

## Risk Assessment

## Vitamin A (Retinol)

### General information

#### Chemistry

The term 'vitamin A' refers to a group of fat-soluble compounds known as 'the retinoids'. Generally, their structure consists of a  $\beta$ -ionone ring, a conjugated isoprenoid side chain and a polar terminal group. The parent compound all-*trans*-retinol and its fatty acid ester derivatives are referred to as pre-formed vitamin A, as opposed to the vitamin A precursors, such as  $\beta$ -carotene. Following the recommendations of the Food Agricultural Organisation (FAO) and World Health Organisation (WHO), estimations of vitamin A requirement and food content take into account the different activities and variable absorption of vitamin A and provitamin A, and are expressed in terms of 'retinol equivalent' (RE) where  $1 \mu\text{g RE} = 1 \mu\text{g retinol} = 1.78 \mu\text{g retinyl palmitate} = 6 \mu\text{g } \beta\text{-carotene} = 12 \mu\text{g other carotenoids with provitamin A activity} = 3.33 \text{ IU vitamin A activity from retinol}$ .

#### Natural occurrence

The primary sources of vitamin A are the precursor compounds, the carotenoids, which are largely plant derived and retinyl esters found in foods of animal origin.

#### Occurrence in food, food supplements and medicines

Foods rich in pre-formed vitamin A (retinol, retinyl esters) include dairy products (90-300  $\mu\text{g RE/kg}$ ), fortified margarine (approximately 330  $\mu\text{g RE/kg}$ ), liver (approximately 3500  $\mu\text{g RE/kg}$ ) and fish oils. Other sources of exposure include single- or multi-vitamin supplements (at levels up to 2400  $\mu\text{g RE}$  per daily dose) and synthetic forms prescribed for therapeutic purposes, e.g. certain skin disorders.

#### Other sources of exposure

No other sources of exposure have been identified.

#### Recommended amounts

The UK RNI ranges set by COMA are 700  $\mu\text{g RE/day}$  for adult males and 600  $\mu\text{g RE/day}$  for adult females (COMA, 1991). The US recommended dietary allowance (RDA) is 1000  $\mu\text{g RE/day}$  for adult males and 800  $\mu\text{g RE/day}$  for adult females. The WHO recommended dietary intake (RDI) is 600  $\mu\text{g RE/day}$  for adult males and 500  $\mu\text{g RE/day}$  for adult females.

## Analysis of tissue levels and vitamin A status

The hepatic concentration of retinyl esters is the most objective measure of vitamin A status, but cannot be readily determined in living individuals. The plasma retinol concentration is under homeostatic control by the synthesis of retinol binding protein, and therefore is an insensitive indicator of status, except in cases of extreme depletion when other signs of deficiency are already evident. Hypervitaminosis A may be characterised by elevated levels of plasma retinyl esters, which normally contribute to <5% of blood vitamin A. Other methods used to determine vitamin A status in humans include dietary assessment, clinical evaluation, retinal function tests and assessment of retinol-binding proteins.

## Brief overview of non-nutritional beneficial effects

Vitamin A is used in the treatment of some skin disorders including acne, psoriasis and ichthyosis. Vitamin A has been suggested to be beneficial when used as a chemo-preventative or adjuvant agent in the treatment of some cancers and may improve lung function in chronic obstructive pulmonary disease. Vitamin A is used as a treatment for abnormal dark-adaptation and is included in eye drops used to treat blurred vision, cataracts, glaucoma, conjunctivitis and dry eyes. Vitamin A (along with zinc) has been suggested to be a useful adjunct in the treatment of anaemia with iron. Vitamin A is also taken as an anti-oxidant dietary supplement.

## Function

Vitamin A is essential to the processes of vision, reproduction, embryonic development, morphogenesis, growth and cellular differentiation. With the exception of the visual process, most processes are related to the control of gene expression, with vitamin A metabolites, such as retinoic acid, acting as nuclear receptor-ligands.

## Deficiency

Vitamin A deficiency is a problem largely in developing countries. It can lead to night blindness and xerophthalmia, growth retardation, keratinisation of epithelia, impaired hearing, taste and smell, increased susceptibility to infection, increased child mortality and reduced male fertility. In pregnancy, vitamin A deficiency can result in malformations in offspring. Deficiency in the developed world is usually limited to those with absorption difficulties, increased susceptibility to opportunistic infections, chronic liver disease and alcoholics.

## Interactions

Vitamin A may potentiate the development of intracranial hypertension when taken in combination with tetracycline and minocycline type antibiotics.

The catabolism of retinol and retinoic acid in the human liver may be mediated by cytochrome P450. Drugs such as ketoconazole, which inhibit cytochrome P450, can significantly increase the half-life of retinoic acid.

Hypervitaminosis A may decrease vitamin C tissue storage. Vitamin A may antagonise the action of vitamin K in blood clotting function.

Hypervitaminosis A may have an anti-thyroid effect. Deficiency in zinc may adversely affect mobilisation of vitamin A from hepatic stores and absorption of vitamin A from the gut.

Vitamin A deficiency may result in impaired iron absorption and decrease its utilisation for erythropoiesis.

Alcohol may potentiate vitamin A-induced hepatotoxicity. Competitive inhibition of alcohol dehydrogenase may lead to decreased synthesis of retinoic acid, resulting in functional vitamin A deficiency, which has been postulated to be involved in foetal alcohol syndrome.

