TOX/2021/57

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

First Draft Statement on the effects of excess Vitamin A on maternal health

1. The Scientific Advisory Committee on Nutrition (SACN) last considered maternal diet and nutrition in relation to offspring health in its reports on 'The influence of maternal, fetal and child nutrition on the development of chronic disease in later life' (SACN, 2011) and on 'Feeding in the first year of life' (SACN, 2018). In the latter report, the impact of breastfeeding on maternal health was also considered.

2. In 2019, SACN agreed to conduct a risk assessment on nutrition and maternal health focusing on maternal outcomes during pregnancy, childbirth and up to 24 months after delivery; this would include the effects of chemical contaminants and excess nutrients in the diet.

3. SACN agreed that, where appropriate, other expert Committees would be consulted and asked to complete relevant risk assessments e.g. in the area of food safety advice. This subject was initially discussed during the horizon scanning item at the January 2020 meeting with a scoping paper being presented to the Committee of Toxicity in July 2020. This included background information on a provisional list of chemicals proposed by SACN. It was noted that the provisional list of chemicals was subject to change following discussion by COT who would be guiding the toxicological risk assessment process: candidate chemicals or chemical classes can be added or removed as the COT considered appropriate. The list was brought back to the COT with additional information in September 2020 (Available: Here). Following a discussion at the COT meeting in September 2020, it was agreed that papers on a number of components should be prioritised and to this end, papers on iodine, vitamin D and dietary supplements have been or will be presented to the Committee. The remaining list of compounds were to be triaged on the basis of toxicity and exposure. This draft statement presents information on the potential effects of excess vitamin A on maternal health.

4. From their conversations on the discussion paper on vitamin A, the Committee noted that for completeness, all the available publications including a paper of Mawson and Croft (2019) had been described. The Committee expressed concern that the COT paper appeared to give greater attention than necessary to a contentious hypothesis by Mawson and Croft, parts of which had already been

discredited. The Committee wished to point out that the presence of this paper in the evidence base should not be misrepresented as indicative of the COT's views and that the final statement on the risks of vitamin A to maternal and fetal health would be entirely based on evaluation of the evidence rather than hypotheses and conjecture in any single paper.

5. Members felt that the exposure data provided needed clarification, especially those on liver consumption, since there were so few consumers recorded and those on butter, since ghee is widely used in some cuisines, but this was not reflected in the data.

6. The Chair questioned why the EFSA upper limit on vitamin A had been used in the risk characterisation but the EVM value had not been discussed and whether there was any known basis for the apparently enhanced adverse effects from retinoid esters such as the proprietary product Accutane compared with dietary vitamin A.

7. A Member asked whether in the UK, as was the practice in the Nordic countries, vitamin A was removed from products such as fish liver oil and liver pate and then was reintroduced at a lower concentration to give the benefits of the vitamin while mitigating its adverse effects.

8. On beta carotene, the Committee decided that the possible increased risks of lung cancer were applicable largely to smokers and that if women who smoked continued to do so during pregnancy then that was in itself a more major health risk than additional exposure to this carotenoid.

9. With regard to other endpoints of possible concern, the Chair suggested the interaction of vitamin A with vitamin D and the possible ensuing effects on maternal and fetal bone mineral density could be investigated.

Questions for the Committee

10. The Committee are asked to consider the following question.

a) Does the Committee have any comments on the content and structure of this draft statement?

Secretariat

November 2021

TOX/2021/57

Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment

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Introduction

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Following discussion of the first prioritisation paper (Here) on substances to be considered for risk assessment by the COT, the Committee decided that Vitamin A should be considered in a single paper.

Current UK Government and International advice

4 UK Government advice as given on the NHS.uk website lists good sources of vitamin A as cheese, eggs, oily fish, fortified low-fat spreads, milk, yoghurt and liver and liver products such as pate (NHS, 2021). Good sources of β-carotene in addition to yellow, red or green (leafy) vegetables are carrots, sweet potatoes, red peppers and spinach are fruit such as mango, papaya and apricots.

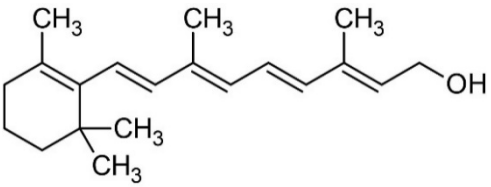
5 UK Government dietary advice, as given on the NHS website recommends that pregnant women, or women thinking about having a baby, should not consume liver or liver products such as pate, or supplements that contain vitamin A, including fish liver oil, unless advised by a GP, to avoid potential harm to the unborn baby.

