OPINION OF THE SCIENTIFIC COMMITTEE ON FOOD ON THE TOLERABLE UPPER INTAKE LEVEL OF PREFORMED VITAMIN A (RETINOL AND RETINYL ESTERS)

(EXPRESSED ON 26 SEPTEMBER 2002)

FOREWORD

This opinion is one in the series of opinions of the Scientific Committee on Food (SCF) on the upper levels of vitamins and minerals. The terms of reference given by the European Commission for this task, the related background and the guidelines used by the Committee to develop tolerable upper intake levels for vitamins and minerals used in this opinion, which were expressed by the SCF on 19 October 2000, are available on the Internet at the pages of the SCF, at the address: http://www.europa.eu.int/comm/food/fs/sc/scf/index_en.html.

1. INTRODUCTION

Vitamin A is a micronutrient essential to most mammalian species. The term vitamin A describes a group of lipid soluble compounds related metabolically to all-*trans*-retinol. In the diet, vitamin A is found in products of animal origin, as retinyl esters, mainly retinyl palmitate. Other esters (oleate, stearate, myristate), and retinol contribute to the dietary vitamin A intake. The forms most commonly found in vitamin supplements or enriched food, are retinyl acetate, retinyl palmitate and retinol. These vitamin A compounds, together with their metabolites, and synthetic derivatives that exhibit the same properties, are called retinoids.

Some carotenoids (α- and β-carotenes, β-cryptoxanthine) can be cleaved into retinol, via an enzymatic process, which occurs mainly in the small intestine, and is readily saturated. The toxicity of carotenoids differs from that of retinoids, and the risks of high intakes of carotenoids are not linked to the adverse effects of retinoids. Consequently, this report will deal only with the effects of retinoids, on the assumption that the pro-vitamin A properties arising from dietary intakes of carotenoids will not contribute significantly to the toxicity of high intakes of vitamin A.

Vitamin A can be expressed on a weight basis as Retinol Equivalents (1 RE = 1 μg retinol) or in International Unit (IU). Both units take into account the vitamin A potency of various esters, according to the conversion factors indicated in the following table:

Molecule	Vitamin A activity in International Units (I.U.)	Vitamin A activity in Retinol Equivalent (R.E.)
Retinol (1 mg)	3330	1000
Retinyl acetate (1 mg)	2900	870
Retinyl palmitate (1 mg)	1830	550

2. NUTRITIONAL BACKGROUND, FUNCTION, METABOLISM AND INTAKE

Dietary vitamin A is absorbed in the upper part of the small intestine, by mechanisms similar to those of lipid absorption. Retinyl esters undergo hydrolysis by pancreatic lipase (short chain esters) and an enzyme in the intestinal brush border (long chain esters). The released retinol is incorporated into mixed micelles and absorbed into enterocytes where it is bound to an intra-cellular protein called CRBPII (cellular retinol binding protein II). The intracellular retinol is re-esterified (largely with palmitic acid), packaged into chylomicrons and released into the general circulation via the lymphatic system. The chylomicrons in the general circulation are hydrolysed by plasma lipoprotein lipase and chylomicron remnants that are rich in retinyl esters are taken up by tissues, which possess specific receptors, mainly the liver. Remnants are degraded within the hepatocytes, and the released retinol is transferred to stellate cells for storage after re-esterification.

The liver is the major storage site for vitamin A, which is mainly localised in lipid droplets of hepatic stellate cells (also known as Ito cells or lipocytes). These droplets, which never fuse into a large vacuole, may almost fill the cell, which thus has a very high, but not unlimited, storage capacity.

In normal conditions, vitamin A is mobilised from the liver stores as retinol, bound to a specific carrier protein, the Retinol-Binding Protein (RBP), and released into the plasma. This mobilisation is highly regulated and ensures a homeostatic control of the plasma retinol concentration, which is maintained at a concentration of about 2 µmol/L, except during extreme hypo- or hyper-vitaminosis A. The usual plasma concentrations of retinoic acids, the active metabolites of retinol, are much lower (about 10 nmol/L), and their regulatory control is not well understood. The retinol-RBP complex is stabilised by forming a complex with transthyretin (pre-albumin).

Retinol bound to RBP enters target tissues, by a mechanism that may involve a specific membrane receptor, although such a receptor has been described only in the pigmented epithelium of the retina. Another hypothesis to explain the specific and regulated delivery of vitamin A to tissues proposes a particular lipid composition of some membrane areas. After internalisation, retinol is usually bound to the intra-cellular binding protein CRBP.

Intracellular retinol can undergo a number of different pathways of metabolism. It can be esterified within the tissue, probably to generate a limited local *in situ* store. Retinol can undergo metabolic activation by oxidation of the side chain into retinal and retinoic acids, which can in turn be further oxidised, probably by cytochrome P450, into 4-hydroxy metabolites (Collins and Mao, 1999). Another metabolic pathway is conjugation with glucuronic acid, which leads to retinoyl- and retinyl glucuronides, the enhanced polarity of which results in their elimination in faeces and urine (reviewed in Olson, 1999). Nearly all of the metabolically inter-related retinoid compounds may exist in an all-*trans* form or as *cis*- or di-*cis*- isomers (mainly at the 9, 11 and 13 positions). Numerous enzymes are involved in these various metabolic pathways, and the relative involvement of each of them is not yet completely understood. Of particular importance are the enzymes involved in the conversion of retinol to retinal and then to retinoic acid, because these enzymes may act as regulators of tissue retinoic acid levels. The reversible interconversion of retinol into retinal can be catalyzed both by the group of short-chain dehydrogenase/reductase and by alcohol dehydrogenases. Conversely, the irreversible oxidation of retinal into retinoic acid can be due to aldehyde dehydrogenases or members of the cytochrome P450 family (Duester, 1996).

Retinal, the initial oxidised metabolite of retinol, is the chromophore of rhodopsin, a visual pigment of the cone cells of the pigmented epithelium of the retina. The photo-induced isomerisation of 11-*cis*-retinal into all-*trans*-retinal is the initial event of the photo-transduction cascade, which ends by the production of a signal to the ocular nerves.

Retinoic acids, both all-*trans*-retinoic acid (TRA) and its 9-*cis* isomer (9CRA) act as regulators of genomic expression; the 13-*cis* isomer (13CRA), which is present in plasma at similar concentrations to TRA, is probably not involved in the actions of vitamin A. Retinoic acids are able to bind to specific nuclear receptors known as RAR and RXR receptors: RARs can bind either TRA or 9CRA, while RXRs bind only 9CRA. Upon ligand binding these nuclear receptors bind to specific response elements on DNA, and thus regulate gene expression. The system is complex due to the existence of several isoforms for RAR and RXR, and because the activated receptors dimerise with themselves, with each other, or with other members of the same superfamily of receptors (namely vitamin D receptor, thyroid hormone receptor, PPAR) before they bind to the DNA response elements. The number of genes known to be regulated by retinoic acids is continually increasing. Retinoic acids are considered as the molecular species responsible for all the functions attributed to vitamin A, with the exception of vision.

Of particular importance in the setting of an upper level is the role of retinoic acids during morphogenesis and embryonic development. It has long been recognised that abnormal fetal development is associated with either insufficient or excessive intakes or vitamin A and related compounds. The role of retinoic acid and its receptors in ontogenesis was confirmed by the finding that RAR-null mutant mice (Lohnes *et al*, 1994) died *in utero* or shortly after birth, and exhibited congenital abnormalities. Moreover, RARs and RXRs show specific spatio-temporal patterns of expression in all developing systems during embryonic development, which suggests that retinoic acid signalling is involved in most, if not all, morphogenetic and patterning processes (Morriss-Kay and Sokolova, 1996).