### Absorption and bioavailability

Approximately 80% of dietary pre-formed vitamin A is absorbed but this may be reduced if diets are low in fat or individuals are suffering from fat malabsorption syndrome. The form of preparation influences the rate and extent of absorption of vitamin A from supplements. Thus, aqueous dispersions and emulsions achieve higher plasma levels, at a faster rate, with lower faecal losses, than oily solutions. The pharmacokinetic handling of retinol from foods, such as liver, differs to that obtained from supplements.

Dietary retinyl ester is released from food by proteolytic digestion and hydrolysed to retinol in the gut.

### Distribution and metabolism

The retinol is taken up into enterocytes, undergoes re-esterification and is incorporated into chylomicra, which are released into the circulation via the lymph. Following the breakdown of chylomicra by serum lipases, the retinyl esters are released, taken up by hepatocytes and re-hydrolysed. The resulting retinol is transferred to the stellate (fat storing) cells and stored in the form of long-chain fatty esters. Approximately 90% of the body's vitamin A is stored in the liver this way. The availability of hepatic stores of vitamin A may be decreased if protein status is low.

Plasma retinol is usually maintained under tight homeostatic control and concentrations do not alter significantly unless hepatic stores are severely depleted. If hepatic storage capacity is exceeded, plasma levels of retinyl ester increase, but plasma levels of retinol itself do not.

Mobilised retinol is transported in plasma bound to retinol-binding protein and transthyretin. Uptake into extra-hepatic tissues occurs via a receptor-mediated process. Once inside the cell, retinol undergoes a complex series of metabolic oxidations, isomerisations and conjugations, most of which are reversible. Several enzymes are involved in these reactions, including cytochromes P450. Cellular binding proteins direct the reactions. Other intracellular binding proteins facilitate transport of specific vitamin A metabolites, such as retinoic acid, into the nucleus of the cell, where they interact with the retinoid nuclear receptors (RARs and RXRs) and participate in the control of gene expression for differentiation and growth.

### Excretion

Oxidised products are excreted in the urine or conjugated with glucuronic acid and excreted in the urine or bile.

## Toxicity

### Human data

#### *Acute toxicity*

Symptoms of acute vitamin A toxicity include abdominal pain, anorexia, vomiting, blurred vision, irritability, headache, and in neonates and infants, bulging of fontanelles. Acute toxicity is associated with doses well in excess of 100,000  $\mu\text{g}$  RE and 10,000  $\mu\text{g}$  RE, in adults and children, respectively. Infants of < 6 months have been shown to develop acute symptoms following a single dose of 7500-15,000  $\mu\text{g}$  RE, whereas a dose of 30,000  $\mu\text{g}$  RE appears to be well-tolerated in older infants (6 and 9 months of age).

#### *Chronic and sub-chronic toxicity*

Symptoms of chronic toxicity include dry thickening of the skin, cracking of lips, conjunctivitis, erythematous eruption, alopecia, reduced bone mineral density, bone joint pain, chronic headache, intracranial hypertension and hepatotoxicity. The onset and severity of toxic manifestations are dependent on dose, duration and the manifestation in question. Damage to the eyes, bone and liver may be permanent, but most other symptoms are reversible. Chronic toxicity in adults is generally attributed to supplemental doses of > 7500-15,000  $\mu\text{g}$  RE/day, over weeks, months or years. However, there have been cases of toxicity associated with lower doses of ~1500-3,000  $\mu\text{g}$  RE/day. Determination of a threshold dose for chronic toxicity may be confounded by pre-existing disease, alcohol abuse, drug therapy and limited knowledge of dietary intake. Extreme vitamin A intolerance may have a genetic basis, although the precise metabolic defect has not yet been elucidated.

Epidemiological data have shown that the risk of hip fracture in postmenopausal women may be doubled when dietary retinol intake is >1500  $\mu\text{g}$  RE/day, compared to an intake of 500  $\mu\text{g}$  RE/day. Other supporting epidemiological data have shown that hip fracture risk is higher in Northern European countries, particularly Scandinavia, where dietary intake of retinol is higher. Differences in fracture are such that Swedish men have a higher hip fracture rate than English or Swiss women.

#### *Developmental toxicity*

There are a number of case reports of vitamin A-associated birth defects. However, only one case involved a vitamin A exposure of < 7500  $\mu\text{g}$  RE/day. Since 1990, seven epidemiological studies (five case-control and two prospective) have been reported. Four studies failed to establish an association between vitamin A exposure and birth defects, whereas three studies determined a teratogenic effect at varying levels.

#### *Supplementation trials*

In the ( $\beta$ -Carotene Retinol Efficacy study) CARET trial, a combination of 7500  $\mu\text{g}$  retinol/day and 30,000  $\mu\text{g}$ /day  $\beta$ -carotene resulted in an increase in lung cancer in male smokers and males who had been exposed to asbestos. However, this and the ABTC study<sup>19</sup> indicated strongly that the  $\beta$ -carotene

<sup>19</sup> See  $\beta$ -carotene risk assessment for details of these studies.

component was responsible for this effect. A chemopreventive study in workers exposed to blue asbestos found that 45/512 subjects taking 7500  $\mu\text{g RE/day}$  elected to change treatment because of associated headaches. A modest increase in skin cancer was apparent in subjects given 7500  $\mu\text{g retinol/day}$  compared to isotretinoin or controls. In a chemopreventative trial, patients with lung cancer or head and neck cancer, were given 90,000  $\mu\text{g RE/day}$  as retinyl palmitate for one year followed by 45,000  $\mu\text{g RE/day}$  for a further year. Side effects including dryness, and itching of skin and bleeding and hair loss were reported by 45%.