6 The World Health Organisation (WHO, 2021) recommend that vitamin A supplementation be given to pregnant women only in areas where vitamin A deficiency is a severe public health problem, to prevent night blindness, ie if ≥5% of women in a population have a history of night blindness in their most recent pregnancy in the previous 3–5 years that ended in a live birth, or if ≥20% of pregnant women have a serum retinol level <0.70 µmol/L. Vitamin A supplementation in HIV-positive pregnant women is not recommended as a public health intervention for reducing the risk of mother-to-child transmission of HIV. Vitamin A supplementation in postpartum women, for the prevention of maternal and infant morbidity and mortality, is also not recommended.

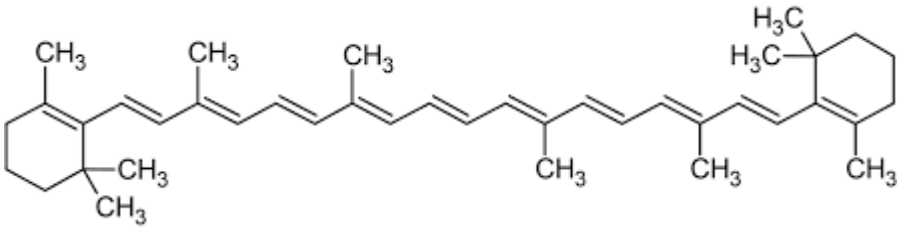
Background

Structure and sources

7 In structure, vitamin A is classified as a retinoid, consisting of an alicyclic β-ionone ring and a 9-carbon-long isoprenoid side chain. These compounds are derived from a family of pro-vitamin A carotenoids, the major source of vitamin A being β-carotene. Retinol (vitamin A_1 or vitamin A alcohol) and 3-dehydroretinol (Vitamin A_2) occur in foods of animal origin and β-carotene is found in red and yellow and leafy green vegetables. (Bowman and Rand, 1980) (Figure 1, Merck Index, 1996).



Retinol



β-carotene

Figure 1: Structure of retinol and β-carotene

8 Other carotenoids, for example, β-cryptoxanthin, α-carotene, lycopene, lutein and zeaxanthin are also present in plants. The first two of these carotenoids have a β-ionone ring at only one end of the molecule and hence yield only one molecule of retinol upon hydrolysis but the latter three are not metabolised to retinol and are therefore not classified as pro-vitamin A carotenoids (Collins and Mao, 1999).

9 Therapeutically useful retinol analogues include the naturally occurring 13-cis-retinoic acid (also known as isotretinoin, used orally to treat severe acne), and the synthetic aromatic retinoids such as etretin and etretinate.

10 The Expert Committee on Vitamins and Minerals (EVM) published a report in 2003 that included a review of Vitamin A. Vitamin A was defined as "… 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." Vitamin A can be expressed on a weight basis as Retinol Equivalents (1 RE = 1 µg retinol) = 1.78 µg retinyl palmitate = 6 µg β-carotene = 12 µg other carotenoids with provitamin A activity = 3.33 International Units (IU)[1] vitamin A activity from retinol" Link here

11 The EVM statement was based on an original detailed review, published in 2002 Link here

Previous evaluations

12 Previous evaluations on Vitamin A have been carried out by EFSA (2002, 2015) and EVM (2002, 2003) EFSA set a Tolerable Upper Intake Level (UL) for preformed vitamin A of 3000 µg RE/day for women of childbearing age and men, based on the risk of hepatotoxicity and teratogenicity, which was proposed to also apply during pregnancy and lactation. EVM (2002, 2003) did not recommend a maximum level of intake but, in the context of bone health, considered that an intake greater that 1500 µg/day was "inappropriate".

13 The COT last considered vitamin A in relation to the diet of infants and children aged 1 – 5 years, in 2017. The Committee concluded that the Tolerable Upper Level (TUL) of 200 µg RE/kg bw/day derived by themselves, based on a LOAEL of 800 µg RE/kg bw/day for an endpoint of bulging fontanelles, "…was appropriate to evaluate the effect of vitamin A exposures in infants. However, no TULs could be established for the ages 12-60 months on the basis of the available data. Comparisons were therefore made with a conservative TUL based on teratogenic effects in adults. High- level consumers are approaching or exceeding levels of vitamin A reported in the literature as causing toxicity and the possibility of adverse effects from these levels cannot be excluded. However, if effects did occur they would only be in a small proportion of consumers. Though the data on liver consumption are limited, frequent consumption could be a cause for concern and the current Government recommendation that infants over six months old should not have more than one portion of liver per week is appropriate."

