defined, the proposed formula for the derivation of a maximum level of biotin in food supplements cannot

be used. Up to now, BgVV/BfR recommended that the maximum level of three-fold the estimated value for adequate intake of DGE should not be exceeded in the daily dose of a food supplement. Based on the estimated values for 1991, this led to a maximum level of 300 µg. If one takes the more recent reference intakes (DGE/ÖGE/SGE/SVE, 2000) as the basis, then the maximum level obtained in this way would be 180 µg biotin.

15.4.1.1 Possible management options

a) Continuation of existing practice

The previous maximum level of 300 µg biotin in the daily dose of a food supplement, which was recommended by BgVV in 1998, has been complied with as far as we know by German manufacturers.

Advantages: No negative experience has been reported. There is no need for a change in the assessment.

Disadvantages: With the publication of the new nutrient reference intakes (DGE/ÖGE/SGE/SVE, 2000) with a reference intake of 30-60 µg/day for adults, there is no longer a basis for setting a maximum level of 300 µg.

A maximum level of 300 µg is higher than the prophylactic daily dose of 200 µg recommended for medicinal products.

b) Maximum level which corresponds to the new reference intake

The reference intake for biotin is in the range of 30-60 µg. Given that average dietary biotin intake equals the amount of the reference intake in the German population, it does not seem necessary to supplement foods to a level above the reference intake.

Advantages: A food supplement in the amount of the upper reference intake (60 µg) is sufficient in order to cover requirements even if the diet should be low in biotin.

There are no identifiable disadvantages for health.

Disadvantages: The reduction of the previous maximum level to one-fifth cannot be justified on health grounds.

c) No setting of maximum levels

Since, according to literature reports, even an intake of 200-fold the reference intake was tolerated without adverse effects, there is no need to set a maximum level.

Advantages: There are no identifiable health advantages. No justification for a maximum level is necessary.

Disadvantages: Because of the lack of data about the long-term tolerance of biotin amounts in the milligram range, it cannot be ruled out with any certainty that adverse effects (e.g. in the regulation of the immune system) could occur.

Food supplements could overlap with medicinal products with respect to the biotin level.

With regard to the use of biotin in multivitamin products, an imbalance in the dose of various vitamins seems to be unphysiological.

d) Maximum level which corresponds to the upper tolerable biotin dose

Advantages: There are no identifiable health advantages.

Disadvantages: A NOAEL which could serve as the basis for this maximum level could not be unequivocally identified.

15.4.2 Derivation of a maximum level for biotin in fortified foods

Since a tolerable upper intake level for daily total intake of biotin cannot be defined, the proposed formula for the derivation of a defined maximum level in fortified foods cannot be used.

15.4.2.1 Possible management options

a) Continuation of existing practice

The Working Group Food Chemistry Experts of the *Länder* and BgVV stated in 1998 that when advertising claims a food has a high vitamin content, at least the recommended daily intake of the vitamin should be contained in the daily portion of the food, but under no circumstances more than three times that amount. No data are available about the extent to which manufacturers have complied with this recommendation.

Advantages: There are no known cases of adverse health effects arising from previous fortification practice.

Disadvantages: There are no nutritionally justifiable grounds for this regulation.

b) Maximum level which corresponds to the new reference intake

As a rule, fortified foods are not the sole dietary source. There are special provisions for foods for special medical purposes.

Advantages: A maximum level in the amount of the reference intake per portion of a fortified food is suited to meet the daily biotin requirement. As a rule, the total biotin daily intake of biotin will then exceed the reference intake for biotin.

Disadvantages: No health disadvantages are expected.

c) No setting of a maximum level

Advantages: There are no identifiable disadvantages for health.

Disadvantages: Depending on the level of biotin addition, adverse health effects can no longer be ruled out.

BfR considers the health risk from the use of biotin in food supplements and the addition of biotin to fortified foods to be low. Given the data available, a risk cannot be ruled out with any certainty, however. For reasons of preventative consumer protection, BfR is of the opinion that Option a) should be consistently selected for food supplements (maximum level 180 µg) and Option b) for fortified foods (60 µg).

15.5 Gaps in knowledge

- Identification of biotin requirements through correlation of intake with changes in biomarkers of biotin supply status.
- Investigation into the importance of the effects of high biotin intakes on the immune system.

15.6 References

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16 Risk Assessment of Vitamin B_{12}

16.1 Summary

The calculations available for the Federal Republic of Germany on vitamin B_{12} intake indicate that on average far more of this vitamin is taken up than is deemed necessary to cover requirements (supply category 4). The biochemical studies undertaken to estimate vitamin B_{12} supply did not point to any occurrence of deficiency conditions. According to the estimation of BfR the use of vitamin B_{12} in food supplements or for food fortification constitutes a minor health risk for the consumer (cf. Table 2).

Up to now, no adverse effects have been described which could be attributed to excessive intake of vitamin B_{12} from foods or supplements which means that no tolerable upper intake level (UL) could be derived. This means that a derivation of safe maximum levels mainly based on toxicological considerations is not suitable and the proposed formula cannot be applied.

For reasons of preventive health protection and because of the existing gaps in knowledge, BfR recommends that the admissible maximum level should not exceed 3 maximum 9 µg vitamin B_{12} per day in food supplements. A limiting of the added vitamin amount is justified because of the absorption capacity which is already limited physiologically. Furthermore, a major increase in recommended daily vitamin intake does not offer any additional nutritional-physiological advantages.

In order to prevent the cumulation of high vitamin doses from various products, an appropriate addition of vitamins in the expected daily portion of fortified foods should not exceed one-fold the recommended daily intake (3 µg). Moreover, it makes sense from a nutritional-physiological angle to limit the addition of vitamins to specific groups of foods.

Recommended intake	3 µg/day	
Intake [µg/day] (NFCS, 1994)	**m**	**f**
Median	6.23	4.37
P 2.5	2.64	1.66
P 97.5	19.3	17.1
Tolerable upper intake level	Not defined (SCF) Database not sufficient No known risk in ordinary doses	
Proposal for **maximum levels** in:		
food supplements	3-9 µg/daily dose	
fortified foods	3 µg/daily portion where appropriate, restriction of addition to specific groups of foods	

16.2 Nutrient description

16.2.1 Characterisation and identification

Vitamin B_{12} or cobalamin is the collective term for a series of different substituted corrinoids with biological activity in human beings. The basic construct is formed by the flat corrin ring system, a porphyrin-like compound consisting of four reduced pyrrol rings with a central cobalt atom. Depending on substitution at the sixth ligand of the cobalt atom, various derivatives are possible like aquo-, nitro-, methyl-, adenosyl-, hydroxo- or cyanocobalamin (Bässler *et al.*, 2002; Forth *et al.*, 1987; Harper *et al.*, 1987). Only *cyanocobalamin* (CAS No.

68-19-9, MG 1355,40; the most stable form of cobalamin (Harper *et al.*, 1987)), and *hydroxocobalamin* (CAS No. 13422-51-0; MG 1346,40) play a role for therapeutic or supplementation purposes.

Under food law vitamin B_{12} is not considered to be a food additive in Germany. In the Ordinance on foods for special dietary purposes (DiätVO), cyanocobalamin and hydroxocobalamin are listed in Annex 9 as admissible vitamin compounds (cf. also Directives of the European Commission 2000/15/EC (of 15 February 2001 on substances that may be added for nutritional purposes to foods for particular nutritional uses) and 2002/46/EC (of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements).

16.2.2 Metabolism, function, requirements

Metabolism: Human beings cannot synthesise vitamin B_{12} de novo and are, therefore, dependent on vitamin B_{12} uptake from food (Bässler *et al.*, 2002). Only specific microorganisms are capable of synthesis. Human beings are not adequately capable of using vitamin B_{12} formed through the bacterial flora in the lower part of the intestinal tract (Bässler *et al.*, 2002; Forth *et al.*, 1987).