## Animal data

### *Acute and chronic toxicity*

Vitamin A toxicity in animals is dependent upon dose, formulation, duration, species, and age. Lethal doses in rat and mouse are  $> 2,500,000 \mu\text{g/kg bw}$ . Chronic toxicity in adult rats begins at about 3000  $\mu\text{g RE/kg bw/day}$ . Younger animals may be more susceptible. Hypervitaminosis A causes anorexia, weight loss, anaemia, cachexia and ultimately death in rats, hamsters, mice and dogs. Chronic manifestations include effects on skin (hair loss, localised erythema and thickened epithelium), effects on internal organs (fatty infiltration of the liver and fatty changes in the heart and kidney, testicular changes, degeneration of myocardial fibres), blood effects (decreased haemoglobin, transient increases in circulating lipids and serum cholesterol, elevated serum triglycerides), skeletal effects (limping gait, spontaneous fractures, hind leg paralysis, increased osteoclast activity, decreased osteoblast activity, bone elongation without thickening, reduced formation of dentine and atrophy of odontoblasts), subcutaneous and intramuscular haemorrhage, hypothermia, internal haemorrhage, and inflammation of nasal passage, gut, and conjunctiva. Hypervitaminosis A results in the development of gallstones in the hamster.

### *Developmental toxicity*

Retinol is teratogenic in laboratory animals, with the following order of increasing susceptibility: rat and mouse, hamster, monkey, and rabbit. The lowest teratogenic doses reported in rat, monkey and rabbit are 35,000, 6000 and 2500  $\mu\text{g RE/kg bw/day}$ , respectively. Malformations are largely analogous to those associated with synthetic retinoid teratogenicity in humans, such as anencephaly, spina bifida, cleft lip, cleft palate, micronathia, microphthalmia, several types of malformation to the ear, teeth, salivary glands and aortic arch, ventricular septal defects, imperforate anus, omphalocele, renal agenesis, polycystic kidney, hydronephrosis, phocomelia, digit malformation, certain defects of the genitalia, pituitary, thyroid, thymus, skull, vertebrae, ribs, and muscles and situs inversus (Hathcock *et al.*, 1990 and references therein). Permanent learning disabilities have been seen in F344 rats born to dams exposed  $\geq 3000 \mu\text{g RE/kg/day}$ .

## Carcinogenicity and genotoxicity

There is no evidence to suggest that retinol or retinyl esters are carcinogenic in laboratory animals and at sub-toxic doses, vitamin A can be highly effective in preventing chemically induced tumours. Epidemiological studies have generally shown a negative association between vitamin A intake and cancer.

The weight of evidence suggests that neither retinol nor retinyl ester are genotoxic.

## Mechanisms of toxicity

Retinoic acid plays an important role in the control of expression of many genes, including *Hox* genes, which are vital for correct positional and sequential development of the embryo. The morphogenic action of retinoic acid is mediated through the actions of retinoic acid receptor (RAR and RXR) bound material on nuclear retinoic acid response elements. The most sensitive exposure period, in both humans and animals, coincides with development and differentiation of the facial neural crest and formation of the branchial apparatus (pharyngeal arches), structures from which most retinoid-associated birth defects are derived. The evidence related to vitamin A in humans remains circumstantial, but there is a strong suggestion that retinoid teratogenicity may be the outcome of an exaggerated normal response, resulting from abnormal, inappropriate or prolonged transcriptional activation of critical genes in target embryonic cells.

Although there is some disagreement in the literature, much of the evidence so far indicates that the teratogenic effects of retinol and other naturally occurring retinoid compounds are due to their conversion to all-*trans*-retinoic acid. Interspecies differences make comparisons of teratogenic potency and extrapolation for human risk assessment difficult.

## Dose response characterisation

Symptoms of chronic toxicity in humans, such as thickening and dryness of the skin, cracking of lips, conjunctivitis, erythematous eruption, alopecia, reduced bone mineral density, bone joint pain, chronic headache, intracranial hypertension and hepatotoxicity, are generally associated with supplemental doses of 7500 µg RE/kg bw/day. However, decreases in bone density and increases in fracture risk have been reported with chronic exposure to lower doses of 1500 to 2000 µg RE/kg bw/day.

In humans, teratogenic effects have been reported at 3000-9000 µg RE/day (equivalent to 50-150 µg RE/kg bw/day). The lowest teratogenic doses reported in rat, monkey and rabbit are 35,000, 6000 and 2500 µg RE/kg/day, respectively.

## Vulnerable groups

Retinol may represent a teratogenic risk, particularly within the first trimester of pregnancy. Other groups potentially vulnerable to vitamin A toxicity include the young; older people; those suffering from osteoporosis, chronic renal failure, diabetes mellitus or under-nutrition; haemodialysis patients and individuals with compromised liver function. An apparent vitamin A intolerance has been observed in some children (Carpenter *et al.*, 1987). It has been suggested that there may be a genetic component to these cases. However, the basis of this has not been defined.