Vitamin A deficiency is rare in the Western world, but is a major problem in developing countries. Specific symptoms associated with deficiency include visual problems such as night blindness and xerophthalmia that may end in irreversible blindness. Other reported effects include growth retardation in children, skin disorders, impaired immune function and congenital malformations of the eyes, lung, cardiovascular and urinary systems if deficiency occurs during pregnancy. However, in humans, these latter symptoms are often associated to a multi-nutrient deficiency and the exact role of vitamin A remains to be ascertained.

In 1992, the Committee determined a population reference intake (700 μg/day for men and 600 μg/day for women) (SCF, 1993). This recommendation is met by the intakes of the general population in developed countries. Intake data for various European countries (Table 1) indicate that the mean intakes are well above the population reference intakes, whereas the median intakes are at or slightly below the population reference intakes. The difference between the mean and median values indicates a skewed distribution of intakes, which arises from the non-uniform distribution of preformed retinol in the food supply, and very high intakes by consumers of foods such as liver.

Table 1. The daily intakes of preformed retinol (retinol and retinyl esters) in EU countries (μg/day)

	Population	n	Method	Supplements	Mean	97.5%
Austria[a]	men + women	2488	24 h recall	Not defined	1120	4230
Germany[b]	men	854			660	
	women	1134			530	
Germany[c]	men	1268		-	2010	4100
	women	1540		-	1710	3440
	men	240		+	2020	
	women	347		+	1790	
Italy[d]	household	2734	7-day record	+	759	4377
Netherlands[e]	household	5958	2-day record	-	891	3230
UK[f]	Men	1087	7-day record	-	1226 (602)	6564
	women	1110		-	1058 (463)	5698
	men	1087		+	1277 (618)	6671
	women	1110		+	1133 (491)	5779

Results are for intake as preformed retinol
[a] Elmadfa *et al* (1998)
[b] Heseker *et al* (1992) - median not mean value; data reported as preformed retinol
[c] Mensink and Ströbel (1999) - these values include retinol equivalents from carotenoids
[d] Turrini (INRAN) - as retinol
[e] Hülshof and Kruizinga (1999)
[f] Gregory *et al* (1990) - values are the mean with the median in parentheses

3. HAZARD IDENTIFICATION

Many cases of acute hypervitaminosis A has been reported in the past 60 years. They have been extensively reviewed by Bauernfeind (1980) who quoted an exhaustive compilation of 385 individual cases up to 1975 (Köerner and Völlm, 1975). These cases mainly concern anecdotal ingestion by adults of large amounts of shark or polar bear liver, suspected to provide more than 2 mg/g of retinol equivalents. More than 100 cases of iatrogenic hypervitaminosis A were reported in children in France and Spain in the early 60s, probably potentiated by high doses of vitamin D. Excessive dosages of vitamin A may result in a number of adverse effects, including skin disorders, nausea, vomiting, bone pain, plus teratogenicity due to retinol and its metabolite TRA (which has been the focus of most previous risk assessments).

The following adverse effects are reviewed separately:

1. Bulging fontanelle in infants/intracranial pressure
2. Hepatotoxicity
3. Effects on bone metabolism
4. Effects on lipid metabolism
5. Teratogenicity.

3.1. Bulging fontanelle in infants/Intracranial hypertension

The prevention of vitamin A deficiency in developing countries has involved the administration to infants and children of a single large dose of vitamin A, or a series of large doses with an interval of 1 month or more between consecutive doses. The development of reversible bulging fontanelle (BF) has been reported in a number of these studies. BF is a clinical symptom, which can be observed in infants at examination. Usually, it is not accompanied by an elevation of intracranial pressure (Agoestina *et al*, 1994), probably because the increased volume of the cerebro-spinal fluid can expand, using

the fontanelles and the un-fused cranial sutures (Humphrey *et al*, 1998; WHO, 1998). The effect is age-dependent with higher doses being without effect in 6 or 9 month old infants (Stabell *et al*, 1995) compared with 6-17 week old infants (De Francisco *et al*, 1993; Baqui *et al*, 1995). Vitamin A induced BF is always rapidly reversible (usually in less than 2 days). BF exclusively concerns a distinct and sensitive sub-population that is neonates and infants of both genders, from birth to 6 to 8 months of age. A recent study by Humphrey *et al* (1998) clearly shows that vitamin A-induced BF is not associated with adverse growth or developmental sequelae.

BF may represent the infant form of the headaches that are frequently reported during hypervitaminosis A in adults, and which may possibly arise from increased intracranial pressure (although it is actually not measured). In older children or in adults, the increased volume of the cerebro-spinal fluid can be linked to an increased intra-cranial pressure (Babikian *et al*, 1994). Excessive vitamin A intakes have been described as one among many possible causal factors of symptomatic intracranial hypertension (Pasquariello *et al*, 1977; Tibbles *et al*, 1972; Gangemi *et al*, 1985).

3.2. Hepatotoxicity

Relatively few chronic toxicity studies on animals have been reported. Leo *et al* (1982) showed that rats receiving 120 μg RE of vitamin A daily for 8 weeks resulted in proliferation of the endoplasmic reticulum and mitochondria enlargement, symptoms which were enhanced when the animals received ethanol simultaneously. An earlier study (Randall, 1951, cited by Santos, 1987) in which rats were fed 5 days a week during 10 months with 3 to 15,000 μg RE of vitamin A/kg of diet did not show any change in body growth and haematological parameters, but liver examination was not performed. Subsequent animal studies have confirmed the effects of vitamin A on the liver and the nature and extent of hepatic damage (Shintaku *et al*, 1998). Lettinga *et al* (1996) have shown that feeding rats during one week with 75,000 μg RE of vitamin A/kg diet (approximately 1500 μg RE per animal, daily) led to activation of Kupffer cells and proliferation of hepatic stellate cells. These events are known to be initial steps of the development of an experimental fibrosis in rats.

The available data on humans are exclusively case reports, either describing a single case, or gathering information obtained in a given hospital. For obvious ethical reason, no experiments have been carried out on humans. In most of the reported cases, the toxicity has been linked to the intake of high doses of vitamin A, over long time periods. Evidence of the causal link between the vitamin and the hepatotoxicity was the improvement of symptoms after withdrawal of vitamin A, and the fact that other possible etiologic causes had been ruled out by experienced clinicians and adequate assays. Furthermore, hepatotoxicity was very frequently associated with elevated retinol and retinyl esters in serum, and histology revealed hepatic stellate cell hyperplasia.

Hepatotoxicity is one of the most severe outcomes of chronic intake of high dosages of vitamin A (Bauernfeind, 1980; Geubel *et al*, 1991; Kowalski *et al*, 1994). The first symptoms of hypervitaminosis A are not hepatic; they vary greatly, according to the severity of the disease, and often include headache, bone and joint pain, nausea and dry skin. Vitamin A induced hepatotoxicity can be diagnosed clinically using signs of hepatomegaly, chronic hepatic disease, ascites, icterus, oedema, oesophageal varices or dermatological lesions. Serum transaminase levels are usually moderately enhanced, and there is often a slight anicteric cholestasis. Histological features of vitamin A-induced hepatotoxicity include hepatic stellate cell hyperplasia and hyperproliferation, as well as collagen diffusion within the space of Disse, which can evolve in a portal hypertension (Guarascio *et al*, 1983; Geubel *et al*, 1991; Jacques *et al*, 1979).