Specific vitamin B_{12}-binding proteins are needed for transport and storage. The protein-bound vitamin B_{12} taken up from food is initially released with the help of gastric acid and the proteolytic enzymes and then bound to so-called R-proteins or haptocorrins. After cleavage of the haptocorrin cobalamin compound by pancreatic trypsin, it is bound to the intrinsic factor which is formed by the parietal cells of the gastric mucosa. This complex of cobalamin and intrinsic factor is bound by receptors in the brush border membrane of enterocytes in the ileum and taken up endocytotically in the cell. Inside the mucosa cell of the ileum, cobalamin is cleaved by the intrinsic factor and bound to the transport protein transcobalamin II. This complex leaves the cell by means of an exocytotic process. Another cobalamin-binding protein ("transcobalamin I") occurs in the liver which permits the storage of larger amounts of cobalamin. Passage through the mucosa cell of the ileum is slow which means that maximum blood concentrations are not reached until about 8 hours after oral administration (Bässler *et al.*, 2002; Forth *et al.*, 1987; Harper *et al.*, 1987).

Absorption is dose-dependent. At growing intake the utilisation of vitamin B_{12} falls as bonding capacity is exceeded (Bässler *et al.*, 2002; Beck, 2001). Adams *et al.* (1971) reported the following dose-dependent absorption rates: 50% in the case of a single dose of 1 µg, 20% of 5 µg and a rate of only 5% for an amount of 25 µg. A series of studies indicate that the normal absorption capacity of the system is around 1.5-2.0 µg/meal (Scott, 1997). This active uptake mechanism is of special importance for the absorption of physiological amounts of cobalamin. In healthy persons after the administration of >10 µg vitamin B_{12} , a maximum amount of only 1.5-2.0 µg is actively absorbed irrespective of the extent to which the dose is increased (Loew *et al.*, 1999; Schümann *et al.*, 1997). The average bioavailability of vitamin B_{12} in healthy individuals is around 50% from natural foods: the absorption rate of "free, crystalline" cobalamin is thought to be higher and is stated as 60-80% in the case of oral administration of 0.5-2 µg (Baik and Russel, 1999; Beck, 2001).

Independently of the active intrinsic factor-dependent process, vitamin B_{12} can also be taken up by passive diffusion in the small intestine. This mechanism is important for the absorption of pharmacological doses of vitamin B_{12}, but is not very effective since only around 1% of the administered dose is absorbed (Bässler *et al.*, 2002; Forth *et al.*, 1987; Schümann *et al.*, 1997). Every day around 3-8 µg cobalamin is excreted with bile of which around 75% is reabsorbed in healthy individuals via enterohepatic circulation. However, the reabsorption rate can be increased to up to 100% at low intake, falling biliary excretion or dwindling

vitamin reserves (Crews *et al.*, 2001). On average cobalamin losses through faeces are 0.4 µg/day.

Vitamin B_{12} is mainly stored in the liver but also in other body organs and tissues like heart, brain and skeletal muscles. The total body store is around 2-5 mg and guarantees a storage stock for approximately 3-5 years. Because of the efficient enterohepatic circulation, a low turnover rate and high storage stocks, a vitamin B_{12}-free diet can be expected to lead to a deficient supply situation at the earliest after around 5 years (Bässler *et al.*, 2002).

There are indications that depending on the dose, high levels of vitamin C could impair the absorption of vitamin B_{12} (Herbert and Jacob, 1974). In the opinion of the American Food and Nutrition Board (FNB, 1998), however, these are not real interactions but merely artefacts. The interactions between soluble dietary fibre and vitamin B_{12} described in animal experiments, too, could not be confirmed up to now without any doubt (FNB, 1998; NN, 1991). Some *medicinal products* can reduce the absorption of vitamin B_{12}. In conjunction with the proton pump inhibitor, omeprazole, a dose-dependent reduction in cyanocobalamin absorption was observed (Marcuard *et al.*, 1994; Schenk *et al.*, 1999). Long-term treatment with metformin (Galligan, 2002) was also reported to influence the vitamin B_{12} status.

Functions: Vitamin B_{12} is involved in various metabolism reactions in the forms of the coenzymes 5-desoxyadenosyl cobalamin and methyl cobalamin. 5-desoxyadenosyl cobalamin catalyses the isomerisation of methyl malonyl-CoA to succinyl-CoA during the degradation of odd-numbered fatty acids and branched-chain amino acids. Methyl cobalamin is involved in the synthesis of methionine from homocysteine as the methyl group carrier. This reaction is linked to the presence of folic acid. Cobalamin is also involved in the synthesis of purine and pyrimidine bases, nucleic acids and proteins (Bässler *et al.*, 2002; Forth *et al.*, 1987).

Requirements: Subject to the precondition of full bioavailability, an amount of less than 1 µg/day can cover the minimum requirements of human beings. DGE recommends a regular daily uptake of **3 µg** vitamin B_{12} from food for children aged 13 upwards and for adolescents and adults (DGE/ÖGE/SGE/SVE, 2000). The following overview gives a summary of the DGE recommendations 2000 (DGE/ÖGE/SGE/SVE, 2000) and the population reference intakes (PRI) of the Scientific Committee on Food (SCF, 1992):

Table 21: Recommended intakes and population reference intakes for vitamin B_{12}

Age (years)	Population Reference Intakes (PRI; µg/day) (SCF, 1992)	DGE recommendation for vitamin B_{12} intake (µg/day) (DGE/ÖGE/SGE/SVE, 2000)
Children		
1 up to under 4 years	0.7	1.0
4 up to under 7 years	0.9	1.5
7 up to under 10 years	1.0	1.8
10 up to under 13 years	1.3	2.0
13 up to under 15 years	1.4	3.0
Adolescents and adults		
15 up to age 65 and over	1.4	3.0
Pregnant women	1.6	3.5
Lactating women	1.9	4.0

16.2.3 Exposure (dietary and other sources, nutritional status)

Sources: A purely vegan diet contains almost no vitamin B_{12}. Vitamin B_{12} is found in significant amounts in foods of animal origin only. The main sources include liver, red muscle meat, fish, eggs and dairy products. Vitamin B_{12} is normally found in these products in a

protein compound mainly in the form of methyl, adenosyl and hydroxocobalamin (Bässler *et al.*, 2002; Farquharson and Adams, 1976; Harper *et al.*, 1987).

In the monograph of the BGA Institute for Medicinal Products from 1989, prophylactic daily doses in the range of **1-10 µg** for substitution in the case of bad dietary habits, malnutrition or to cover requirements are described as adequate (BGA, 1989). Furthermore, there are medicinal products with levels of cyanocobalamin of 1-300 µg/day for oral application (BPI, 2003).

Nutritional status:
Dietary intake: According to the re-evaluation of the *National Food Consumption Survey (NFCS)* (DGE, 1996) the average daily vitamin B_{12} intake in the Federal Republic is 6.6 µg for men and 5.3 µg for women. In all age groups average intake was far higher than the reference intakes and fluctuated between 156-254% of the DGE recommendations. The highest intake values, measured against the 97.5 percentile, were found amongst 51-64-year-old men (VERA-Schriftenreihe, 1995). The nutrition survey carried out by way of supplement to the *Federal Health Survey* in 1998 (Mensink *et al.*, 1999) reported good vitamin B_{12} supply, too. In comparison with the DGE recommendations a median for vitamin B_{12} was determined of around 245% for men and of 155% for women. The 25 and 75 percentiles were around 175% and 320% for men and 120 and 210% for women. In the case of the *EPIC Study* ("European Investigation into Cancer and Nutrition" (Schulze *et al.*, 2001)) carried out in Heidelberg and Potsdam between 1996 and 1998, the average vitamin B_{12} intake of men was 7-8 µg/day and of women around 4 µg/day. The 90 percentile for men and women was 13 and 9 µg/day respectively.