## Genetic variations

No genetic variations that increase the likelihood of vitamin A toxicity have been identified.

## Studies of particular importance in the risk assessment

(For full review see <http://www.food.gov.uk/science/ouradvisors/vitandmin/evmpapers> or the enclosed CD)

### Human studies – developmental toxicity

Retinoids are recognised animal teratogens, and isotretinoin (13,*cis*-retinoic acid) is a known human teratogen; therefore, developmental toxicity is critical to the risk assessment of vitamin A.

*Martinez-Frias and Salvador, 1990*

This paper reported the results of a retrospective epidemiological study of prenatal exposure to high doses of vitamin A, using data from a Spanish hospital-based, case control registry (Spanish Collaborative study of Congenital Malformations, a surveillance programme which monitored  $> 10^6$  births). Newborns from 58 hospitals were examined for malformations during the first 3 days of life, and their mothers asked an open-ended question about drug exposures during pregnancy. The mothers were not directly asked about vitamin use. The investigators studied 12,625 cases and 12,525 controls, becoming 11,293 and 11,193, respectively, after eliminations for chromosomal malformations. They considered only women who had used 3000  $\mu\text{g}$  RE/day or more of retinyl palmitate, either alone or as a multivitamin. No information on dietary intake was provided. The number of exposures was very low (16 cases, 14 controls). There was no overall association between vitamin A exposure total supplementary vitamin A and birth defects (odds ratio (OR) =1.1 [95% CI 0.5, 2.5]  $p=0.4$ ). However, for retinyl palmitate exposure alone, (10, cases, 1 control), the odds ratio was 9.9 [95% CI 1.4, 430.1]  $p= 0.006$ ). The OR for exposure to vitamin A in multivitamin complexes (6 cases, 13 controls) was not significantly different to 1 (OR= 0.5 [95% CI 0.2, 1.3]  $p=0.08$ ). The OR for exposures of  $<12,000$   $\mu\text{g}$  RE/day was 0.5 [95% CI 0.1, 1.6]  $p=0.15$  (5 cases, 10 controls) and for exposures to  $> 12,000$   $\mu\text{g}$  RE/day, OR=2.7 [95% CI, 0.8, 11.7]  $p=0.06$  (11 cases, 4 controls). The authors concluded that their results suggested that a teratogenic effect might exist at high levels of exposure. However, their dose-response data are also consistent with no effect. Furthermore, the number of subjects exposed was very small and some exposures may have occurred too late on in pregnancy to be relevant to the birth defects found.

*Werler et al., 1990*

This paper reports a retrospective case control study from the Slone Epidemiology Unit Birth Defects Study, a multi-hospital surveillance programme. The aim of the study was to evaluate the relationship between vitamin A supplementation during pregnancy and malformations of structures derived, at least in part, from the cranial neural crest.

Birth defects were identified from hospital records. Women were interviewed within 6 months of delivery and asked whether they had taken any vitamins, iron or folic acid during the 6 months prior to or at any time during pregnancy. No data on vitamin A dose, number of supplements or diet were collected. Cases were 2,658 infants with primarily craniofacial and cardiac malformations. Controls were 2,609 infants with other malformations. Mothers of 6 controls used vitamin A supplements in each of the of the first 3 lunar months of pregnancy compared to mothers of 14, 14 and 10 cases in lunar months 1, 2 and 3, respectively. Relative risk estimates were 2.5 [95% CI 1.0, 6.2] for lunar month 1, 2.3 [95% CI 0.9, 5.8] for lunar month 2 and 1.6 [95% CI 0.6, 4.5] for lunar month 3. No statistically significant association between vitamin A exposure and birth defects was made. However, the numbers of cases and controls exposed to vitamin A supplements were small. The authors also examined specific common defects

and found no statistically significant positive association with vitamin A supplementation (95% CIs included 1). The authors also compared the risk to those taking multi-vitamins with those taking single vitamin A supplements. The risk was higher but did not achieve statistical significance.

*Rothman et al., 1995*

This paper reports a prospective study from an open prenatal screening programme at Boston University, in which 22,748 pregnant women were identified during prenatal screening. Nurse interviewers obtained information on diet, medication and illness during the first trimester and on family medical history. Information on pregnancy outcomes was obtained from obstetricians or from the mothers themselves. Three hundred and thirty-nine babies were born with birth defects and of these, 121 were identified as having defects originating in the cranial neural crest. The prevalence ratio of babies with defects born to women who had consumed  $> 4500 \mu\text{g RE/day}$  pre-formed vitamin A from food and supplements compared to those born to mothers who consumed  $\leq 1500 \mu\text{g RE/day}$  was 3.5 [95% CI 1.7, 7.3]. For vitamin A from supplements alone, the prevalence ratio among babies born to women who consumed  $\geq 3,000 \mu\text{g RE/day}$  compared to those born to mothers who consumed  $\leq 1500 \mu\text{g RE/day}$  was 4.8 [95% CI 2.2, 10.5]. Using a smoothed regression curve, the authors suggested a threshold of near  $3000 \mu\text{g RE/day}$  of supplemental vitamin A.

There has been some criticism of the design of this study and the analysis of the data. This study had no geographical or other population base and no denominator population, which may have introduced bias to the results. Furthermore, physicians identified malformations in only 76.5% of cases. In the remaining cases, the mother supplied information relating to incidence of malformation, so that there is uncertainty that all malformations were correctly classified.