In many cases, the hepatotoxicity is reversible after the withdrawal of vitamin A, which results in slow (up to several years) normalisation of the biochemical indexes. However, in some patients the liver disease progresses after vitamin A withdrawal from steatosis or fibrosis into micronodular cirrhosis, development of which can be fatal. The hepatotoxicity can be potentiated by various pathological conditions, including hypertriglyceridemia (Ellis *et al*, 1986), chronic alcohol intake (Leo and Lieber, 1999), and pre-existing liver disease (Russell *et al*, 1974). It is difficult to estimate quantitatively the proportion of vitamin A-induced hepatotoxicity which is reversible upon withdrawal. In the most comprehensive available set of data, 41 patients were diagnosed with a vitamin A-induced hepatic pathology, at various levels of severity; nine (22%) died in less than 2 years following diagnosis and progression of the disease was demonstrated in 3 others.

Mechanisms of hepatic effects are linked to overload of the storage capacity of the liver for vitamin A. The liver then becomes unable to take up newly-absorbed retinyl esters, and possibly releases retinol unbound to RBP. Non-specific delivery of retinol, which has surface-active properties, may produce membrane damage and lysosomal rupture (Ellis *et al*, 1986).

In addition, the vitamin A-loaded hepatic stellate cells may fill the sinusoidal space and thus obstruct the blood flow and create portal hypertension (Russell *et al*, 1974; Hruban *et al*, 1974). Hepatic stellate cells are also involved in the synthesis of extracellular matrix proteins, and upon activation, they exhibit a "myofibroblast-like" phenotype (Davis *et al*, 1987; Svegliati-Baroni *et al*, 2001), producing collagen type III, and promoting fibrosis and potential cirrhosis.

3.3. Bone metabolism

Histopathological changes in animal bone following very high vitamin A doses (up to 13,500 μg RE per animal) have been reviewed in Hathcock *et al* (1990) and have been shown to lead to bone fragility and spontaneous fractures (Nieman and Obbink, 1954). Similar bone lesions have been described in rats following retinoic acid administration (Dhem and Goret-Nicaise, 1984), and in the rabbit following intra-articular injection of 30,000 μg RE of retinyl palmitate (Lapadula *et al*, 1995).

Several isolated cases of skeletal problems in children with severe hypervitaminosis A have been reported (reviewed in Biesalski, 1989). Bone symptoms involve a decrease in density, osteoporotic changes and cortical thickening of the long tubular bones, leading to retarded growth. Freudenheim *et al* (1986) measured bone mineral content in a 4 year clinical trial in women receiving or not receiving calcium supplementation. Dietary intake was determined by a single 24-hour record and used to determine any dietary factors affecting the results. A highly significant effect of vitamin A was reported at only one site, the ulna, and only in women taking calcium supplements; this finding is difficult to interpret as it seems to have arisen largely due to a single individual with a very high vitamin A intake (4300 μg RE) and who showed very rapid bone loss. Sowers and Wallace (1990) reported no relationship between vitamin A intake or serum retinol concentration and radial bone mass or fracture history in a group of 246 postmenopausal women. A brief report by Theiler *et al* (1995) suggested that chronic vitamin A intoxication in adults might be related to osteoarthritis. Houtkooper *et al* (1995) analysed the influence of various factors, including nutrient intake, on annual rates of change in bone mineral density in a group of 66 pre-menopausal women who were taking calcium supplements. There was a slight loss of bone, measured at a number of sites, during the 18 months of the study, which was within the measurement errors of the techniques available. At one of the measured sites there was an indication that high intakes of vitamin A were associated with less loss of bone. No association was found between serum retinyl esters and reduced bone density in the 1988-1994 United Kingdom National Health and Nutrition Survey (Ballew *et al*, 2001), although serum retinyl esters reflect recent intake and are not a good indicator of vitamin A status.

There were no changes in serum markers of skeletal turnover (bone-specific alkaline phosphatase, N-telopeptide of type 1 collagen and osteoclastin) in a group of 40 male volunteers given 7.6 mg vitamin A as retinyl palmitate per day for 6 weeks (Kawahara *et al*, 2002). Such serum measurements were considered by the authors to be sensitive markers of bone turnover as they show larger and more rapid changes to therapeutic treatments than would be found with measurements of bone mineral density. However the authors concluded that whether long-term vitamin A supplementation might have adverse skeletal effect remains to be determined.

A nested case-control study (Melhus *et al*, 1998) has investigated the vitamin A intake (which was divided into 4 bands of <0.5, 0.5-1.0, 1.0-1.5 and >1.5 mg/day) by 247 women with a hip fracture and 873 controls from a group of 66,651 Swedish women in a mammography study cohort. The dietary intake of pre-formed retinol, was associated, in a dose-dependent manner, with a higher risk of hip fracture; both univariate and multivariate analysis showed a significant ($P<0.01$) 1.5- to 1.6-fold increase in risk per mg retinol consumed daily. An associated cohort study indicated that similar intakes of retinol reduced bone density (Melhus *et al*, 1998). Analysis of data from the Nurses' Health study in the US (Feskanich *et al*, 2002) reported 603 hip fractures a total of 72,337 women who had been studied for up to 18 years, with an increased risk attributable to total retinol (vitamin A) intake and retinol intake but not beta-carotene intake. The total vitamin A and retinol intakes were divided into quintiles and there was a significantly elevated relative risk of 1.48 and 1.89 respectively in the highest quintiles of intake (>3000 and >2000 μg RE per day respectively) compared with the lowest quintiles. Multivariate analysis revealed highly significant trends of increased risk for both total vitamin A ($P=0.003$) and retinol ($P\leq0.001$) for total intake (food plus supplements) but not for food only ($P=0.24$ and 0.05 respectively). The lower statistical power for the food only data may have been related to differences in the precision of the intake estimates, which were based on a semi-quantitative food frequency questionnaire, and brand-specific information on vitamin preparations. Hormone replacement therapy appeared to reduce the relative risk in post-menopausal women.

These findings may have a mechanistic explanation, related to a possible effect of retinoic acid in regulating the expression of genes, since both osteoblasts and osteoclasts express RARs and RXRs (Saneshige *et al*, 1995). Retinoic acid inhibits osteoblast differentiation (Cohen-Tanugi and Forest, 1998) and stimulates osteoclast formation and bone resorption (Scheven and Hamilton, 1990; Kindmark *et al*, 1995). A molecular interaction of vitamin A and vitamin D could also be responsible for the antagonism of vitamin A towards the action of vitamin D reported in rats (Rohde *et al*, 1999). A recent trial on 9 human healthy volunteers receiving either 15 mg of retinyl palmitate (8250 μg RE), or 2 μg of 1,25$(OH)_2D_3$ vitamin D, or a mixture of both, indicated that retinyl palmitate antagonizes the rapid calcium response to physiological levels of vitamin D (Johansson and Melhus, 2001). These data suggest that excessive vitamin A may increase bone resorption and decrease bone formation (Binkley and Krueger, 2000).

3.4. Lipid metabolism

Several reports suggest that retinoic acids increase plasma triacylglycerol concentrations in humans. Long-term intakes of moderate doses of retinol have been shown to increase circulating concentrations of both triacylglycerols and cholesterol (Cartmel *et al*, 1999). A population of 2297 subjects, with a moderate risk of skin cancer (actinic keratoses), received 7500 μg RE/day of retinol for approximately 4 years in a placebo-controlled trial. The treated group exhibited a small (2-3%) increase in cholesterol concentration. Serum cholesterol is a known risk factor for cardiovascular diseases, and even a small increase in concentration would represent an increase in risk.