Functions

14 Retinol performs many important physiological functions in animals. It is involved with the synthesis of collagen and elastin fibres by fibroblasts, as well as the processes of cell division, and differentiation and the functioning of skin and mucous membranes. Vitamin A positively influences the development of the skeleton by regulating the activities of osteoblasts and osteoclasts. It decreases the secretion of thyroxine from the thyroid gland by suppressing production of thyrotropin by the pituitary. Vitamin A also stimulates the immune system and hence improves resistance to infections. (Rutkowski and Grzegorczyk, 2012)

15 Vitamin A is an antioxidant: the conjugated C = C bonds in the side chain are oxidised by reactive oxygen species (ROS) and free radicals and thus protect those bonds in the polyunsaturated fatty acids in cell membrane lipids. This also protects against neoplastic transformation (promotion) induced by uncontrolled oxidation of the glycosyl residues of proteins in cell membranes. The antioxidative function also stabilises thiol groups (–SH) of membrane proteins and suppresses oxidatively stimulated expression of the c-myc oncogene. Epithelial cells in particular are protected by vitamin A, including tissues of the nasal and throat cavity, oesophagus, stomach, intestines, respiratory tract, bladder, and prostate. (Rutkowski and Grzegorczyk, 2012)

16 Retinol is oxidised to retinal (vitamin A aldehyde or retinaldehyde) which, as its 11-cis isomer, functions as an essential component in the process of visual signal transduction in the retina, in the pigment rhodopsin in the rods as well as the pigments in the cones. (EVM, 2002).

17 Retinal is further oxidised to retinoic acid and thence undergoes further side-chain isomerisation and oxidation into a range of different products. Retinoic acid

has pleiotropic effects in development and is well documented in the literature as a teratogen (Collins and Mao, 1999).

18 Retinoids are used clinically to treat a range of disorders from skin lesions and cancers and a range of synthetic analogues with enhanced receptor specificities and pharmacokinetic profiles has been developed in order to maximise the benefits of treatment while ameliorating their toxicity. Barnard *et al* (2009) reviewed the design and structure of a wide range of synthetic retinoids with modified beta ionone heads, isoprenoid chains and hydrophilic end groups to explore this pharmacological space.

Mechanism of action

19 The majority of the effects of ingested vitamin A are thought to be mediated by the action of retinoic acid. Since retinoic acid is produced endogenously and combines with specific nuclear receptor proteins that bind to DNA and regulate the expression of various genes, it is classified as a hormone. It is a ligand for specific nuclear receptors, the most studied of which are retinoic acid receptor (RAR) or retinoid X receptor (RXR), that regulate the transcription of numerous target genes, as homo- or hetero-dimers. More than 500 genes are known to be regulated by retinoic acid, many of which control embryonic development. Retinoic acid signalling is turned off by ligand degradation by CYP450 enzymes, such as CYP26A1.

20 Das et al. (2013) and Huang *et al.* (2014) detail the molecular biology and functions of the retinoid receptors. In the presence of retinoids, the holo-receptor binds to the chromosomal retinoic acid response element (RARE) and activates histone acetyl transferases (HATs). This results in histone acetylation, which opens the chromatin so that transcription can take place. In the absence of ligands, the apo receptor pair binds with the RARE in a complex with corepressors and activates histone deacetylase (HDAC). As a result, histone deacetylation prevails causing chromatin condensation and gene silencing. A critical role for RA during somite development was uncovered by studies showing that RA regulates the expression of the transcription factor Cdx1, a factor crucial for the somatic expression of multiple Hox genes that control the sequence of skeletal development. RA also has a crucial role in ensuring the suppression of left-right asymmetries during developmental pattern formation in embryos. RA is not produced by all cells of the body at all stages of development but is produced in a unique spatiotemporal pattern which orchestrates development.