*Khoury et al., 1996*

This is a communication (letter), in response to the publication of the Rothman study, from investigators reporting re-examination of data from a large population-based case-control study of major birth defects, conducted by the Centres for Disease Control. Using the same classification of defect as in the Rothman *et al.* study, the authors found no overall increased risk of birth defects and no increased risk of cranial neural crest defects among users of vitamin A and/or multi-vitamin supplements (OR = 0.6 [95% CI 0.28, 1.29] and 0.69 [95% CI 0.24, 1.91], respectively). However, no data were collected on vitamin A dosage although the authors noted that most multi-vitamins and supplements during the study period were expected to contain  $< 2400 \mu\text{g RE}$  pre-formed vitamin A (which is less than the  $3000 \mu\text{g RE/day}$  suggested as a threshold by the Rothman study).

*Shaw et al., 1996*

This is a communication (letter) also in response to the publication of the Rothman study. The authors re-examined geographical population-based surveillance data from previously published studies of oral cleft and conotruncal defects of the heart (both structures associated with the cranial neural crest). The authors assumed that most preparations contained pre-formed vitamin A rather than  $\beta$ -carotene, that single supplements would have contained at least  $3000 \mu\text{g RE}$  and that combination exposures of single and multi-vitamin supplements would exceed  $3000 \mu\text{g RE}$ . There was no increased risk of either birth defect associated with the use of vitamin A supplements (OR = 0.55 [95% CI 0.21, 1.5] and OR = 0.0 [95% CI 0.0, 2.2], oral cleft and conotruncal heart defect studies, respectively). These data were limited in that interviews with the mothers were conducted an average of 3.5 years after delivery and lacked specific information regarding vitamin A dosage.

*Mills et al., 1997*

This paper describes a geographically based case-control study (encompassing all pregnancies in California and Illinois between 1985-87) to examine whether moderate doses of vitamin A are associated with teratogenicity. The study group included women whose pregnancies resulted in offspring with neural tube defects (n=548), offspring with major malformations other than neural tube defects (n=387) and normal controls (n=573). Study participants gave telephone interviews to trained interviewers between 1 and 5 months after the detection of the congenital anomaly, in order to estimate periconceptional vitamin A exposures from supplements, fortified cereals, meat and offal consumption. The proportion of women consuming 2400–7500 µg RE was no greater in either of the malformation groups than in the control group. Women exposed to > 2400 µg RE/day and > 3000 µg RE/day had odds ratios for major malformations of 0.79 [95% CI 0.4, 1.53] and 0.73 [95% CI 0.27, 1.96], respectively when compared to women consuming < 1500 µg RE/day (the result for neural tube defects was similar). Consequently, no association was made between periconceptual exposure to vitamin A at doses > 3000 µg RE/day. However, unlike the study by Rothman *et al.*, dietary recall was many months after the critical period of pregnancy.

*Mastroiacovo et al., 1999*

This paper describes a multi-centre prospective controlled study to evaluate whether foetuses exposed during organogenesis to high doses of vitamin A have a higher risk of major malformations than the general population. Thirteen European Teratology Information Services (all part of ENTIS – European Network Teratology Service) counselled women during pregnancy. Data were collected on 423 pregnancies exposed to a maternal daily dose of at least 3000 µg supplemental RE during the first nine completed weeks of gestation. 394 women were followed up by telephone interview until the first few weeks after expected delivery date using standardised procedures. Information about malformations was obtained from mothers or doctors. No information on dietary intake was obtained. The occurrence of major structural malformations, excluding chromosomal and genetic diseases, was evaluated in 311 infants exposed to a median daily dose of 15,000 (range 3000-90,000) µg RE/day. Only three infants with major malformations were reported and no congenital malformations were reported among 120 infants exposed to > 15,000 µg RE/day. The birth prevalence ratios of major malformations compared to two internal control groups (i) exposed to high levels of vitamin A later on in pregnancy and (ii) non-teratogenic agent exposures were 0.28 [95% CI 0.06-1.23] and 0.50 [95% CI 0.14, 1.76], respectively. Consequently, this study provided no evidence that there was an increased risk of major malformations associated with exposure to high doses of vitamin A during organogenesis. However, the sample size in this study was not large enough to enable the detection of differences in risk of less than 2.76.

### Human studies – general toxicity

*Wald et al., 1985*

In a randomised double-blind study, 376 people were studied and allocated to 1 of 7 dose regimens: 0, 3000, 4054, 6757, 8108, 9460 or 10,810 µg RE/day, for 6 months. The study was designed to investigate the effect of supplementation on serum retinol levels. The only adverse effects noted related to skin and mucous membranes with 6% of the subjects receiving 4054 µg RE or more reporting skin dryness and itching at their second clinic visit compared to 3% of subjects receiving 0 or 3000 µg RE/day.

*Hathcock et al., 1990*

This paper is a review of vitamin A-related chronic and sub-chronic toxicity data in humans. Clinical case studies reviewed supported the view that exposure to doses  $\geq 30,000 \mu\text{g RE/day}$  for short periods (days/few weeks) or exposure to  $7500 - 15,000 \mu\text{g RE/day}$  for several months or more can produce multiple adverse effects. Most evidence for toxicity at the lower end of this range was from reports of individuals who had concomitant hepatic damage from other contributing factors. Furthermore, information on dietary intake was not always available. The authors concluded that the data available were not sufficient to provide a specific minimum threshold for adverse effects but that  $7500 \mu\text{g RE/day}$  was nutritionally excessive and carried some risk of toxicity. Similarly, data did not permit identification of a safe upper limit for the intake range. It was noted that an intake of  $3000 \mu\text{g RE/day}$  was more than adequate for good nutrition but was low enough to avoid toxicity in most people. However the effects of vitamin A between  $3000 \mu\text{g RE/day}$  and  $7500 \mu\text{g RE/day}$  could not be predicted.