3.5. Teratogenesis

The teratogenic effects of retinoic acids, the active oxidized metabolites of vitamin A, have been known for a long time and documented both in animals and in humans. Children exposed *in utero* to isotretinoin (13CRA) exhibit a pattern of congenital malformations, known as “the retinoic acid syndrome”, which includes defects of the craniofacies (small or absent external ears and auditory canals, cleft palate, micrognathia, low set ears), of the central nervous system (micro- or anopthalmia, cerebellar or cortical defects, microcephaly), of the thymus and of the cardiovascular system (transposition of the heart vessels, aortic arch hypoplasia, ventricular septal defects) (Lammer *et al*, 1985; Chan *et al*, 1996; Sinning, 1998). The risk of these defects was 25 times higher in the exposed children, and even greater when neuropsychological dysfunctions were assessed (Adams and Lammer, 1991). This last outcome could be related to an abnormal development of specific brain structures, which has been documented in rodents (Holson *et al*, 1997a, b and c). Most of these anatomical defects appear to be associated with alterations in the migration of cells from the neural crest (Morriss-Kay *et al*, 1993). The gestational period at which exposure occurred is of critical importance in the generation of these effects. In animals, the extent and nature of the defects resulting from the same dose of the same retinoid varies according to the gestational day of exposure (Holson *et al*, 1997a, b and c; Shenefelt, 1972). In humans, the critical period seems to be between the second and the fifth week of pregnancy, although it is generally stated that caution should be taken from the very beginning and up to the 60th day of pregnancy.

Birth defects similar to those observed following therapeutic use of isotretinoin or other retinoids have been described in approximately 20 women who had ingested vitamin A during the early weeks of their pregnancies (reviewed in Biesalski, 1989). These were separate case reports, in which the exposure to vitamin A occurred via supplements. Although the data represent a series of anecdotal cases, they confirm the link between excessive vitamin A intake and teratogenesis, which has been clearly documented in various animal species, including mice, rabbits, rats (Piersma *et al*, 1996) and non human primates. Large species differences in susceptibility exist, for example, the teratogenicity of 13CRA in mice is 20-fold less than in the Cynomolgus monkey, and 100-fold less than in humans (Hummler *et al*, 1990; Public Affairs Committee of the Teratology Society, 1990). The Cynomolgus monkey is the most sensitive animal species studied to date.

An important question is whether the pre-existing body stores affect the intake-response relation for teratogenicity. It is possible that the risk of the malformations could be higher in individuals with high body loads, but data from experiments on rats do not support this hypothesis. Biesalski *et al* (1996) did not find teratogenicity in rats fed a very high vitamin A diet before mating, and Piersma *et al* (1996) reported that the background level of circulating retinol or liver vitamin A did not affect the teratogenic potential of a single dose of retinyl palmitate. This indicates that a high vitamin A dose would have a similar teratogenic potential in a woman with good liver stores and in a vitamin A-deficient mother.

Several epidemiological studies have been designed to investigate the relationship between vitamin A intake and teratogenesis in humans. Five case-control studies have been published since 1990, in which the intake of vitamin A has been estimated retrospectively, both in controls and in mothers of malformed babies (Martines-Frias and Salvador, 1990; Werler *et al*, 1990; Botto *et al*, 1996; Mills *et al*, 1996; Shaw *et al*, 1997). The design of these studies varies, especially as regards the classification of the observed malformations, the numbers of women studied and the statistical power, and the collection of data on vitamin A consumption. The studies and data are summarised in Table 2.

Table 2. Epidemiological case-control studies that investigated the association between vitamin A intake and foetal malformations

Population		Results		Comments	Ref.
Cases (n)	**Controls (n)**	**Exposure to vitamin A**	**Odds ratio (95% confidence interval)**		
11,193	11,293	>3,000 µg RE/day >12,000 µg RE/day	1.1 (0.5 - 2.5) 2.7 (0.8 - 11.7)	only 11 cases and 4 controls at high exposure level	Martines-Frias and Salvador, 1990
2,658	2,609	during the 1st month	2.5 (1.0 - 6.2)	no information on vitamin A doses well-characterized neural crest-malformations	Werler *et al*, 1990
		2nd month	2.3 (0.9 - 5.8)		
		3rd month	1.6 (0.6 - 4.5)		
158	3026	Use of multivitamin Supplement	0.57 (0.33 - 1.00)	focus on conotruncal defects only	Botto *et al*, 1996
548 (NTD)	573	>2,400 µg RE/day from supplements	NTD: 0.91 (0.31 - 3.68) others: 1.05 (0.51 - 2.18)	Consumption of liver does not increase the risk	Mills *et al*, 1996
387 (others)		>3,000 µg RE/day from food and supplements	NTD: 0.73 (0.40 - 1.53) others: 0.92 (0.40 - 2.11)		
426	432	0-2999 µg RE/day	1.0 (reference)	NTD (neural tube defects) only; vitamin A from food and supplements	Shaw *et al*, 1997
16	12	3000-4499 µg RE/day	1.4 (0.6 - 2.8)		
6	7	>4500 µg RE/day	0.9 (0.3 - 2.5)		

NTD: neural tube defect.

Two prospective studies have been performed. Rothman *et al* (1995) recruited more than 22,748 pregnant women into a prospective study in which their intake of vitamin A, through both diet and supplements, was assessed by questionnaire for each of the 12 weeks since their last menstrual period. Information on pregnancy outcomes was obtained through obstetricians or mothers, without direct examination of the children. The birth defects that were reported in 339 children were then classified, and 121 malformations appeared to be of cranial-neural-crest origin. Rothman and colleagues reported that a daily intake exceeding 3000 µg RE of supplemental vitamin A significantly increased the risk of malformations. The percentages of babies with cranial-neural-crest defects were 0.52 and 1.06 in women with intakes from food of 0-1500 µg RE/day and more than 3000 µg RE/day respectively. A greater difference was found when the comparison was based on reported intakes from supplements with values of 0.46 and 2.21 percent in women with intakes of 0-1500 µg RE/day and more than 3000 µg RE/day respectively (giving a prevalence ratio of 4.8 with 95 percent confidence intervals of 2.2 to 10.5). These differences were based on small number of babies in each of the high intake sub-groups, i.e. 2 babies after >3000 µg RE/day from food and 7 babies after >3000 µg RE/day from supplements. Analysis of the potential vulnerable period in babies whose mothers had an intake >3000 µg RE/day showed that the prevalence of cranial-neural-crest defects was 4.8%, 3.8% and 0% when the high intake was only during 2 weeks before conception (n=2/42), only before week 7 of pregnancy (n=3/80) and only after week 6 (n=0/70) respectively. The overall conclusion of the study (see quantitative considerations below) was different from those of the retrospective studies. This paper has been criticised, particularly in relation to possible

misclassification of the malformations. Only 76.5% of the pregnancy outcomes were assessed by physicians, and the remainder were based on information provided by the mother. The birth defects were classified independently by two researchers, who were blind to the intake estimates, using a classification scheme that focussed on cranial-neural-crest and other likely vitamin A related defects.