21 In preparation for implantation of the fertilised ovum, progesterone released from the corpus luteum causes the cells of the superficial layer of the endometrium to enlarge and compact. These cells are known as decidual cells because they are shed after birth and the process is decidual transformation (Bowman and Rand, 1980). Ozaki et al (2017) showed that decidual transformation of human endometrial stromal cells (HESCs) resulted in reprogramming of the retinoic acid signalling and metabolic pathways. Differentiating HESCs downregulate the intracellular carrier proteins CRABP2 and FABP5 that are responsible for transfer and binding of retinoic acid to the nuclear receptors RAR and PPARβ/δ, respectively. The expression of the RAR receptor, which mediates the pro-apoptotic effects of retinoic acid, was also inhibited. The PPARβ/δ receptor, which transduces the differentiation responses of retinoic acid, was upregulated. Decidualisation was also associated with increased

expression of retinol-binding protein 4 (RBP4) and various enzymes involved in the metabolism of retinoic acid and retinal, including CYP26A1, DHRS3, and RDH12. Exposure of differentiating HESCs to retinoic acid or retinal reversed the inhibition of the CRABP2-RAR pathway, perturbed the expression of decidual marker genes and triggered cell death. The authors concluded that the data showed that decidualizing HESCs silence retinoic acid signalling by downregulating key cytoplasmic binding proteins and by increasing retinoid metabolism. However, excessive retinoic acid exposure is toxic for decidual cells and triggers a response that may lead to pregnancy failure.

22 Both deficiency and excess of retinoic acid causes the ectopic induction and the down regulation of many genes as a prelude to changing the anatomy of the embryo. For example, excess RA causes the chick limb bud to develop six digits instead of the normal three. This effect is elicited by the induction of a nested set of Hox genes, the fibroblast growth factor-4 gene, the bone morphogenetic protein-2 gene, the sonic hedgehog gene, and others forming interacting networks of genes to control outgrowth in the three axes of the limb. Conversely, quail embryos deficient in retinoic acid have down-regulated sonic hedgehog, fibroblast growth factor-4, engrailed and others, but also express ectopically induced genes such as Wnt-7a, probably by the down-regulation of a repressor.

23 The hindbrain of the embryo is also profoundly affected. RA administration to mouse embryos induced the hindbrain Hox genes in an altered expression pattern which resulted in an altered anatomy, with the seven structures in this tissue known as rhabdomeres developing in the wrong order, leading to malfunction of the whole brain region.

24 Carazo et al (2021) reviewed the forms, sources, kinetics, detection, function, deficiency, therapeutic use and toxicity of vitamin A. They concluded that “Given the importance of vitamin A in multiple crucial physiological processes, its deficiency can pose a serious health challenge, even leading to death in the most serious cases. At the same time, it can lead to serious health issues in high-dose situations.”

ADME

25 EFSA (2015) state that preformed vitamin A is efficiently absorbed (70–90 %). The absorption of β-carotene appears to be highly variable (5–65 %), depending on food- and diet-related factors, genetic characteristics and the health status of the subject

26 Spiegler et al. (2012) reviewed the absorption, distribution, metabolism and excretion of vitamin A. Nearly all retinyl esters in the diet are hydrolysed to retinol in the intestinal lumen. Retinol is absorbed by intestinal epithelial cells, where it is re-esterified to long-chain fatty acids, primarily by the enzyme lecithin:retinol acyltransferase (LRAT), which is widely expressed in tissues, and is incorporated into chylomicra, which circulate in the intestinal lymph before moving into the general

circulation. Once in the general circulation, lipoprotein lipase (LPL), which is bound to the luminal surface of the vascular endothelium, catalyses the lipolysis of triglycerides to generate free fatty acids and chylomicron remnants. Chylomicron remnants are cleared mainly by the liver, but extrahepatic uptake of the remnants may be important in the delivery of vitamin A to mammary tissue, bone marrow, adipose tissue, and spleen. Retinyl esters in serum are normally below 0.2 µmol/L in the fasting state but they increase significantly after a large influx of vitamin A, such as occurs after a vitamin A–rich meal.

27 Once taken up by the hepatocytes, retinyl esters are again hydrolysed to retinol to be transferred to stellate cells and then re-esterified by LRAT for storage. Alternatively, retinol can bind to retinol-binding protein (RBP) and be secreted into the bloodstream as a 1:1 molar complex with the serum protein transthyretin. RBP thus functions to mobilise hepatic retinoid stores and deliver retinol to peripheral tissues and developing embryos. In fasting conditions, retinol-RBP accounts for approximately 95-99% of all serum retinoids. Upon vitamin A intake, the concentration of retinoids in chylomicrons and chylomicron remnants can greatly exceed that of plasma retinol. Blood levels of retinol-RBP in both humans and animals are tightly controlled, except in extreme cases of insufficient intake of vitamin A, protein, calories and zinc; or in response to hormonal factors, stress; and in certain disease states.