### Human studies – bone toxicity<sup>20</sup>

*Freudenheim et al., 1986*

In a 4 year clinical trial, the effect of usual intakes of energy and 14 nutrients on single-photon absorptiometric measurement of mineral content in arm bones was assessed in 99 women aged 35-60 given either a calcium supplement or placebo. In the post-menopausal women of the treatment group, there was an inverse correlation between vitamin A intake and the rate of change in ulna bone mineral content. In a single patient receiving a high supplemental dose (average intake  $4392 \mu\text{g RE/day}$ ) bone loss was very rapid with no other reason apparent.

*Sowers and Wallace, 1990*

Vitamin A intake, serum retinol concentrations, radial bone mass and fracture history were evaluated in 246 postmenopausal women. More than 36% of the population used a vitamin A supplement, with 8% using a supplement containing  $> 2000 \mu\text{g RE/day}$ . No relationship was observed between radial bone mass and fracture history and vitamin A intake or serum retinol. No statistically significant relationship was observed between serum retinol and bone mass after adjustment for factors associated with bone mass such as age, when the population was stratified by supplement use. When serum retinol values were divided into tertiles, no relationship was observed with bone mass when the comparison included adjustment for age, muscle area and use of thiazide anti-hypertensives. However, it has been noted (Binkley and Krueger, 2000) that this study had inadequate power to test an association between bone mass and vitamin A intakes  $> 2000 \mu\text{g RE/day}$  (only 8% of the study population had intakes exceeding this level); 36% of the population were aged  $< 60$  years of age and were therefore likely to be heterogenous with regards to oestrogen depletion bone loss; and the site where bone mass was measured, i.e. central radius, is considered less responsive to change.

<sup>20</sup> A cohort study of serum retinol levels and fracture risk in 266 men followed up over 30 years was recently published by K. Michaëlsson *et al.* (N. Engl. J. Med. 2003; **348**: 287-294). This study was published after the cut off date of 31 December 2002 for data to be considered by the EVM and it has therefore not been used for risk assessment. This prospective cohort study showed a statistically significant association between serum retinol levels and risk of fracture. The relative risk for all fractures in men in the highest quintile of serum retinol compared to middle quintile was 1.64 (95% CI, 1.12-2.41).

*Houtkooper et al., 1995*

The relationships between total energy intake, nutrient intake, body composition and exercise group status with rates of change in bone mineral density were measured in 66 pre-menopausal women taking calcium supplements. Nutrients were not significant variables in regression models predicting bone mineral density slopes at any femur site, but retinol intake was associated with decreased bone mineral density.

*Melhus et al., 1998*

This paper reported two studies, a randomly selected cross-sectional study, involving 175 females (28-74 years) and a nested case-control study, involving 247 women (40-60 years), who had first hip fracture 2-64 months after enrolment, and 873 age-matched controls (selected from a mammography study cohort). Retinol intake was estimated from dietary records and a food-frequency questionnaire. There was no reported use of vitamin A supplements.

In multivariate analysis, intake of pre-formed retinol was negatively associated with bone mineral density. For intake greater than 1,500  $\mu\text{g RE/day}$ , compared with less than 500  $\mu\text{g/day}$ , bone mineral density was reduced by 10% at the femoral neck ( $p=0.05$ ), 14% at the lumbar spine ( $p=0.001$ ) and 6% for the total body ( $p=0.009$ ). The risk for hip fracture was apparently doubled (odds ratio 2.1 [95% CI 1.1-4.0]). For every 1000 (g increase in daily intake, risk of hip fracture increased by 68% [95% CI 18-140%,  $p=0.006$ ]. Smoking was found to be a confounding factor.

The authors suggested that their study was limited by the possibility of information bias resulting from questioning case-patients after hip fracture had occurred, as was done for some covariates such as physical activity. In addition, data on thyroid hormone therapy and family history of osteoporosis were not available. Furthermore, the authors could not rule out confounding influences of an unidentified dietary factor but suggested that the possibility of a high degree of random error in the assessment of retinol intake might lead to an underestimation of the true risk of hip-fracture associated with high levels.

The rationale for this study was based on the findings of the MEDOS study group (Johnell *et al.*, 1992) that hip fracture rates varied across Europe, being 11 and 7 fold higher for women and men respectively in Northern Europe than in Southern Europe, particularly in Sweden and Norway. The difference in European rates was sufficiently marked that fracture rates were higher in Swedish men than in Swiss or English women. The authors further noted that the difference in incidence was higher between countries than between sexes suggesting that an important genetic or environmental factor was involved. Known risk factors were not thought to explain the finding. Melton (1995) also reported that hip fracture rates were higher in Scandinavia than in comparable populations in North America. When dietary patterns in Europe were compared in different European towns, retinol intakes were found to be 6 fold higher in Scandinavia, compared to Southern Europe (Cruz *et al.*, 1991).