The second prospective study (Mastroiacovo *et al*, 1999) collected data on 423 babies exposed for at last one week during the first 9 weeks of pregnancy to high doses of vitamin A (3000 μg RE or more) and evaluated the outcome data. The mothers were recruited following referral to 13 European Teratology Information Services for advice about the possible risk associated with their high intakes of vitamin A. In this study, only a low incidence of major malformations was reported (3 out of 311 exposed pregnancies). Based on the prevalence rate in the study of Rothman *et al* (1995) a total of 7 babies would have been predicted to have suffered malformations. Although the confidence intervals for this result overlapped with the confidence intervals for the data from the study of Rothman *et al* (1995), the authors pointed out that the cases were at intakes of 7500, 9000 and 15,000 μg RE per day, and that no abnormalities were reported in 120 women with intakes reported to exceed 15,000 μg RE per day. No evidence was found of an increased risk of major malformation in babies exposed to vitamin A in early pregnancy when compared to those exposed later (i.e. after the 9th week of pregnancy) (rate ratio: 0.28 [95% confidence intervals 0.06-1.23]). There was no evidence of increased risk when the data for the group exposed to vitamin A were compared to a group referred for advice because of exposure to non-teratogenic compounds (rate ratio: 0.50 [CI 0.14-1.76]). The possibility of misclassification of the malformations was not directly considered, but all cases of congenital anomaly, neonatal problems or prolonged stay in hospital were followed up with the attending paediatrician.

A clinical trial has been carried out in Hungary (Dudas and Czeisel, 1992) in which a supplement of 1800 μg RE vitamin A did not increase the incidence of foetal malformations. However this study does not negate the findings of Rothman *et al* (1995) because of the low dose used; also no conclusions can be drawn with respect to the incidence of neural tube defects, because folic acid was administered simultaneously with vitamin A.

4. DOSE-RESPONSE ASSESSMENT

4.1. Bulging fontanelle in infants/Intracranial hypertension

The available data on bulging fontanelle (BF) come from intervention studies on large human populations of healthy infants (more than 100 subjects), with a placebo group, in which all the events of BF have been recorded. The route of exposure (*per os*) and the chemical form and intake conditions (and therefore the bioavailability) were similar in all studies. The data only concern the effects of acute or sub-acute dosages.

Vitamin A-induced BF is reported regularly, and usually occurs in a small proportion of treated infants less than 6 months of age. The proportion affected increases when the same infants receive further doses. The administered dose-effect relationship is clearer when the effect of cumulative dose is considered. BF has been reported in young infants given doses of 15,000 μg RE at 6, 10 and 14 weeks of age (De Francisco *et al*, 1993), or 7500 μg RE at 6, 12 and 17 weeks of age (Baqui *et al*, 1995). Conversely, BF was not reported when 30,000 μg RE was given at both 6 and 9 months.

4.2. Hepatotoxicity

In humans, the available data clearly suggest that the occurrence of toxic symptoms depends both on the vitamin A dose taken on a regular basis, and on the duration of this intake. The most extensive report (Geubel *et al*, 1991), included 41 cases, but reliable intake information was available on only 29 patients who had a mean daily intake of 28,770 μg RE (range, 6,000-120,000 μg RE). The duration of high intake averaged 7.17 ± 1.21 years (range 0.2-15 years). Interestingly, these authors reported that the most severely affected subjects, i.e. those with cirrhosis (n=13), had consumed significantly more vitamin A, both daily and in total, than the patients without cirrhosis. The lowest continuous daily consumption in patients with cirrhosis was 7500 μg RE/day taken over 6 years. A similar case (7500 μg RE/day for 6 years) has been reported more recently (Kowalski *et al*, 1994), in which progressive liver failure led to death of the patient. Cases of hepatotoxicity have not been reported below 7500 μg RE/day, and it can be hypothesized that this value might be the upper threshold of the storage capabilities of the liver. It is not known if a dose lower than 7500 μg RE/day could induce hepatotoxicity if taken for more than 6 years, but such low intakes may not been considered by physicians when they attempted to identify the cause of their patient's liver disease.

Differential sensitivity to vitamin A-induced hepatotoxicity has been considered by several authors. On a weight basis, it does not seem that children (more than one year old) are more sensitive than adults (reviewed in Hathcock *et al*, 1990). In elderly people (64-88 years old) plasma retinyl esters and retinol values were correlated to their supplemental vitamin A intakes (up to 14,100 μg RE/day for 5 years), but not to liver function tests (Stauber *et al*, 1991).

4.3. Bone metabolism

The risk for hip fracture in Swedish women (Melhus *et al*, 1998) is doubled for retinol intake greater than 1500 μg RE/day as compared to intakes less than 480 μg RE/day. Based on univariate analysis, the relative risks at intakes of 500-1000 μg/day, 1000-1500 μg/day and >1500 μg/day, compared with individuals with intakes <500 μg/day, were 0.93 (0.61-1.41), 1.27 (0.80-2.02) and 1.95 (1.15-2.11) respectively. The intake was from dietary sources and therefore it is possible that the effects detected may have arisen from unrecognised confounding; however the mechanistic data on the actions of retinoic acid on bone metabolism are consistent with the reported relationship. An intake of 1500 μg RE/day is close to the PRI (600 μg RE/day for women) and lower than the actual intakes for a substantial proportion of the population (see Table 1). A similar dose response relationship was reported by Feskanich *et al* (2002) in data from a large cohort of women in the US, studied over a period of 18 years. The cohort was divided into quintiles for total vitamin A intake (<1250, 1250-1699, 1700-2249, 2250-2999, >3000 μg RE daily) and also for retinol intake (<500, 500-849, 850-1299, 1300-1999, >2000). Significant trends were apparent between relative risk and the intakes from food and supplements of total vitamin A and also of retinol. A significant increase in relative risk was reported using a multivariate analysis for the two highest quintiles of retinol intakes (1300-1999 and >2000 μg RE/day) compared with the lowest quintile (<500 μg RE/day). The trend analyses for retinol from food and supplements (P≤0.001) compared with food only (P=0.05) indicates an important contribution from supplements and this would be less likely to be affected by dietary confounding than the data from the study of Melhus *et al* (1998). Therefore, both of these major epidemiology studies indicate an increased risk of bone fracture over an intake range similar to that normally consumed from food and supplements (Table 1).

4.4. Lipid metabolism

Patients given 7500 μg RE/day for approximately 4 years exhibited a small (2-3%) increased in cholesterol concentration (Cartmel *et al*, 1999). A similar study, conducted on 146 patients with retinitis pigmentosa during 12 years failed to show any adverse effect of 4500 μg RE/day (Sibulesky *et al*, 1999).

4.5. Teratogenesis

Several methods have been used to assess the dose-response relationship for the risk of high intakes of vitamin A during pregnancy.

4.5.1. Analysis of the relationship between vitamin A intake and the occurrence of birth defects

Dose-response relationships could be examined on the basis of the published case reports, but the available information is usually limited, and relates to supplemental intake only. The 18 cases reviewed in Biesalski (1989) had ingested daily between 5400 and 45,000 μg RE, over periods of several weeks or months, usually starting before pregnancy. Based on the data from studies in pregnant animals, teratogenesis could result from either a single dose or a limited number of doses, and there is a report of congenital malformation after ingestion of a single dose of approximately 300,000 μg RE (Mounoud *et al*, 1975). The absence of anecdotal case reports at lower intakes of vitamin A cannot be taken as evidence of an absence of risk: babies with cranial-neural-crest defects are born to women with normal intakes of vitamin A, and clinicians would not suspect a cause-effect relationship at doses close to normal intakes. Establishment of the dose-response relationship requires the analysis of data from epidemiology studies.