28 Spiegler et al. (2012) also stated that the mechanisms that regulate the secretion of the complex retinol-RBP from the liver have yet to be fully elucidated. Tissue uptake of vitamin A seems to be mediated by Stra6 (“Stimulated by retinoic acid 6”), the receptor for the circulating complex where this is expressed, but this uptake mechanism is unclear. Stra6 may also act as a cytokine receptor to transduce signalling by holo-RBP and regulate insulin response. In addition, Stra6 may also mediate the efflux of retinol from the cell and thus act as a bi-directional transporter of retinol, with intracellular retinol concentration determining influx or efflux dominates. In some tissues with high retinoid content, such as skin and liver, Stra6 is expressed at very low levels and other mechanisms such as spontaneous transfer of free retinol across the phospholipid bilayer, may regulate retinol uptake. Even in the fasting state there are low concentrations of RE that are associated with circulating lipoproteins (in VLDL and LDL) and small amounts of circulating retinoic acid bound to albumin.

29 Within cells, retinol is reversibly oxidized to retinal by members of the alcohol dehydrogenases, medium-chain dehydrogenase/reductases, retinol dehydrogenases and short-chain dehydrogenase/reductases. Retinal is further oxidized to retinoic acid by retinal dehydrogenases. Several intracellular binding proteins for retinol, retinal and retinoic acid have been identified and characterised, including cellular retinol-binding proteins I, II and III, cellular retinaldehyde binding protein and cellular retinoic acid-binding proteins I and II. Each of these retinoid-binding proteins has a distinct expression pattern and plays a specific role in vitamin A transport and metabolism.

30 Willhite et a.l (1990, from abstract) found that a single application of 17 µg/kg or 8.7 mg/kg of radiolabelled all-trans-[10,11-$^{3}H_{2}$]-retinoic acid dissolved in acetone to shaved dorsal hamster skin was rapidly absorbed and showed a dose-dependent

rate of elimination. An equation describing a two-compartment open model with a very brief lag time and first-order uptake and elimination was used to describe the central plasma compartment kinetics. Unchanged all-*trans*-retinoic acid represented up to 4% of the total circulating radioactivity. Peak circulating concentrations of parent all-*trans*-retinoic acid were less than those observed after an equivalent oral dose, but prolonged absorption from the skin contributed to high total bioavailability of retinoid applied topically. Topical administration to intact skin of up to three consecutive doses of 10.5 mg/kg/d all-trans-retinoic acid or a single 5 mg/kg dose of etretinate (Ro 10-9359) during a critical stage of embryogenesis in hamsters caused erythema and/or dose-dependent epidermal hyperplasia at the site of application but failed to induce a significant teratogenic response. Topical application of 0.01-1.0 mg/kg of artificial carotenoid Ro 13-6298 resulted in dose-dependent mucocutaneous toxicity and an increase in the numbers of dead embryos and malformed offspring. The marked skin toxicity and attenuated concentrations in maternal blood, compared to the oral route, limited the amounts of retinoid that reached the hamster embryo. Therefore, it was considered more important to compare the absorbed dose than the applied dose, when interpreting the bioassays. The data suggested that in human skin, toxicity limits the amounts of retinoid that can be applied during pregnancy and subsequently reaches the embryo whereas in the rodent, overt skin toxicity under continued dosing could increase the penetration .

31 Retinol metabolites are excreted mainly in the urine (38 to 60 %), but also in faeces (18 to 37 %) and breath (18 to 30 %). Retinol is metabolised in the liver to numerous products, some of which are conjugated with glucuronic acid or taurine for excretion in bile and the amount of retinol metabolites excreted in bile increases as the liver retinol exceeds a critical concentration. Excretion of labelled retinol metabolites in bile of rats fed increasing amounts of retinol traced by [3H]-retinyl acetate was constant when hepatic retinol concentrations were low (≤ 32 µg/g (112 nmol/g) and increased rapidly (by eight-fold) as liver retinol concentration increased, up to a plateau at hepatic retinol concentration ≥ 140 µg/g (490 nmol/g) This increased biliary excretion may serve as a protective mechanism for reducing the risk of excess storage of vitamin A. (EFSA 2015)

Acute and chronic toxicity

32 Acute clinical features of vitamin A toxicity in age groups other than infants are lethargy, pain in the joints, dry skin, headache and nausea and vomiting, although these vary depending on severity. More severe signs that can diagnose hypervitaminosis A clinically include alopecia, drowsiness, liver and bone damage and visual problems (Loughrill 2016, SCF 2002). In infants, the major symptom of toxicity is bulging fontanelles.