No reliable information is available about the proportion or level of vitamin B_{12} intake from fortified foods and food supplements.

Vitamin B_{12} plasma concentrations: In the VERA Study plasma vitamin B_{12} concentrations were determined by using a radioimmunology method in a representative random sample of over 18-year-olds (VERA-Schriftenreihe, 1992). In this case a reference value of 136 pmol/L was used to assess vitamin B_{12} status. In the overall random sample a mean value of 302 pmol/L and a median of 275 pmol/L were determined. The concentrations were below the reference value only in the case of 4.3% of the total random sample. By way of comparison, a higher prevalence of low vitamin values was determined in the age group of over-65-year-old men. All the same, a normal vitamin B_{12} plasma level does not rule out depleted tissue stores.

The calculations available for the Federal Republic on vitamin B_{12} intake indicate that on average far more is ingested than is deemed necessary to meet requirements (supply category 4). The biochemical studies undertaken to estimate vitamin B_{12} supply do not provide any evidence of deficiency conditions.

16.3 Risk characterisation

16.3.1 Hazard characterisation (LOAEL, NOAEL)

There are no reports of adverse effects in conjunction with excess vitamin B_{12} intake (BGA, 1989; Forth *et al.*, 1987). The American Food and Nutrition Board (FNB, 1998) and the Scientific Committee on Food of the European Commission (SCF, 2000) also indicated that up to now no adverse effects have been reported that could be attributed to increased vitamin B_{12} uptake from foods or supplements. Hence, it was not possible for either body to establish a **LOAEL** (Lowest observed adverse effect level) or a **NOAEL** (No observed adverse effect level) which could be used as the basis for deriving a **tolerable upper intake level (UL**). However, there are individual case reports about side effects (Braun-Falco and

Lincke, 1976; James and Warin, 1971; Pevny *et al.*, 1977), which were not, however, considered suitable for the derivation of a LOAEL. DGE (DGE/ÖGE/SGE/SVE, 2000) indicated that even in the case of very high vitamin B_{12} intake (pharmacological doses of up to 5 mg) no side effects had been observed.

16.3.2 Deficiency, possible risk groups

16.3.2.1 Deficiency

There are various possible causes of vitamin B_{12} hypovitaminosis, for instance (Bächli and Fehr, 1999; Carmel, 2000; Herold, 1987):

- Inadequate vitamin B_{12} intake, long-term bad dietary habits/malnutrition (e.g. vegans);
- Reduced ability to absorb protein-bound cobalamin/impaired release of cobalamin from food, "food-cobalamin malabsorption" (e.g. in the case of hypochlorhydria, use of acid-suppressive drugs, gastritis/helicobacter pylori infection, pancreatic insufficiency);
- Lack of the intrinsic factor (e.g. in the case of pernicious anaemia or after gastrectomy);
- Inadequate absorption (e.g. after ileum resection, in the case of diseases like Crohn's);
- Increased consumption (in the case of bacterial overgrowth or diphyllobothriasis);
- Other malabsorption conditions (e.g. HIV infection, multiple sclerosis);
- Inborn errors of B12 transport and metabolism (like e.g. transcobalamin deficiency, Imerslund-Gräsbeck Syndrome).

The most frequent causes are "*food-cobalamin malabsorption*" and *pernicious anaemia*. In the case of "food-cobalamin malabsorption" this disorder only affects the uptake of the vitamin bound to food proteins; the absorption of free, crystalline vitamin, by contrast, is not impaired. Pernicious anaemia, the prevalence of which is estimated to be around 3% in the white population, is an autoimmune disease triggered by auto-antibodies to parietal cells and the intrinsic factor. This form impedes absorption of both the bound and the free vitamin (Carmel, 2000; Stopek, 2000).

The typical symptoms of manifest vitamin B_{12} deficiency include:

(1) *haematological* (e.g. ineffective erythropoesis with macrocytic anaemia and megaloblasts in the peripheral blood smear);

(2) *neurological/psychiatric* (e.g. funicular myelosis with paraesthesis and polyneuropathy, memory disorders, apathy, depression etc.);

(3) *gastrointestinal disorders* (e.g. glossopyrosis, loss of appetite, constipation).

In terms of differential diagnosis it should be borne in mind that the haematological changes in the case of a folic acid deficiency can scarcely be distinguished from those caused by a vitamin B_{12} deficiency ("masking" of a vitamin B_{12} deficiency).

16.3.2.2 Possible risk groups for deficiency

Most forms of vitamin B_{12} deficiency occur in conjunction with congenital or acquired diseases. Cases of a purely diet driven vitamin B_{12} deficiency are rare. The possible risk groups for insufficient dietary intake under discussion are indicated below.

- Since vitamin B_{12} does not occur in foods of plant origin, a possible risk group are people who have followed a *vegan or strictly vegetarian* diet over a longer period (Miller *et al.*, 1991).

- Various authors report a growing prevalence of lower cobalamin values in the serum in *older people* (Bächli and Fehr, 1999; Baik and Russel, 1999; Carmel, 1997; FNB, 1998). According to Baik and Russell (1999), 10-15% of the over 60s seem to suffer from a vitamin B_{12} deficiency.

  The calculations available for the Federal Republic indicate adequate intake in all age groups. Nevertheless, the VERA Study reported a higher prevalence of low vitamin values measured in men over 65 (VERA-Schriftenreihe, 1992). Since the vitamin B_{12} intake of these individuals is, however, comparable with that of other age groups (DGE, 1996), these results cannot be attributed to inadequate vitamin B_{12} intake. Similar results were provided by a study from the Netherlands involving people aged between 74 and 80 (Van Asselt *et al.*, 1998). Despite adequate intake above the recommended vitamin B_{12} intake, the clinical-chemical parameters in 23.8% of cases pointed to a "mild cobalamin deficiency" (plasma cobalamin <260 pmol/L).

  The observations are not interpreted as "natural, age-related physiological changes". Possible causes are a decline in gastric acid production and the presence of atrophic gastritis and/or the presence of a helicobacter infection leading to reduced absorption of the protein-bound cobalamin which occurs naturally in foods (Bächli and Fehr, 1999; Baik and Russel, 1999; Carmel, 1997; FNB, 1998).

  According to DGE (DGE/ÖGE/SGE/SVE, 2000) around 30% of the over 65s develop atrophic gastritis. It, therefore, recommends that older people with atrophic gastritis should have an additional vitamin B_{12} intake in the form of supplements. Other authors attribute low serum cobalamin levels in older patients to around 43% to a "*food cobalamin malabsorption*" (Stopek, 2000).

  From these findings it can be concluded that a threshold supply situation in older people can also be attributed to restricted absorption in conjunction with an underlying *disease coupled with adequate intake*. This would mean that "age" alone or "older people" per se could not be classified as fundamental risk groups. Based on their own studies, some experts do not however rule out that a cobalamin deficiency could possibly be more prevalent amongst older people in particular - contrary to the expectations from the intake survey.
- Some authors fear that growing *fortification with folic acid* could lead to a higher risk of "masking" of an, as yet, undiscovered vitamin B_{12} deficiency (Baik and Russel, 1999; Rasmussen *et al.*, 2001).

16.3.3 Excessive intake, possible risk groups

16.3.3.1 Excessive intake

Vitamin B_{12} has a low toxicity. There are no known cases of intoxications or overdose symptoms (BGA, 1989; Forth *et al.*, 1987). SCF (2000) reports that patients who were given high dose oral vitamin B_{12} therapy (up to 1000 µg/day) over a longer period did not develop any side effects.