4.5.2. Analysis of the results of epidemiological studies

No association has been found in the majority of case-control studies between daily doses of vitamin A of 3000 μg RE or less and foetal malformation. However, in each of these studies, the number of women consuming high amounts of vitamin A was too limited to give a reliable estimate of a safe intake value. It is possible that a meta-analysis could be of value, but to combine the data for high exposure individuals would probably require access to the original databases.

The prospective study of Rothman *et al* (1995) was large enough to stratify the population according to the vitamin A intake. Moreover, the origin of the vitamin A intake (supplement or food) was available for all subjects. The authors found that the women taking daily more than 4500 μg RE of total vitamin A (from food and supplement) had a 3.5 times higher risk of giving birth to a child with cranial-neural-crest defects, than mothers ingesting less than 1500 μg RE/day. When the analysis was restricted to the supplemental intake of vitamin A only, the relative risk for mothers ingesting more than 3000 μg RE/day was 4.8 higher than those ingesting 1500 μg RE/day. The authors fitted a regression curve to their data, which indicated a rise in the ratio of prevalence of birth defects associated to the cranial-neural crest at doses greater than 3000 μg RE/day of vitamin A (food and supplement). The conclusions of the study remained the same when several potential confounding factors were considered.

The quantitative conclusion from the Rothman's study was that 3000 μg RE/day of supplemental vitamin A can be considered as a threshold for teratogenicity, and this has been discussed extensively. Khoury *et al* (1996), for example reported that 2400 μg RE/day of supplemental vitamin A did not increase the risk of birth defects, but this report, which lacks details does not contradict the conclusion from the study of Rothman *et al* (1995). Similarly, in the study of Duda and Czeisel (1992) a dose of 1800 μg RE/day was given to pregnant women without observing any increase in birth defects, but there are methodological uncertainties in the report, and the data are consistent with the conclusion of Rothman *et al* (1995). The study that contradicts the dose-response model reported in the paper of Rothman *et al* (1995) is that of Mastroiacovo *et al* (1999). These authors reported only three cases of malformations out of 423 pregnancies (311 births) exposed to vitamin A, at levels above 3000 μg RE/day. Although the number of women recruited was considerably smaller than in the Rothman *et al* (1995) study, they all had high intakes because they had been referred for advice about the possible risk associated with their high intakes. The number of women exposed to more than 6000 μg RE/day was twice as high as in the Rothman's study. Moreover no malformations were observed in the babies from 120 women who were exposed to more than 15,000 μg RE/day. The main weakness of this study is its statistical power: the sample size had only 80% power to detect an increased risk higher than 2.76. However, it seems reasonable to conclude from the data of Mastroiacovo *et al* (1999) that a daily intake of 3000 μg RE/day would be associated with a low or negligible risk of teratogenicity.

4.5.3. Analysis based on circulating concentrations of active metabolites

A "metabolic" approach has been proposed based on the hypothesis that the plasma concentrations of retinol and its metabolites following different doses of vitamin A are predictive of the teratogenic risk.

This approach was developed using data from animal studies. Ritchie *et al* (1998) quantified the teratogenic potencies of retinoids on cultured rat embryos, and compared them with circulating concentrations of the same metabolites *in vivo* after administration of a teratogenic dose of vitamin A. The hypothesis was that malformations would only be induced if the threshold concentrations were exceeded. Their conclusion was that plasma retinol was the best predictor of teratogenicity, and that an intake of 7500 μg RE/day of vitamin A would be unlikely to generate teratogenic plasma concentrations of retinoids. However, they pointed out several pitfalls in this analysis. Species differences, protein binding and transfer to the embryo were not taken into account and this prevented recommendation of this method to predict the teratogenicity of vitamin A in humans.

Data on Cynomolgus monkeys (Wiegand *et al*, 1998, and unpublished results) indicate that a dose of 2250 μg RE/kg body weight daily as retinyl palmitate from the 16th to the 27th day of gestation did not produce any malformations of the pups, as compared with controls fed a diet providing 300 μg RE/kg body weight. The authors extrapolated these data to humans on the basis that the dose-responses for the teratogenicity of isotretinoin (CRA) appeared similar in Cynomolgus monkeys and humans, and that there is similar conversion of CRA to TRA in both species. They conclude that a daily intake of 9000 μg RE should be considered non-teratogenic in humans.

A similar method has been used in humans by Miller *et al* (1998), who determined the baseline circulating concentrations of retinoic acids, and then compared them to the increases observed following intakes of known doses of vitamin A. Plasma concentrations in pregnant women with normal pregnancy outcomes, were 0.81 to 2.40 ng/mL for TRA, 0.81 to 4.90 ng/mL for 13CRA and 0.97 to 7.86 ng/mL for 4-oxo-13CRA (Miller *et al*, 1998). These authors concluded that that a meal containing 3000 or 9000 μg RE of vitamin A would only slightly increase the peak plasma concentrations of TRA and of 13CRA; the peak values would still be within the range of the reference values. Other authors have shown that 15,000 μg RE/day for a period of 20 days increased the level of retinoic acids or metabolites by a factor 2 to 7 (Eckhoff and Nau, 1990). Buss *et al* (1994) reported higher plasma concentrations;

after a single dose of 45,000 μg RE, the maximum plasma concentration of TRA increased 35 times if a water miscible supplement was given but only 1.6 times after the same dose in cooked liver. Increases in the peak concentrations of 13CRA and 4-oxo-13CRA acid were 26 and 10 times, after supplement intake, and 10 and 5 times after liver intake, respectively. This study showed that the matrix in which the vitamin is present can influence the plasma concentration profiles of retinol and its active metabolites. However, a recent study (van Vliet *et al*, 2001) showed higher serum levels of TRA when 15,000 μg RE of vitamin A had been taken in liver paste than in an oil-based supplement. These two studies indicate that the rate of absorption may influence the plasma concentration-time curves of the active metabolites.

This method has a number of problems that prevent its use for deriving safe levels of vitamin A intake. Important issues requiring resolution include:

i. The nature of the ultimate teratogenic metabolite(s) of vitamin A, and the importance of circulating TRA 13CRA and 9CRA. Numerous derivatives of retinoic acid can be found in plasma: TRA can be isomerized *in vivo* to 9CRA and 13CRA, oxidized to 4-oxo-TRA and then converted to oxo-13CRA, conjugated with glucuronic acid and metabolised by other minor pathways. Moreover, it is likely that retinoic acid metabolites can be generated locally within tissues, so that circulating concentrations may not reflect tissue levels.

ii. The metabolic inter-conversions of the different bioactive metabolites of retinol. The teratogenic potency of different retinoic acid derivatives is difficult to assess unequivocally, because they are interconnected by metabolic pathways, for which there are large interspecies differences.

iii. The link between circulating concentrations and foetal exposure including the unique conformation of the human placenta. Embryotoxic doses of vitamin A in rabbits are associated with low plasma but high embryonic concentrations of TRA (Tzimas *et al*, 1996). The limited teratogenicity of 13CRA in mice may be due to the very low placental transfer of this derivative in this animal species. Conversely, 13CRA is a potent teratogen in humans, probably due to its metabolism to TRA, either before or after placental transfer (Creech-Kraft *et al*, 1989). The placental transfer differs between the chemical structures of closely related retinoic acid derivatives, as shown by various embryo/maternal plasma ratio, reported by Nau (1995). Thus, the differing placental structure between animal species, including human, is likely to be a critical parameter in the teratogenicity of vitamin A, although this has been poorly addressed until now.