33 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.

Some adverse effects, for example hepatotoxicity, are regarded as reversible with withdrawal of the vitamin but others, such as deficits in the eyes and bone, are not. (Kamm, 1982).

34 Penniston and Tamuihardjo (2006) found that few human studies had looked at the acute effects of a large dose of vitamin A on circulating vitamin A concentrations. Evidence suggested that sub-toxicity without clinical signs of toxicity may be a growing concern, because intake from preformed sources of vitamin A often exceeded the recommended dietary allowances (RDA) for adults, especially in developed countries. Osteoporosis and hip fracture have been associated with preformed vitamin A intakes of only twice the current RDA. Assessing vitamin A status in cases of sub-toxicity or toxicity is complicated because serum retinol concentrations are non-sensitive indicators in this range of liver vitamin A reserves.

Reproductive effects of vitamin A

35 It is now generally believed that all-*trans* retinoic acid (AT-RA) supports both male and female reproduction as well as embryonic development. (Zile, 1998, Clagett-Dame and DeLuca 2002**,** Clagett-Dame and Knutson, 2011,). This conclusion is based on the ability of RA to reverse most reproductive and developmental blocks found in vitamin A deficiency induced in experimental animals either by nutritional or genetic means, and the fact that the majority of embryonic defects arising from vitamin A deficiency are also observed in retinoic acid receptor null mutants. The differential activity of cytochrome P450 (CYP)26 enzymes in tissues is a key regulatory mechanism. If severely vitamin A-deficient pregnant rats are given small amounts of carotene or limiting quantities of RA early in organogenesis, embryos form but show a collection of defects called the vitamin A deficiency syndrome or late vitamin A deficiency. Vitamin A is essential for the maintenance of the male genital tract and spermatogenesis and participates in a signalling mechanism that initiates meiosis in the female gonad during embryogenesis, and in the male gonad postnatally. Both nutritional and genetic approaches have been used to elucidate the vitamin A-dependent pathways upon which these processes depend. Retinoids are extensively involved in embryogenesis and fetal development.

36 The teratogenic effects of retinoic acid have been documented both in animals and in humans (Zile 1998). Retinoic acid induces differential patterns of malformations in mammalian embryos based on the different stages of embryonic development Children exposed *in utero* to isotretinoin (used primarily to treat severe acne) have been found to exhibit congenital malformations, known as “the retinoic acid syndrome” (Collins and Mao, 1999). The malformations include effects on the central nervous system (hydrocephalus, anencephaly, exencephaly, spina bifida), eyes (anophthalmia, microphthalmia, defects of the retina), face (harelip, cleft palate, brachygnathia, hypoplastic maxilla), dentition, ear (absent or deformed), limb (phocomelia), urinogenital system (hypoplastic kidney, polycystic kidney, absent/hypoplastic genitalia), heart (incomplete ventricular septation, transposition of the great vessels, double aortic arch, hypoplastic aortic valves), thyroid gland (hypoplasia), and the axial skeleton (vertebral and rib fusions, extra vertebrae and ribs, hypoplastic tail). (Maden, 2001).

Animal studies

37 Within embryos of experimental animals, too little or too much vitamin A/RA causes malformations. Rat fetuses in mothers reared on vitamin A-deficient diets show a range of anomalies known as “fetal vitamin A deficiency” (VAD) syndrome, which comprises defects of hind-brain, eye, ear, heart, lung, diaphragm, kidney, testis, limbs, and skeleton. Mice with compound null mutations of RA nuclear receptors and RA-synthesizing enzymes also have malformations resembling the VAD syndrome. Excess vitamin A/RA in humans and animal models causes malformations resembling the fetal VAD syndrome. Lee *et al.* (2012) investigated the effect of excess retinoic acid on the development of rodent embryonic kidneys. It was stated that both vitamin A excess and deficiency were known to lead to lack of kidney development (bilateral renal agenesis) in the hamster and the mouse before there was any morphologically identifiable precursor of the organ present. The authors presented evidence that, paradoxically, the malformations observed following maternal high dose (100 mg/kg bw) RA may have been due to RA deficiency at a crucial stage in development. The mechanism appeared to be RA-induced inhibition of its own endogenous synthesis and increased expression of RA-metabolising CYP isoforms. Pleiotropic mutations resulted, many of which were ameliorated by supplementation with a lower dose of RA given to the mother after fetal clearance of the original high dose.