16.3.3.2 Possible risk groups in conjunction with the growing use of cyanocobalamin

Normally cyanocobalamin is not detectable in plasma but can be detected in the case of higher cyanide exposure (smoking, specific plant components). The current data situation does not permit any assessment of the risk from the oral administration of cyanocobalamin.

16.4 Tolerable upper intake level for vitamin B_{12}

Up to now, no adverse effects have been reported in conjunction with excessive vitamin B_{12} intake. The Nordic Council (2001) has merely derived a **UL (upper intake level)**[7] of **100 µg**. By contrast, other bodies like SCF[8] (2000), FNB[9] (1998) and EVM[10] (FSA, 2003) were of the opinion that the data situation did not suffice for the derivation of a UL.

This then rules out the derivation of safe maximum levels mainly based on toxicological considerations. For these reasons it makes sense to orientate them towards known (nutritional) physiological aspects:

Reference values for recommended vitamin B_{12} intake: Values of this kind are available for vitamin B_{12}. The reference intakes (PRI, recommendation) defined for intake meet, in terms of definition, the requirements of almost all persons in a defined group of a healthy population. They take into account the current level of knowledge about a balanced diet and the prevention of food-related diseases. They should protect almost all people in a respective group from food-related health damage. Furthermore, they can create a certain body reserve. A daily nutrient intake on the recommended level means inadequate supply is very unlikely. With the diet common in central Europe, vitamin B_{12} levels are reached which are far higher than daily requirements (DGE/ÖGE/SGE/SVE, 2000).

Specificities of the vitamin B_{12} metabolism: Given the high storage stocks and the low turnover rate, a deficient supply situation is only to be expected at the earliest after around 5 years even in the case of a vitamin B_{12}-free diet (Bässler *et al.*, 2002). The higher the added single dose, the lower the bioavailability of vitamin B_{12}. The amount which can be absorbed by a healthy individual is already limited in physiological terms and cannot be increased at will; i.e. even after administration of >10 µg vitamin B_{12} a maximum amount of 2 µg is actively absorbed. This means that exceeding this amount cannot be expected to bring any additional benefits for the healthy population.

Substitution – safeguarding the covering of requirements: Prophylactic daily doses in the 1-10 µg range are considered adequate to compensate for bad dietary habits and to cover requirements (BGA, 1989). This recommendation correlates with the "physiological" dose range for vitamin B_{12}, which according to Loew et. al., (1999) is maximum 10 µg.

16.4.1 Derivation of a maximum level for vitamin B_{12} in food supplements

Up to now, BfR was of the opinion that food supplements should not contain more than three-fold the daily intake for vitamin B_{12} recommended by DGE and proposed a maximum level of **9 µg** (BgVV, 1998). Taking into account the current data situation and the above comment, there is no need to deviate from this proposal. It is indeed the case that up to now no risks have been reported in conjunction with the oral administration of even larger amounts of vitamin B_{12}. However, in healthy individuals there are no signs of benefits either.

It has already been pointed out that various bodies like SCF (2000) and FNB (1998) were not able to derive a UL because of the inadequate data situation. The Nordic Council (2001) did indeed set a UL of 100 µg; however it is not known on what this value is based.

[7] UL = Upper intake level = the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population.

[8] UL = Tolerable upper intake level = the maximum level of total chronic daily intake of a nutrient (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans.

[9] UL = Tolerable upper intake level = the highest level of a daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals.

[10] UL = Safer upper level = represents an intake that can be consumed daily over lifetime without significant risk to health.

Consequently, the formula which was used for other micronutrients in our report to calculate maximum levels for vitamin B_{12} cannot be used here.

16.4.1.1 Possible management options

Bearing in mind the current data situation and the above comments, the following management options are proposed:

a) Continuation of existing practice with an upper level of maximum 9 µg vitamin B_{12} in food supplements per daily dose

   Advantages: Under the existing practice we do not know of any side effects. The value is oriented towards nutritional-physiological requirements and is based on "three-fold" the DGE recommendations (DGE/ÖGE/SGE/SVE, 2000).

   Disadvantages: Given the current supply situation in Germany, no disadvantages would be expected in terms of preventive consumer protection.

b) Limiting the maximum level to one-fold the reference intakes with an upper level of maximum 3 µg vitamin B_{12} in food supplements per daily dose

   Advantages: This level is oriented towards nutritional-physiological needs in line with the DGE recommendations (DGE/ÖGE/SGE/SVE, 2000). No side effects or risks are to be expected from this maximum level.

   Disadvantages: Bearing in mind the current supply situation in Germany, no disadvantages would be expected in respect of preventive consumer protection.

c) No restriction on the admissible maximum level in food supplements with minimum levels >9 µg per daily dose

   Advantages: There are no known advantages.

   Disadvantages: There are no signs of a benefit in healthy individuals. Since absorption capacity is already limited physiologically, it cannot be increased in an arbitrary manner through higher doses either. The inadequate data situation, which prevented SCF and other bodies from setting a **UL**, does not mean that higher levels might not be linked to a health risk. This option would also violate the precautionary principle.

16.4.2 Derivation of a maximum level for vitamin B_{12} in fortified foods

In line with the comments of the Working Group Food Chemistry Experts of the federal states and BgVV (ALS, 1988; 1998), appropriate vitamin addition to the recommended daily portion should not exceed three-fold the recommended vitamin intake (corresponding to **9 µg**). Bearing in mind the above comments and in order to avoid the cumulation of high vitamin doses from various products, BfR believes it is now reasonable to limit an appropriate vitamin fortification in the expected daily portion to one-fold the recommended daily intake (**3 µg**). Limiting the amount of vitamin added is justified because of the absorption capacity which is already limited physiologically.

Furthermore, it seems to make sense on nutritional-physiological grounds to limit vitamin B_{12} supplementation to certain food groups. All the same, it would have to be required that foods considered for fortification are indeed consumed in a significant manner by the individuals who have been identified as possible risk groups in order to contribute to covering their

nutrient requirements. Consequently, the fortification of some foods of plant origin with vitamin B_{12} could make sense for those individuals who follow a strict vegetarian diet. In order to allay the fear that the growing fortification of foods with folic acid for the purposes of preventing neural tube defects could encourage a "masked" vitamin B_{12} deficiency, it seems advantageous to accept the addition of vitamin B_{12} for those products which are also fortified with folic acid. Fortification of foods of animal origin, which already make a significant contribution in a natural manner to vitamin B_{12} supply, should in principle be ruled out.

16.4.2.1 Possible management options

Bearing in mind these comments, the following management options are proposed:

a) Continuation of existing practice with an upper level of maximum 9 µg vitamin B_{12} per recommended daily dose

Advantages: We are not aware of any side effects in conjunction with existing practice. The value is oriented towards nutritional-physiological requirements and is based on "three-fold" the DGE recommendations (DGE/ÖGE/SGE/SVE, 2000).

Disadvantages: As a rule fortified foods are consumed in an uncontrolled manner without any fixed daily portions which means that, depending on choice of food and eating habits, a cumulation of high vitamin levels cannot be ruled out and vitamin B_{12} intake could reach an order of magnitude at which the absorption capacity has already been exhausted. No "additional" nutritional benefit would be expected.

b) Restricting the maximum level to one-fold the reference intakes with an upper level of maximum 3 µg vitamin B_{12} per recommended daily dose

Advantages: This level is oriented towards nutritional-physiological requirements in accordance with the DGE recommendations (DGE/ÖGE/SGE/SVE, 2000). No side effects or risks are to be expected from this maximum level. The risk of a cumulation of high vitamin doses from various products is lower than in Option a).