*Ballew et al., 2001*

The association between fasting serum levels of retinyl esters and bone mineral density (BMD) was studied in 5790 non-pregnant participants aged 20 years or older. Data were also collected on age, body mass index, smoking, alcohol consumption, use of dietary supplements, diabetes, physical activity and, in women, use of oral contraceptives or oestrogen replacement therapy, menopausal status and parity. These covariates were controlled for using multiple linear regression. The study showed no significant association between fasting serum retinyl esters and BMD, as assessed at the femoral neck, trochanter, intertrochanter and total hip.

*Feskanich et al., 2002*

As part of the Nurses' Health Study, the relationship between high vitamin A intake from food and supplements and hip fracture was assessed. The study included 72,337 post-menopausal women aged 34-77 and the period 1980 to 1998 was considered in the analysis. During this period, 603 hip fractures occurred resulting from low or moderate trauma. After controlling for confounding factors, women in the highest quintile of vitamin A intake ( $\geq 3000 \mu\text{g RE/day}$ ) had a significantly elevated relative risk (RR) for hip fractures (RR, 1.48; 95% CI 1.05 to 2.07; P for trend 0.03) compared to women in the lowest quintile of intake ( $<1250 \mu\text{g RE/day}$ ). The increased risk was primarily attributable to retinol (RR, 1.89; 95% CI, 1.33-2.68). The association of retinol with hip fracture was reduced in women taking postmenopausal oestrogens.  $\beta$ -Carotene did not contribute to fracture risk. Women currently taking a specific vitamin A supplement had a non-significant 40% increased risk of hip fracture (RR, 1.4; 95% CI 0.99-1.99) compared to those not taking the supplement. Among women not taking supplements, retinol from food was significantly associated with fracture risk (RR, 1.69, 95% CI 1.05-2.74).

Overall the authors concluded that long-term intake of diet high in retinol may promote the development of osteoporotic hip fractures in women and that the amount of retinol in supplements and fortified foods may need to be reassessed. The authors noted that the study cohort was largely comprised of white women and that the findings were not necessarily applicable to other ethnic groups.

*Promislow et al., 2002*

The association between BMD and bone loss, and total and supplemental retinol intake was investigated in 570 women and 388 men, aged 55 to 92 at completion of the study. Dietary intake of vitamin A was assessed by dietary questionnaire over a four year period. This ended four years before the start of a 4-year period in which annual measurements of BMD and bone loss were made. Retinol intake was associated with decreased BMD and increased bone loss at total intakes above  $840 \mu\text{g/day}$ , even after adjustment for age, body mass index, weight change, calcium intake, years menopausal (women), use of steroids, cigarettes, alcohol, thiazides, thyroid hormones and supplemental retinol. Supplemental retinol use was also associated with decreased BMD and increased bone loss.

## Animal data

### *Developmental toxicity*

Vitamin A has been shown to be teratogenic in animals. The data are summarised below:

*Hendrickx et al., 1997a,b; Wiegand et al., 1998 and Miller et al., 1998*

Hendrickx *et al.* (1997a,b), Wiegand *et al.* (1998) and Miller *et al.* (1998), reported a teratogenicity study in which dose-related increased rates of abortions and malformations were observed in Cynomolgus monkeys administered oral doses, during early pregnancy (gestation days 16-27), of 0, 2250, 6000, 12,000 and 24,000  $\mu\text{g RE/kg bw/day}$  retinyl palmitate. Incidences of malformation were 1/21 and 5/11 at doses of 6000 and 24,000  $\mu\text{g RE/kg bw/day}$ , respectively (intermediate dose not reported). The higher dose affected structures derived from the cranial neural crest. These malformations were similar to those observed with isotretinoin (13-*cis*-retinoic acid) in both monkeys and humans. The spectrum of defects differed slightly to that observed in isotretinoin syndrome in that there was a higher frequency of abnormality of craniofacial structures, thymus and heart defects were less severe and there were no brain malformations. Maternal toxicity was observed (nature not specified).

*Bone toxicity**Leelaprute et al., 1973*

Gross bone lesions characterised by resorption of parts of the pelvis, fibulae, and scapulae with bone thinning were observed in growing female rats (initial average weight 147g) treated with 7500-22,500  $\mu\text{g}$  RE/day retinol as palmitate or retinol (vitamin A alcohol) for 17 days. Soft tissue calcification also occurred. The animals were given the doses either orally or by intraperitoneal injection. Intraperitoneal administration was associated with greater retinol toxicity, though not with vitamin A palmitate.

*Frankel et al., 1986*

A single oral dose of 82,000  $\mu\text{g}$  RE/kg bw given to adult rats, had no effect on biologically active parathyroid hormone (bioactive PTH) concentrations. Secretions of bioactive PTH were not altered by incubation of rat thyroparathyroid complexes with retinol *in vitro*. In 3-week-old rats given 15,000  $\mu\text{g}$  RE, 3 times a week for 6 weeks, osteoclast numbers were higher and osteoid lower than in the controls. Serum bioactive PTH was not detectable and serum 25-hydroxyvitamin D was significantly lower than in controls. At 7500  $\mu\text{g}$  RE, 3 times a week for 3 weeks, serum bioactive PTH was suppressed to undetectable levels but there was no effect on serum 25-hydroxyvitamin D. Serum calcium and 25-hydroxyvitamin D levels were lower in vitamin D intoxicated rats which were also given 7500  $\mu\text{g}$  RE, 3 times a week.