38 The potential adverse effects of retinoids have been assessed in animal studies using both oral and dermal routes of exposure.

Dermal exposure

39 A technical report in 2012 by the US National Toxicology Program Link here quotes a study by Seegmiller et al (1990) in which time-mated Sprague-Dawley rats were administered retinoic acid topically to clipped intact dorsal skin on gestational days 11 to 14 at 12, 100, or 250 mg/kg bw. Maternal weight gain, pup weight, number of resorptions, number of fetuses with gross malformations, and skeletal and organ anomalies were determined. Dams treated dermally with retinoic acid exhibited skin lesions at the site of application from gestational day 15, and most dams showed vaginal bleeding by day 16. Approximately 20% did not survive to day 19. Maternal weight gains in the treated groups were decreased by approximately 50% relative to control animals at the lowest dose, with essentially no weight gain at the intermediate- and high-dose levels. Decreases in fetal weights at the two higher dose levels were significant, but there were no differences from controls in the number of resorptions or malformation frequencies.

Oral exposure

40 Schnorr et al (2011) dosed rats with vitamin A at 375, 750 and 7500 μg RE/kg and observed an increase of oxidative damage markers in the reproductive tissues and plasma of dams. The activity of glutathione-S-transferase was modulated by

vitamin A supplementation, increasing in the liver of dams and decreasing in the kidneys of mothers and offspring. In pups, supplementation decreased the total antioxidant potential of the liver as well as the superoxide dismutase/catalase activity ratio in the kidney. Lipoperoxidation increased in male offspring but decreased in female pups. Although no clear explanation was given for the sex difference in response, the authors suggested that male offspring were more susceptible to free radical injury than were females. The results suggested that excessive vitamin A intake during gestation and lactation might be toxic for mothers with adverse effects for the developing offspring.

Human studies

Teratogenicity- Food and food supplements.

41 Van den Berg *et al* (1996) assessed the distribution of dietary vitamin A intake among Dutch women aged 16-50 and among pregnant women, to evaluate the effect of the use of a vitamin A (1200 RE)-containing multivitamin supplement in terms of nutritional and teratogenic risk. Data from the 2nd Dutch national food consumption survey (1992) were used to calculate the vitamin A intake among 1725 16-50 year old women and 58 pregnant women with and without simulation of the use of a supplement containing 1200 RE vitamin A. Average vitamin A intake, based on a two-day dietary record method, was 850 RE for the 16-50 year old non-pregnant (NP) women (RDA: 800 RE), and 990 RE for the pregnant (P) women (RDA: 1000 RE), respectively. Consuming liver on one of the survey days resulted in 60% of the women in this subgroup exceeding 3000 RE, and in 23% of the cases intakes were > 7500 RE. Those not consuming liver or liver products on the survey days had average intakes (NP: 540 RE; P: 720 RE). About 70% of the non-liver consumers had intakes below the RDA. Including the daily use of a vitamin A containing multivitamin supplement with 1200 RE resulted in intakes > RDA, while only in 2% (NP), and 3% (P) of the cases the intake exceeded the 3000 RE but was less than 7500 RE/day.

42 Werler et al (1990) used data from a case-control study to assess the maternal use of vitamin A supplements alone and vitamin A-containing multivitamin supplements in relation to the occurrence of certain birth defects involving structures derived, at least in part, from cranial neural crest cells. The cases were 2,658 infants with such defects (primarily craniofacial and cardiac malformations) with the controls being 2,609 infants with other malformations. Vitamin A supplementation was defined as daily use for at least 7 days of retinol alone or with vitamin D, or of fish oils. Information on vitamin A dose and nutrition was not available. The mothers of the six controls used vitamin A supplements in each of the first trimester of pregnancy in comparison to the mothers of 15, 14, and 10 cases in months 1, 2, and 3, respectively. Risk estimates relative to controls and 95% confidence intervals were 2.5 (1.0-6.2) for month one, 2.3 (0.9-5.8) for month two, and 1.6 (0.6-4.5) for month three. However, the findings were considered tentative because no dose information was available, only small numbers of cases and controls were exposed to vitamin A supplements, and relative risk estimates were not statistically significant.