Disadvantages: There are no identifiable disadvantages.

c) Restricting fortification to specific groups of foods:

- No fortification of foods of animal origin, which already make a significant contribution in a natural way to vitamin B_{12} supply
- Limiting fortification to certain foods of plant origin which are eaten by possible risk groups (e.g. vegans) and could make a significant contribution to vitamin B_{12} supply
- Vitamin B_{12} "co-fortification" with foods fortified with folic acid

Advantages: Since the supply situation of the German population can largely be considered to be secured, there is not initially any need for fortification for nutritional-physiological reasons. Uncontrolled fortification, which could be linked to the risk of an imbalance, could be further prevented. Possible risk groups for vitamin B_{12} deficiency (e.g. vegans) could be reached in a more efficient manner through targeted fortification. The coupling of vitamin B_{12} fortification to existing folic acid fortification makes sense in the context of the risk of a "masked" vitamin B_{12} deficiency from the nutritional-physiological angle. Bearing in mind the current supply situation in Germany, no risks would be expected in conjunction with preventive consumer health protection.

Disadvantages: There are no identifiable disadvantages; all the same, further special provisions would be necessary.

According to BfR there is a low risk of adverse effects in conjunction with the use of vitamin B_{12} in food supplements and for food fortification.

Up to now, there are no known cases of adverse effects from excessive vitamin B_{12} which means that a derivation of maximum safe levels (UL) mainly based on toxicological considerations is not appropriate. Therefore, the derivation of maximum levels for vitamin B_{12} in foods should mainly be undertaken on the basis of known (nutritional) physiological aspects.

Of the above-mentioned management options, Options a) and b) (3-9 µg/daily dose) are recommended for food supplements and Option b) (3 µg/recommended daily dose) in combination with Option c) (if necessary restricting fortification to specific groups of food) is recommended for fortified foods.

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17 Risk Assessment of Vitamin C

17.1 Summary

The surveys available for Germany on vitamin C uptake indicate that the large majority of the population reaches an intake level needed to cover requirements. The plasma studies conducted confirm that more than 75% of the population has a vitamin C plasma level which reaches the kidney's reabsorption limit. The studies did not reveal any signs of deficiency conditions or inadequate vitamin C intake (supply category 3/4). The target parameter for recommended intake is a preventive plasma level of 50 µmol/L and saturation of immunocompetent cells. Both parameters can be achieved with a total daily intake of 100 mg vitamin C. The uptake of vitamin C is linked to a moderate health risk because of faecal and renal excretion of any excess (cf. Table 2). The Scientific Panel on Dietetic Products, Nutrition and Allergies of EFSA was not able to derive a **UL** because of the shortage of data about very high doses of vitamin C. Nevertheless, it believes that total daily intake of 1 g vitamin C is safe. According to BfR, growing use of vitamin C in food supplements or for the purposes of food fortification could lead, in an unknown percentage of the population with increased oxalate excretion, to elevated excretion which could increase the risk of the development of kidney stones.

Against this backdrop BfR recommends the maximum level of 225 mg vitamin C per daily portion in food supplements.

Since vitamin intake in excess of requirements does not offer any additional nutritional-physiological advantages, it is advised that only one-fold amount the recommended intake, i.e. 100 mg, be used in the expected daily portion of a food for the fortification of conventional foods with vitamin C.

Recommended intake	100 mg/day	
Intake [mg/day] (NFCS, 1994)	**m**	**f**
Median	75.5	86.1
P 2.5	20.4	20.3
P 97.5	270	282
Tolerable upper intake level	Not defined (EFSA) Database not sufficient	
Proposal for **maximum levels** in: food supplements	225 mg/daily dose	
fortified foods	100 mg/daily portion	

17.2 Nutrient description

17.2.1 Characterisation and identification

Vitamin C is the water soluble γ-lacton of 2-keto-L-gulonic acid, L-ascorbic acid (CAS No. 50-81-7) or its anion L-ascorbate. Because of its endiol grouping, L-ascorbic acid is a strong reduction agent which is oxidised enzymatically under release of hydrogen to dehydro-L-ascorbic acid (DHA). The reversibility of this redox reaction is responsible for the antioxidative property of vitamin C. Ascorbic acid reacts through disassociation of the two enolic hydroxyl groups as a diprotic acid which forms salts. Sodium, calcium and magnesium salts are the most important. Crystalline ascorbic acid is stable. When dissolved it is sensitive, however, to light and oxygen. In an alkaline milieu and in the presence of transition metal traces (in particular Cu), ascorbic acid is destroyed oxidatively. The isomers D-

ascorbic acid, L- and D-isoascorbic acid are biochemically inactive (Jakubke and Jeschkeit, 1975).

In Germany the following additive substances are in general authorised for the vitamin supplementation of foods (Ordinance on vitaminised foods, Ordinance on foods for special dietary uses):

- Sodium-L-ascorbate
- Potassium-L-ascorbate and calcium-L-ascorbate
- 6-palmitoyl-L-ascorbic acid

L-ascorbic acid is also listed in Annex 9 of the Ordinance on foods for special dietary uses (DiätVO).

The same compounds are also mentioned in *Directive 2000/15/EC of the Commission of 15 February 2001 on substances that may be added for specific nutritional purpose in foods for particular nutritional uses* and Directive 2002/46/EC on food supplements and are also authorised as antioxidation agents for technological purposes (see references, Additives Marketing Ordinance – ZVerkV).

17.2.2 Metabolism, function, requirements

Metabolism: Ascorbic acid is produced in mammalian metabolism via the glucuronate pathway from glucose. Human beings, anthropoid apes and guinea pigs are defect mutants for the gene of the enzyme L-gluconolactone-oxidase and cannot, therefore, synthesise vitamin C themselves. They are dependent on exogenous vitamin C intake from food. Low doses of L-ascorbic acid are actively absorbed in the human duodenum and in the proximal jejunum by the transport proteins SCVT1 and SCVT2 (MacDonald *et al.*, 2002) . When high doses are administered, there is also passive uptake by diffusion. The oxidised form dehydro-ascorbic acid, which can be reversibly reduced in the metabolism by glutathione, is passively absorbed (Bässler *et al.*, 2002). The active transport of L-ascorbic acid is sodium-dependent and follows a saturation kinetic. Overall the absorption rate of vitamin C falls as individual doses increase since the small intestine cells reduce the expression of the vitamin C receptor in the presence of high concentrations of vitamin C (MacDonald *et al.*, 2002). At a dose of 180 mg/day, 80-90% of vitamin C is absorbed, at a dose of 1 g/day around 65-75% and at 12 g only 16% (BPI, 2000). The non-absorbed portion is degraded by the colon flora partly into organic acids and CO_2. At levels of 25-75 mg per meal, vitamin C promotes the absorption of iron in the small intestine.

Human beings do not have any special stores for vitamin C. The total body pool at full saturation is 1.5 to maximum 3 g. The pituitary gland, brain, adrenal cortex, leukocytes, crystalline lens, liver, spleen, stomach and pancreas are particularly rich in vitamin C. In the leukocytes vitamin C is mainly localised in the cell plasma. The blood plasma level of vitamin C fluctuates between 0.8-1.4 mg/dl, whereby ascorbic acid is present to around 24% bound to the protein. The turnover is around 60 mg/day depending on pool size and daily intake. It is influenced by stress, smoking and chronic disease. Depending on intake the biological half-life varies between 10 and 30 days. The reduction of the total body pool to levels below 300 mg leads to deficiency symptoms. Scurvy is well known as a classical vitamin C deficiency disease (Bässler *et al.*, 2002).