The authors considered that the skeletal changes caused by high levels of vitamin A were independent of the effects on PTH but could be caused by the changes in vitamin D metabolites. However, these pathological changes could be modified by secondary changes in calcium metabolism and in the metabolism of calcium-regulating hormones.

*Hough et al., 1988*

Young rats (initial weight 100g) were treated with 3000 or 7500  $\mu\text{g}$  RE/day retinyl palmitate for 21 days by stomach tube. Tibial histomorphometry revealed increased bone resorption (increased osteoclast size and number) and reduced bone formation. There was also a paucity of trabecular surfaces covered with osteoid. Spontaneous limb fractures and increased skeletal turnover (as measured by serum alkaline phosphatase and urinary hydroxyproline excretion) were also observed in the high dose group. Serum calcium and magnesium levels were unremarkable but serum phosphorus levels were significantly elevated in the control animals. Circulating levels of the potent bone resorbers, PTH, 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D were comparable in the treated animals and the controls suggesting that vitamin A was having a direct effect on bone.

## Exposure assessment

Total exposure/intake:

Food Median: 520  $\mu\text{g RE/day}$ <sup>21</sup> (from 1986/87 NDNS)  
97.5th percentile: 6050  $\mu\text{g RE/day}$

Supplements Up to 2400  $\mu\text{g RE/day}$  (Annex 4)

Estimated maximum intake:  $6050 + 2400 = 8450 \mu\text{g RE/day}$

High intake groups include people who consume liver and liver products regularly.

## Risk assessment

Acute vitamin A toxicity in humans is rare, but is more likely to occur following ingestion of high dose supplements, rather than following high intakes of vitamin A from food. Vitamin A accumulates in the body and, therefore, individuals who have regular high daily intakes of vitamin A might suffer adverse effects from chronic hypervitaminosis A. Although most manifestations of chronic vitamin A toxicity are reversible on cessation of dose, permanent damage to liver, bone and vision, and chronic muscular and skeletal pain may occur in some cases.

Epidemiological studies have indicated that exposure to high levels of vitamin A during pregnancy might increase the risk of birth defects. The available data do not allow identification of a threshold dose, although one study has suggested that effects may occur at modest intakes. Vitamin A has also been shown to be teratogenic in animals.

Recent epidemiological data have indicated that post-menopausal women with long-term high intakes of vitamin A have an increased risk of hip-bone fracture. Other supporting epidemiological data have indicated that this effect may occur in men as well as women. These findings are supported by animal data, which have indicated that retinol has a direct effect on bone, possibly via an interaction with vitamin D, and an effect on parathyroid hormone and therefore calcium metabolism.

### ESTABLISHMENT OF GUIDANCE LEVEL

It is not possible to establish a Safe Upper Level for vitamin A. There are two threads of evidence regarding potential adverse effects of vitamin A, one on teratogenicity and one on the risk of bone fracture, which suggest different levels of intake at which adverse effects may occur. Both of these ranges appear to overlap with dietary intakes of vitamin A.

<sup>21</sup> The median intake, rather than the mean, is provided because the mean appears artificially high. It is skewed by the individuals who have a very high vitamin A intake from liver and its products.

A number of epidemiological studies have suggested that high doses of vitamin A may be teratogenic, i.e. that they could cause malformations in the unborn child. The precise threshold for this effect is uncertain. The study by Rothman *et al.* reports that supplemental doses of  $> 3000 \mu\text{g RE/day}$ , in addition to the diet, may be teratogenic; this is the lowest dose level associated with such an effect. It was also noted that a small excess of cranial defects was found in the offspring of women consuming  $5000 \mu\text{g RE/day}$  from food and supplements, compared to those taking  $1660 \mu\text{g RE/day}$ . Other studies indicate that the threshold may be higher. However, given the severity of the effect, it is prudent to take  $3000 \mu\text{g RE/day}$  as the threshold for teratogenicity. The Group note and endorse the current advice that women who are pregnant or who wish to become pregnant should not take dietary supplements containing vitamin A except on medical advice.

In studies of long-term dietary intake, vitamin A has been associated with decreased bone density and increased risk of hip fracture. This finding is supported by investigations in laboratory animals in which vitamin A has been reported to affect calcium metabolism as well as to have a direct effect on bone. Other supportive epidemiological data suggest that the effect may also occur in men since fracture risk is increased in both males and females in Scandinavian countries, where retinol intake is also higher than in Southern Europe.

The risk of hip fracture is a continuous graded response associated with exposure levels that include average dietary intakes. It is not possible to identify an intake that is without some degree of risk. However, the available data indicate that total intakes greater than  $1500 \mu\text{g RE/day}$  may be inappropriate. This corresponds to  $25 \mu\text{g RE/kg bw/day}$  in a 60 kg adult.

Data on retinol intakes from food and supplements suggest that high level consumers of liver and liver products and/or supplements may exceed intakes at which adverse effects have been reported in the literature. It should also be noted that dietary supplements may contain 20-100% more vitamin A than is stated on the label, due to the practice of using 'overages' within the food supplements industry to ensure that the product contains no less than the stated content of the vitamin throughout its shelf life. This may be particularly important given that the effect on fracture risk appears to be a graded response, with the risk of fracture increasing with increased intake.

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