The correlation between the occurrence of a given retinoic acid metabolite and the teratogenic potency has not been established clearly, even in animal studies. Studies on rats or mice had suggested that there was a correlation between the AUC (area under the concentration-time curve) for these metabolites in plasma and the teratogenicity of retinoids (Nau, 1990). However, it is likely that the duration of the exposure at potentially teratogenic concentrations is also of crucial importance, because the embryo should be exposed during a time long enough to generate malformations (possibly 12 to 24 hours in the human species; Ritchie *et al*, 1998). Tembe *et al* (1996) reported different plasma concentration-response relationships in studies on the plasma kinetics and teratogenicity of TRA in rats given a single dose or the same total amount in divided doses over a period of a few hours. This study raises further doubts about risk assessments based on circulating plasma concentrations of TRA and its metabolites.

Because of these difficulties, the concentrations and/or the kinetics of the circulating retinoids following the intake of a given vitamin A dose cannot be used at the present time as the basis for deriving a tolerable upper intake level.

4.6. Selection of effect(s) on which to base the upper level

A number of adverse effects have been reported at intakes of preformed vitamin A above the population reference intake. The lowest doses reported to produce the different effects are:

Bulging fontanelle	7500 μg RE (as a single dose in infants)
Hepatotoxicity	7500 μg RE/day for 6 years
Bone density/fracture	1500 μg RE/day (trend analyses do not show a threshold)
Lipid metabolism	7500 μg RE/day for 4 years (but a minor change only)
Teratogenicity	>3000 μg RE/day (based on Rothman *et al*, 1995)

It is clear from this that the hazards and their associated doses are different for different groups of the population. In addition the severity of the adverse effect varies from minor to irreversible.

The associations between vitamin A and bone mineral density and the risk of bone fracture (Melhus *et al*, 1998; Feskanich *et al*, 2002) were reported at lower intakes than the other adverse effects listed above. The associations were based on the analysis of data for women in Sweden aged 40-76 years, and for women in the US aged 34-77 years. Middle aged and elderly women probably represent the most sensitive group for such effects. However, the dose-response arose from normal dietary intakes and non-prescription use of supplements, so that it is not possible to establish a clear tolerable upper intake level. Both studies indicate that intakes as low as 1500 μg RE/day might pose a risk. Whether the same dose-response would apply to men is not known. The statistical analyses showed a relationship after multivariate analysis and the possible impact of correction for confounding on the data is of concern, especially at such low relative risks.

Previous evaluations of the risk of high intakes of vitamin A have concentrated on teratogenicity, because this is an irreversible form of toxicity that occurs at low intakes. As indicated above, a tolerable upper intake level based on this effect would also allow for other adverse effects, with the possible exception of changes in bone mineral density and the risk of bone fracture.

5. DERIVATION OF A TOLERABLE UPPER INTAKE LEVEL

Determining an upper level for preformed vitamin A is difficult, because any proposal has to take into account the narrow margin between the population reference intake and the intakes associated with adverse effects.

The findings on bone density and the risk of fracture were reported at lower daily intakes than other adverse effects. However, it was considered that the currently available data did not provide sufficient evidence of causality, and were not appropriate for establishing a tolerable upper level.

The teratogenic potential of vitamin A has received the most attention in previous evaluations, probably because of the severe and irreversible nature of this form of toxicity. A clear dose-response has been provided in the paper of Rothman *et al* (1995), but this was derived by fitting a curve to the available data that included a very large number of subjects from the at risk group of the population, but only a few cases at intakes above the suggested threshold of 3000 μg RE/day. The study of Mastroiacovo *et al* (1999), indicated that the threshold could be at higher intakes. In consequence the establishment of a clear threshold for teratogenicity is difficult, but a cautious approach would be to use the low value from the study of Rothman *et al* (1995). An uncertainty factor is not considered necessary, because the data from other studies indicated that the true threshold for an effect could be higher than this value. Based on these studies a tolerable upper level of 3000 μg RE/day is suggested for all women of child-bearing age (because the risk occurs very early in pregnancy). This value is 2.5-fold lower than the daily intake that might cause hepatotoxicity in women during chronic intake. The study of Rothman *et al* (1995) estimated the intakes of preformed vitamin A from all sources, and therefore the tolerable upper level applies to intakes from both foods and supplements.

Although teratogenicity is only relevant to women of child-bearing age, the upper level of 3000 μg RE/day is appropriate for men, and for infants and children after correction for differences in metabolic rate, because it is 2.5-fold lower than the lowest daily intake that has been associated with hepatotoxicity during chronic intake. This upper level does not apply to postmenopausal women, who represent the group at greatest risk of bone fracture, because it may not provide an adequate margin of safety in relation to the possible decrease in bone density and the risk of bone fracture. Further data to clarify the possible contributions of confounding to the reported increase in risk of bone fracture would provide greater confidence in a true cause-effect relationship at such low levels of intake.

Because the tolerable upper intake level relates to the risk of hepatotoxicity as well as effects produced during reproduction, it applies to intakes during pregnancy and lactation.

The tolerable upper level for children is based on the value of 3000 μg RE/day for adults, with correction for differences in basal metabolic rate compared to adults using scaling according to body surface area (body weight$^{0.75}$).

Age (years)	Tolerable Upper Intake Level (UL) for preformed vitamin A (retinol and retinyl esters) (µg RE/day)
1- 3	800
4 - 6	1100
7- 10	1500
11-14	2000
15- 17	2600
Adults+	3000

+ Women of child-bearing age and men (see text for advice concerning postmenopausal women).

6. RISK CHARACTERISATION

1. The tolerable upper level applies to both dietary and supplemental intakes of vitamin A.

2. The 97.5 percentile intake for adults in most of Europe is greater than 3000 µg RE/day.

3. Because alterations of embryogenesis may occur following a single or a small number of doses of vitamin A, for women of child bearing age the upper level should be compared with intake estimates that reflect short-term, rather than long term exposure.

4. The current recommendations that women who are planning to become pregnant or who are pregnant should not consume cooked animal livers (SCF, 1992) should be maintained.

5. Because the tolerable upper level may not adequately address the possible risk of bone fracture in particularly vulnerable groups, it would be advisable for postmenopausal women, who are at greater risk of osteoporosis and fracture, to restrict their intake to 1500 µg RE/day.

6. Because the current intakes may exceed the tolerable upper level, careful consideration should be given to the appropriateness of the enrichment of human foods with vitamin A, and to the potential effects on human exposure of the addition of vitamin A to animal feed.

7. RECOMMENDATIONS

1. The possible link between bone density, the risk of fracture and vitamin A intake should be reviewed when further data become available.
2. Ideally, resolution of the issue of bone mineral density and the risk of fracture should be studied by a prospective study, in which the effects of age on the risk, and also of confounding variables are taken into account in the study design. It is recognised that such a study would require a very large population and prolonged treatment and follow up.

8. REFERENCES

Adams J and Lammer EJ (1991). Relationship between dysmorphology and neuro-psychological function in children exposed to isotretinoin in utero. In: Functional Neuroteratology of Short-term Exposure to Drugs. Fujii T, Boer GJ (eds.), pp. 159-170.

Agoestina T, Humphrey JH, Taylor GA, Usman A, Subardja D, Hidayat S, Nurachim N, Wu L, Friedman DS, West KP, Sommer A (1994). Safety of one 52-mumol (50,000 IU) oral dose of vitamin A administered to neonates. Bull WHO 72: 859-868.