In human beings L-ascorbic acid is either degraded reversibly into dehydro-ascorbic acid or into oxalic acid (around 40%), L-threonic acid, L-xylosis and ascorbic acid-2-sulphate and eliminated renally. Vitamin C is excreted in urine when the total body pool considerably exceeds 1500 mg or the plasma concentration considerably exceeds the reabsorption capacity of the kidneys (kidney threshold) which is around 1 mg/dl. Below that concentration

vitamin C is completely reabsorbed in the proximal tubule. In the event of a deficiency the tubular reabsorption rate increases which means there is a certain adjustment to the body state. Normally, around 3% of the orally taken vitamin C is excreted in faeces, either unchanged or as metabolites. In the event of higher doses a large proportion is eliminated faecally in an unchanged manner (Bässler *et al.*, 2002; Blanchard *et al.*, 1997; Blanchard, 1991).

Function: Because of its redox potential vitamin C is involved as a cofactor in numerous enzyme reactions (collagen formation, catecholamine synthesis, hydroxylation of steroids etc.). In a series of hydroxylation reactions, which use vitamin C as a non-specific co-factor, it may be replaced by other reduction agents. As a radical scavenger it is involved in the detoxication of oxygen radicals and in the detoxication of zenobiotics through P450 enzymes. As a reduction agent, vitamin C is also involved in monooxygenase and dioxygenase reactions. In this way it influences the synthesis of various regulated peptide hormones (bombesin, calcitonin, cholecystokinin, GRF, TRH, melanotropin, ocytocin, vasopressin etc.) and of other degradation processes. The formation of hepatotoxic and carcinogenic nitrosamines from dietary nitrite and secondary amines is inhibited by vitamin C (Bässler *et al.*, 2002). Ascorbic acid improves the absorption of non-haem irons by reducing Fe^{3+} to Fe^{2+}. The antioxidative functions of vitamin C have a close biochemical interaction with those of vitamins E and A and of carotinoids (BPI, 2000). The antioxidative properties of vitamin C play a major role both in cellular as well as in humoral immune defence (Bässler *et al.*, 2002).

Interactions: The interactions between vitamin C and transition metals like iron, copper and also zinc have still to be clarified. There are numerous publications on this which address, in most cases, interactions between vitamin C and iron and copper. Vitamin C also interacts with vitamin E. Brown *et al.* (1997) examined the effects of high vitamin E doses on plasma ascorbate levels and peroxidation susceptibility of erythrocytes: in 50 non-smokers who were given vitamin E doses of 560 and 1050 mg/day the plasma ascorbate level fell after 20 weeks by 33 and 40%. At the same time, the peroxidation susceptibility of their erythrocytes rose by 42% (Brown *et al.*, 1997).

Requirements: In healthy adults the metabolic vitamin C losses are between 5 and 45 mg/day (Jacob and Sotoudeh, 2002). Bearing in mind preventive physiological effects, the Deutsche Gesellschaft für Ernährung (DGE/ÖGE/SGE/SVE, 2000) recommends total daily vitamin C intake of 100 mg for adults. Depending on their age, the recommendations for children are between 50-100 mg daily. In the case of a saturated vitamin C pool, higher vitamin C intakes lead to downregulation of the vitamin C transporter in the small intestine cells and, what's more, to increased renal excretion. A daily intake of 10 mg ascorbic acid already suffices to avoid vitamin C deficiency syndromes (scurvy). The importance of vitamin C as a protective antioxidant in conjunction with cell-mediated immune defence as well as epidemiological studies on reducing disease risks, which result from a sub-optimum antioxidant status, have led in recent years to an increase in recommended intakes to 100 mg/day. At an intake of 100 mg vitamin C per day saturation concentrations are reached in the neutrophiles, monocytes and lymphocytes. Complete saturation of the blood plasma is reached at a daily intake of 1000 mg vitamin C per day (Levine *et al.*, 1999). The desirable plasma levels of 50 µmol/L are reached and the immune cells are saturated with a daily intake of 100 mg vitamin C (DGE/ÖGE/SGE/SVE, 2000). The recommended intake for infants is calculated from the vitamin C content in human milk, which is 6.5 mg/100 g. The following table gives the D-A-CH reference intakes (DGE/ÖGE/SGE/SVE, 2000) and the population reference intakes (PRI) of the Scientific Committee on Food (SCF) (SCF, 1993).

Table 22: Recommended intakes and population reference intakes for vitamin C

Age (years)	Population Reference Intakes (PRI) (SCF, 1993) (mg/day)	Reference intakes (DGE/ÖGE/SGE/SVE, 2000) (mg/day)
Infants		
0 up to 12 months	20	50-55
Children		
1 up to under 4 years	25	60
4 up to under 7 years	25	70
7 up to under 10 years	30	80
10 up to under 13 years	35	90
13 up to under 15 years	40	100
Adolescents and adults	45	100
Pregnant women	55	110
Lactating women	70	150

17.2.3 Exposure (dietary and other sources, nutritional status)

Sources: The main natural sources of vitamin C are fresh fruit and vegetables, in particular peppers, citric fruits, berries and potatoes. Depending on storage and processing conditions, there are losses during industrial and kitchen preparation. The use of vitamin C as an antioxidant additive in drinks and the additional supplementation of fruit juices, nectars and lemonades are other important sources of vitamin C (Bässler *et al.*, 2002).

From the Summary of Product Characteristics (SPC) for medicinal products, it can be seen that vitamin C-containing medicinal products are on sale with doses between 500-1000 mg/day for oral administration (BPI, 2000). In the BGA monograph for vitamin C (BGA, 1992) doses of 225-1000 mg/day are indicated for the treatment of acute deficiency conditions and of 50-225 mg/day for prophylaxis.

Nutritional status:
Dietary intake: The median and the 2.5.-97.5 percentiles of daily vitamin C intake are 75.5 (20.4-270) and 86.1 (20.3-282) mg respectively for men (n=854) and women (n=1134) in the VERA Study (1985-1988) (Heseker *et al.*, 1992). The average daily intake of vitamin C, using the estimated food consumption data for 1993, was 108.1 mg for men and 105.7 mg for women (DGE, 2000). According to the data from the Nutrition Survey conducted within the framework of the Federal Health Survey 1998, vitamin C intake is far higher amongst male and female takers of supplement than amongst non-takers. The median and interquartile range (25-75 percentile) of vitamin C intake per day was 129.4 (91.0-180.3) and 130.8 (95.9-175.9) mg for male and female non-takers compared with 178.2 (128.2-308.5) and 177.8 (125.0-285.4) mg for regular takers of supplements. If one adds intake from supplements in the case of regular supplement takers, this reduces the percentage which still is below the reference intakes for men to 13.2% and for women to 13.2% too. In comparison the proportion of people (as a percentage) whose daily vitamin C intake was below the DGE reference, was 32.7 for male and 28.7 for female non-takers (Mensink *et al.*, 2002).

Vitamin C plasma level: Plasma concentrations below and up to 0.2 mg/dl (approx. 10 µmol/L) have been linked to the onset of the vitamin C deficiency disease, scurvy (BGA, 1992) and plasma levels above 0.8 mg/dl (45 µmol/L) have been described as normal for healthy people (Bässler *et al.*, 2002). In the VERA Study the vitamin C plasma level was photometrically recorded in a representative random sample of over 18 year-olds (Heseker *et al.*, 1992). In the overall random sample a mean value was measured of 76 µmol/L (approx. 1.5 mg/dl) and a median of 76.1 µmol/L. The measured values of the 2.5 and 97.5 percentiles were 25.6 (0.45 mg/dl) and 121 µmol/L (2.3 mg/dl). Only 4% of the examined persons had plasma values below 30 µmol/L (<0.53 mg/dl). None of the measured values was in the scurvy-suspicious range of 10 µmol/L (0.17 mg/dl). The fact that only 6.7% of the

examined persons had a measured value below the reference intake of 36.8 µmol/L (approx. 0.65 mg/dl) used in the study also testifies to the good vitamin C supply situation within the Federal German population.

17.3 Risk characterisation

17.3.1 Hazard characterisation (LOAEL, NOAEL)

Because of the faecal and renal excretion of excess amounts, ascorbic acid is a vitamin with very low toxicity. Even the consumption of high doses only displays a low side effect potential (Johnston, 1999). After single doses of more than 3 g, short-lived osmotic diarrhoea, accompanied by corresponding abdominal symptoms, occurs because of microbial degradation in the intestines (almost always at doses of 10 g) (Bässler *et al.*, 2002; Cameron and Campbell, 1974; Wandzilak *et al.*, 1994). A mean dose of 3 g/day was identified as the **LOAEL** (Lowest observed adverse effect level) based on a few studies with a few test persons. The **NOAEL** (No observed adverse effect level) was set at a portion of 2 g/day (FNB, 2000).

Hazard potential results from elevated oxalic acid excretions (from 1000 mg/day for healthy people and from 500 mg/day for people with a predisposition to kidney stones). This includes patients with rare primary hyperoxaluria, patients with chronic intestinal diseases like Crohn's disease and patients with extensive intestinal rechapter who may develop secondary hyperoxaluria. There are contradictory statements about the dose-response relationship (Levine *et al.*, 1996; Urivetsky *et al.*, 1992; Wandzilak *et al.*, 1994). In the depletion-repletion studies by Levine *et al.* (1996) with vitamin C in seven healthy volunteers, pharmacokinetic data were collected on the bioavailability and excretion of urate and oxalate following different oral and intravenous doses. 100% bioavailability was found from a single dose of 200 mg upwards, which fell to below 50% following a single dose of 1250 mg. At a daily oral vitamin C dose of 1000 mg, urate excretion was between 700 and 1100 mg/24 hours. Patients with secondary gout developed kidney stones in 50% of cases when urate excretion was higher than 1100 mg/24 hours. At a daily dose of under 1000 mg vitamin C, oxalate excretion was 30 to 50 mg/24 hours. Compared with the low doses the 1000 mg dose led to a significant increase both in urate and oxalate excretion. However, these levels are still at the upper level of the norm (Levine *et al.*, 1996). Although the high vitamin C doses were well tolerated clinically by all test persons without any side effects (no diarrhoea), sensitive individuals with hyperoxaluria may face a higher risk of the development of kidney stones. That's why the same authors advocate in a review that the upper level for vitamin C should be 1000 mg (Levine *et al.*, 1999).

In the prospective cohort studies of the Harvard School of Public Health involving 45,251 men and 85,557 women (Physician Health Study and Nurse Health Study) with no previous history of kidney stones, no increased risk was identified at a daily intake of 1500 mg and more vitamin C compared to those with a low intake (<250 mg). According to this, no elevated risk of kidney stones is to be expected in conjunction with high vitamin C intake by the healthy population (Curhan *et al.*, 1996; 1999). Gerster (1997) came to the same conclusion in an overview of several clinical intervention studies and prospective studies including the NHS/PHS studies of the Harvard School of Public Health (Gerster, 1997).

Besides antioxidative effects, prooxidative effects of vitamin C were also identified by Podmore *et al.* (1998b) in a human study with 30 test persons from a doses of 500 mg/day upwards. We do not know to what extent these effects are of relevance for health. In an aqueous solution vitamin C, in combination with lipid hydroperoxides which are formed, for instance, during the action of free radicals on unsaturated fatty acids, led to electrophilic compounds which are considered to be genotoxic (Lee *et al.*, 2001). However, no statements

can be made on whether the observed reaction is possible in the human body and what conditions would be the prerequisites for this.

Vitamin C promotes iron uptake in the intestines. For people with hereditary haemochromatosis there is, therefore, a risk of iron overcharge in conjunction with vitamin C supplementation (McLaran *et al.*, 1982).

17.3.2 Deficiency, possible risk groups

Clinically manifest vitamin C deficiency diseases (like scurvy in adults or Balrow's disease in children) develop insidiously over several months from a condition of latent vitamin C deficiency (BGA, 1992). One main cause for vitamin C hypovitaminosis is inadequate intake which can occur, for instance, in conjunction with malnutrition, bad dietary habits or lengthier absorption disorders as accompanying symptoms to stomach and intestinal diseases.

The need for vitamin C is elevated during pregnancy and during lactation periods, through regular smoking and during re-convalescence after operations and disease.

A plasma concentration below 10 µmol/L (0.17 mg/dl) is considered to be clinical evidence of a manifest vitamin C deficiency state. Non-specific early symptoms already occur in plasma concentrations around 20 µmol/L and take the form of reduced physical performance, increased exhaustion and irritability. In the case of ongoing insufficient dietary intake there may be elevated capillary fragility, reduced resistance to infection, gingivitis, squamous mucosa and skin bleeding (BGA, 1992).

Clinically relevant vitamin C deficiency can only be observed in isolated cases in the Federal Republic since insufficient dietary intake is rare. Although no undersupplied group could be identified in the VERA Study, there is, nevertheless, in men over the age of 55 a higher prevalence (14%) of lower plasma levels below the reference intake. Furthermore, the group of men aged between 35 and 44 has been observed to have an elevated prevalence (17.4%) of lower vitamin C plasma levels (<36.8 µmol/L) (Heseker *et al.*, 1992).

The observations are not interpreted as gender-specific or age-related natural differences but are clearly linked to diet and cigarette consumption. The additional vitamin C requirement caused by cigarette consumption is around 40 mg daily.

Other groups with elevated vitamin C requirements are pregnant women and lactating mothers. The plasma level of pregnant women falls during pregnancy whereas the foetal plasma level is around 50% higher than the maternal level (DGE/ÖGE/SGE/SVE, 2000). The DGE recommendation is to counteract the reduction in the maternal vitamin C body pool caused by the foetus through additional daily intake of 10 mg. It is assumed that lactating mothers have a daily loss of 50 mg vitamin C in 750 ml human milk which has to be offset through additional dietary intake.

The calculations available for the Federal Republic for vitamin C intake indicate than on average the recommended vitamin C intake level of 100 mg per day is considerably exceeded although there are men and women who ingest less than the recommended amount. The plasma studies conducted confirm that more than 75% of the population has vitamin C plasma levels which are in the range of the kidney threshold. In the case of around 6.7% of the population, particularly amongst older men and smokers, there is a low risk of deficiency (category 3/4).

17.3.3 Excessive intake, possible risk groups

There are no known cases of hypervitaminosis in conjunction with vitamin C. The accompanying symptoms to the consumption of very high doses are increasing oxalate excretion with the risk of kidney stones, increasing uric acid excretion, potential prooxidative effects, elevated iron absorption and acid-related dental enamel corrosion (FNB, 2000).

The risk groups for vitamin C intake beyond the recommended level include people with congenital metabolism defects (thalassamia, haemochromatosis and glucose-6-phosphate-dehydrogenase deficiency) and patients with secondary hyperoxaluria (through intestinal hyperabsorption of oxalate or secondary gout) (FNB, 2000).