Babikian P, Corbett J, Bell W (1994). Idiopathic intracranial hypertension in children: the Iowa experience. J Child Neurol 9: 144-149.

Ballew C, Galuska D, Gillespie C (2001). High serum retinyl esters are not associated with reduced bone mineral density in the Third National Health and Nutrition Examination Survey 1988-1994. J Bone Mineral Res 16: 2306-2312.

Baqui AH, de Francisco A, Arifeen SE, Siddique AK, Sack RB (1995). Bulging fontanelle after supplementation with 25,000 IU of vitamin A in infancy using immunization contacts. Acta Pediatr 84: 863-866.

Bauernfeind JC (1980). The safe use of vitamin A. A report of the International Vitamin A Consultative Group (IVACG). The Nutrition Foundation, Washington D.C., pp. 1-44.

Biesalski HK (1989). Comparative assessment of the toxicology of vitamin A and retinoids in man. Toxicology 57: 117-161.

Biesalski HK, Hemmes C, El Hanafy M, Weiser H, Zschaebitz H, Stofft E (1996). Long-term administration of high dose vitamin A to rats does not cause fetal malformations: macroscopic, skeletal and physico-chemical findings. J Nutr 126: 973-983.

Binkley N, Krueger D (2000) Hypervitaminosis A and bone. Nutr Rev 58: 138-144.

Botto LD, Khoury MJ, Mulinare J, Erickson JD (1996). Periconceptional multivitamin use and the occurrence of conotruncal heart defects: results from a population-based, case-control study. Pediatrics 98: 911-917.

Buss NE, Tembe EA, Prendergast BD, Renwick AG, George CF (1994). The teratogenic metabolites of vitamin A in women following supplements and liver. Hum Exp Toxicol 13: 33-43.

Cartmel B, Moon TE, Levine N (1999). Effects of long-term intake of retinol on selected clinical and laboratory indexes. Am J Clin Nutr 69: 937-943.

Chan A, Hanna M, Abbott M, Keane RJ (1996). Oral retinoids and pregnancy. Med J Australia 165: 164-167.

Cohen-Tanugi A and Forest N (1998). Retinoic acid suppresses the osteogenic differentiation capacity of murine osteoblast-like 3/A/1D-1M cell cultures. Differentiation 63: 115-123.

Collins MD and Mao GE (1999). Teratology of retinoids. Ann Rev Pharmacol Toxicol 39: 399-430.

Creech-Kraft J, Nau H, Lammer E, Olney A (1989). Embryonic retinoid concentrations after maternal intake of isotretinoin. N Engl J Med 321: 262.

Davis BH, Pratt BM, Madei JA (1987). Retinol and extra-cellular collagen matrices modulate hepatic Ito cell collagen phenotype and cellular retinol-binding protein levels. J Biol Chem 262: 280-286.

De Francisco A, Chakraborty J, Chowdhury HR, Yunus M, Baqui AH, Siddique AK, Sack RB (1993). Acute toxicity of vitamin A given with vaccines in infancy. Lancet 342: 526-527.

Dhem A and Goret-Nicaise M (1984). Effects of retinoic acid on rat bone. Fd Chem Toxic 22: 199-206.

Dudas I and Czeisel AE (1992). Use of 6,000 IU Vitamin A during early pregnancy without teratogenic effect. Teratology 45: 335-336.

Duester G (1996). Involvement of alcohol dehydrogenase, short-chain dehydrogenase, and cytochrome P-450 in the control of retinoid signaling by activation of retinoic acid synthesis. Biochemistry 35: 12221-12227.

Eckhoff C and Nau H (1990). Vitamin A supplementation increases levels of retinoic acid compounds in human plasma: possible implications for teratogenesis. Arch Toxicol 64: 502-503.

Ellis JK, Russell RM, Makrauer FL, Schaefer EJ (1986). Increased risk for vitamin A toxicity in severe hypertriglyceridemia. Ann Int Med 105: 877-879.

Elmadfa I, Burger P, Derndorfer E, Kiefer I, Kunze M, König J, Leimüller G, Manafi M, Mecl M, Papathanasiou V, Rust P, Vojir F, Wagner K-H, Zarfl B (1998). Austrian Study on Nutritional Status (ASNS). Österreichischer Ernährungsbericht. Bundesministerium für Gesundheit, Arbeit und Soziales. Wien.

Feskanich D, Singh V, Willett WC, Colditz GA (2002). Vitamin A intake and hip fractures among postmenopausal women. JAMA 287: 47-54.

Freudenheim JL, Johnson NE, Smith EL (1986). Relationships between usual nutrient intake and bone-mineral content of women 35-65 years of age: longitudinal and cross-sectional analysis. Am J Clin Nutr 44: 863-876.

Geubel AP, De Galocsy C, Alves N, Rahier J, Dive C (1991). Liver damage caused by therapeutic vitamin A administration: estimate of dose-related toxicity in 41 cases. Gastroenterology 100: 1701-1709.

Gangemi M, Maiuri F, Di Martino L, Pettoello M (1985). Intracranial hypertension due to acute vitamin A intoxication. Acta Neurol 7: 27-31.

Gregory J, Foster K, Tyler HA, Wiseman M (1990). The dietary and nutritional survey of British adults. ISBN 0 11 691300 2. London: HMSO.

Guarascio P, Portmann B, Visco G, Williams R (1983). Liver damage with reversible portal hypertension from vitamin A intoxication: demonstration of Ito cells. J Clin Pathol 36: 769-771.

Hathcock J, Hattan DG, Jenkins MY, McDonald JT, Sundaresan PR, Wilkening VL (1990). Evaluation of vitamin A toxicity. Am J Clin Nutr 52: 183-202.

Heseker H, Adolf T, Eberhardt W, Hartmann S, Herwig A, Kübler W, Matiaske B, Moch KJ, Nitsche A, Schneider R, Zipp A (1992). Lebensmittel- und Nährstoffaufnahme Erwachsener in der Bundesrepublik Deutschland. VERA- Schriftenreihe Band III, Wiss. Fachverlag Dr. Fleck, Niederkleen.

Holson RR, Gazzara RA, Ferguson SA, Ali SF, Laborde JB, Adams J (1997a). Gestational retinoic acid exposure: a sensitive period for effects on neonatal mortality and cerebellar development. Neurotoxicol Teratol 19: 335-346.

Holson RR, Gazzara RA, Ferguson SA, Adams J (1997b). A behavioral and neuroanatomical investigation of the lethality caused by gestational day 11-13 retinoic acid exposure. Neurotoxicol Teratol 19: 347-353.

Holson RR, Gazzara RA, Ferguson SA, Adams J (1997c). Behavioral effects of low-dose gestational day 11-13 retinoic acid exposure. Neurotoxicol Teratol 19: 355-362.

Houtkooper LB, Ritenbaugh C, Aickin M, Lohman TG, Going SB, Weber JL, Greaves KA, Boyden TW, Pamenter RW, Hall MC (1995). Nutrients, body composition and exercise are related to change in bone mineral density in premenopausal women. J Nutr 125: 1229-1237.

Hruban Z, Russell RM, Boyer JL, Glagov S, Bageri SA (1974). Ultrastructural changes in livers of two patients with hypervitaminosis A. Am J Pathol 76: 451-468.

Hülshof KFAM and Fruizinga AG (1999). Third Dutch National Food Consumption Survey

(DNFCS-3) 1997-1998. TNO Zeist, The Netherlands.