17.4 Tolerable upper intake level for vitamin C

The American Food and Nutrition Board (FNB) has set a value of 3000 mg/day as the LOAEL (lowest observed adverse effect level) and derived a UL (tolerable upper intake level) of 2000 mg/day for adults (aged 19 and over) For children from 1-3 years, 4-8 years and 9-13 years, the ULs are 400, 650 and 1200 mg/day respectively. For adolescents aged between 14 and 18 a **UL** was set of 1800 mg/day. The side effects on which the derivation of the UL is based, are osmotic diarrhoea and gastrointestinal disorders (FNB, 2000). The Nordic Council of Ministers (Food) has, by contrast, proposed an upper safe intake level for adults of 1000 mg for vitamin C (Nordic Council, 2001). The Scientific Panel on Dietetic Products, Nutrition and Allergies of EFSA (the successor to SCF) was not able up to now to derive a UL for vitamin C because of the shortage of data on the dose-response relationships between high doses of vitamin C and observed effects in adults, children and older people. Based on the available studies the Panel believes that the daily consumption of vitamin C supplements up to 1 g per day is safe (EFSA). Also the Expert Group on Vitamins and Minerals (EVM) in the United Kingdom has recommended a so-called guidance level for food supplements of 1000 mg from the angle of maximum tolerance. Because of the inadequate data this body was not able to derive a UL for total intake (Food Standards Agency, 2003). There is, more particularly, a lack of clarity about which dose upwards of 1000 mg leads to gastrointestinal side effects. In a small placebo-controlled, double-blind study, 2 out of 9 healthy test persons already suffered osmotic diarrhoea at a dose of 2000 mg (Johnston *et al.*, 1992). Nor is it clear whether potential prooxidative effects of vitamin C are relevant for the entire population (EFSA, 2004; Halliwell, 1996).

In the opinion of BfR there is uncertainty about the derivation of upper safe daily intake levels of vitamin C. The LOAEL derived up to now only takes account of gastrointestinal side effects but not other hazard potentials of relevance to health. In the case of the latter there are still considerable gaps in knowledge about the dose-response relationships and causality. For the following reasons, maximum levels for the use of vitamin C in food supplements and for food fortification should be oriented, on precautionary grounds, towards the integrated consideration of recommended intake, bioavailability, renal excretion threshold and the saturation of immunocompetent cells in blood plasma and not towards toxicological considerations.

Turnover and excretion metabolism: At a daily dose of 150 mg vitamin C, the saturation concentration in the blood plasma is reached around 80 µmol/L (1.4 mg/dl) (Halliwell, 2000). The neutrophile leukocytes are already saturated at a daily dose of 100 mg with a concentration of 1.3 µmol/L (0.023 mg/ml). Above a plasma concentration of 45 µmol/L (0.8 mg/dl), the reabsorption capacity of the kidneys is increasingly exceeded (Bässler *et al.*, 2002). From a daily intake of 200 mg/day and above, the excretion of vitamin C in urine increases dramatically (DGE/ÖGE/SGE/SVE, 2000). With a plasma level of around 50 µmol/L (0.88 mg/dl), a metabolic turnover of around 50 mg/day is measured. The saturated body pool of 3 g can be maintained in purely arithmetic terms with daily intakes of 100 mg.

Substitution and covering requirements: Daily doses of 100-150 mg are sufficient to compensate for bad dietary habits and to cover requirements (Blanchard *et al.*, 1997). These levels already reflect the increased requirements of pregnant women, lactating women and smokers. These figures on requirements also take into account the safeguarding of cellular immunodefence.

The UL set by FNB for adults (19 years and above) of 2000 mg vitamin C per day seems to be too high because of the risk of elevated oxalate excretion in predisposed individuals. Given this uncertainty, BfR is of the opinion that these risk groups should definitely be taken into account.

Scientific opinion differs about vitamin C intake in high single doses which extends far beyond nutritional requirements (100 mg/day for adults; 150 mg/day for smokers) for the purposes of influencing various cancer diseases and other diseases possibly associated with antioxidant status. Intervention studies with high supplement doses of vitamin C have not yet identified any positive effect on cancer or cardiovascular diseases (Blanchard *et al.*, 1997; Byers and Guerrero, 1994; Mayne, 1997; Podmore *et al.*, 1998a). Because of the pharmacokinetics there is no reason either for doses above 200 mg (Blanchard *et al.*, 1997: Levine *et al.*, 1999; Loria *et al.*, 2000). Gram doses can, therefore, only be justified as individual therapeutic measures under medical supervision. The setting of a maximum level taking into account requirements, bioavailability, renal excretion threshold and intracellular saturation of immune cells also serves to protect, on precautionary grounds, sensitive individuals and does away with the need for warnings.

Derivation of a maximum level for vitamin C in food supplements

a) Continuation of existing practice

   Retention of the maximum level of **225 mg** vitamin C per daily portion food supplement proposed by BgVV (BgVV, 1998).

   Advantages: No side effects have been reported with the practice up to now. No health risks are to be expected for the consumer and there is still a clear demarcation between food supplements and vitamin C-containing medicinal products for the treatment of deficiency conditions (daily dose from 225 mg). There is no need for warnings.

   Disadvantages: The maximum level is not based on a quantitative risk assessment but is oriented towards the integrated consideration of recommended intake, bioavailability, renal excretion threshold and saturation of the immunocompetent cells in the blood plasma.

b) Setting an admissible daily dose at 430 mg using the UL of 2000 mg/day of the American FNB taking into consideration the proposed calculation

   If the proposed calculation is used to determine the tolerable level (TL) of **vitamin C** in single dietary supplements, then this leads to the following level for **adults**:

$$\frac{2000 \text{ mg} * (\text{UL}) - 282 \text{ mg (DINF)}}{4 \text{ (MEF)}} = 429.5 \text{ mg (TL)}$$

* FNB, 2000

Legend:

UL	=	Tolerable Upper Intake Level (SCF) usually referring to the daily total intake
DINF	=	Dietary Intake by Normal Food (95. or 97.5 percentile)
MEF	=	Estimated Number of Consumed Products
TL	=	Tolerable Level in a single dietary supplement or fortified food

Advantages: There are no identifiable risks for the consumer. This derived tolerable dose is below the threshold considered safe for normal, healthy adults of 1000 mg, upwards of which oxalate and urate excretion increases as does the risk of the development of kidney stones.

Disadvantages: This value only applies to adults. For children and adolescents, correspondingly lower maximum levels were derived analog to the ULs. Especially sensitive risk groups like patients with haemochromatosis, glucose-6-phosphate-dehydrogenase deficiency, primary and secondary hyperoxaluria or kidney diseases would be at risk.

17.4.2 Derivation of the maximum level for vitamin C in conventional foods

a) Continuation of existing practice

In line with the Ordinance on Vitaminised Foods, fortification of foods with vitamin C is permitted without it making any explicit mention of upper levels.

Advantages: There are no identifiable health advantages.

Disadvantages: As a rule, fortified foods are consumed in an uncontrolled manner without any fixed daily portions which means that as a consequence of cumulation depending on choice of food and eating habits, vitamin C intake can reach a level which could be considered harmful for the above-mentioned risk groups.

b) Limiting fortification of conventional foods to 100 mg vitamin C in the daily portion of a food

Advantages: The one-fold recommended daily dose is oriented towards nutritional-physiological aspects as well as towards preventive health protection since this, rather than increasing fortification of foods, takes into account the fact that they are, as a rule, consumed in an uncontrolled manner without fixed daily portions.

Disadvantages: There are no identifiable health disadvantages.

In the estimation of BfR there is a moderate health risk for consumers in conjunction with the fortification of foods with vitamin C because of the faecal and renal excretion of excesses. It is recommended that the setting of maximum levels should be oriented towards the actual supply status of the population, to the physiological excretion threshold and to recommended daily requirements. For the upper levels in foods BfR prefers Option a) and for the fortification of foods Option b).

17.5 Gaps in knowledge

- There are no data available for children and adolescents concerning vitamin C uptake from food supplements in order to be able to estimate their contribution to vitamin C supply.

- Uncertainties concerning the derivation of the LOAEL and NOAEL because of gastrointestinal side effects are based, in some cases, on the lack of systematic tolerance studies with a sufficient number of test persons.
- The clinical relevance of other side effects discussed like the risk of the development of kidney stones or the possible prooxidative effects in specific risk groups require further clarification.
- Impact of fortification practice and supplement offering on actual vitamin C intake.

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