43 Rothman et al (1995) obtained vitamin A supplement data on 22,748 pregnant women when they had screening for maternal serum alpha-fetoprotein or underwent amniocentesis. Information on the outcomes of pregnancy was obtained from the obstetricians who delivered the babies or from the women themselves. Of the 22,748 women, 339 had babies with birth defects; 121 of these babies had defects occurring in sites that originated in the cranial neural crest. For defects associated with cranial-neural crest tissue, the ratio of the prevalence among the babies born to women who consumed more than 4500 μg RE of preformed vitamin A per day from food and supplements to the prevalence among the babies whose mothers consumed 1500 μg RE or less per day was 3.5 (95 percent confidence interval, 1.7 to 7.3). For vitamin A from supplements alone, the ratio of the prevalence among the babies born to women who consumed more than 3000 μg RE per day to that among the babies whose mothers consumed 4500 μg RE or less per day was 4.8 (95 percent confidence interval, 2.2 to 10.5). Using a smoothed regression curve, an apparent threshold was identified near 3000 μg RE per day of supplemental vitamin A. The increased frequency of defects was concentrated among the babies born to women who had consumed high levels of vitamin A before the seventh week of gestation. The authors concluded that among the babies born to women who took more than 3000 μg RE of preformed vitamin A per day in the form of supplements, about 1 infant in 57 had a malformation attributable to the supplement.

44 Azaïs-Braesco and Pascal (2000) reviewed reported cases of teratogenicity associated with high intakes of vitamin A in pregnancy. Up to 20 case reports of the relationship between high vitamin A intake and an adverse pregnancy outcome in humans had been published over the preceding 30 years; however, the authors found these to be of limited use for establishing a quantitative link between vitamin A intake and teratogenic events. The malformations observed were not always consistent with the retinoic acid syndrome, thus calling their true origin into question. The authors found five case-control studies since 1990 retrospectively estimated the intake of vitamin A in control subjects and mothers of malformed babies (see Table 1 below). The design of these studies varied in the classification of malformations, statistical power, and vitamin A consumption data. In most cases, no association was found between moderate doses of vitamin A (<3000 μg RE) and fetal malformations. Moreover, the number of women consuming high amounts of vitamin A was too limited to be statistically significant. Only one prospective study, that of Rothman (1995) had been conducted and the results were inconsistent with the retrospective studies, showing that an intake exceeding 3000 μg RE significantly increased the risk of malformations (prevalence ratio: 4.8; 95% CI: 2.2,10.5). However, the latter paper had been largely criticized in relation to a suspected misclassification of the malformations, but the authors felt it should not be ignored. Another clinical trial had been carried out in Hungary in which a supplement of 1800 μg RE vitamin A did not increase the incidence of fetal malformations, but since folic acid was administered simultaneously with vitamin A only limited conclusions could be drawn regarding the incidence of neural tube defects.

Table1. Case-controlled studies identified by Azaïs-Braesco and Pascal (2000)

Number of Cases	Number of Controls	Exposure (μg RE/day)	Odds ratio for defects (95% confidence interval)	Reference
11193	11293	> 3000 > 13000	2.7 (0.8, 11.7) 1.1 (0.5, 2.5)	Biesalski HK, 1989
2658	2609	No information on the vitamin A doses	2.3 (0.9, 5.8) 1.6 (0.6, 4.5) 2.5 (1.0, 6.2) NTDs	Martines-Frias *et al.,* 1990
158	3026	Multivitamin supplements: No information	0.57 (0.33, 1.00) Conotruncal defects	Werler et al., (1990)
548 (NTDs)	573	> 2400 >3000	NTDs: 0.91 (0.31, 3.68) Other defects: 1.05 (0.51, NTDs: 0.73 (0.40, 1.53) Other defects: 0.92 (0.40, 2.11)	Botto et al., 1996
426 16 6	432 12 7	0–3333 3000–4500 >4500	1.0 1.4 (0.6, 2.8) NTDs only	Mills et al., 1996

NTD, neural tube defect. Conotruncal refers to the outflow region of the developing heart.

45 The pharmacokinetics of vitamin A have been investigated in the context of the reported adverse effects.

46 Buss et al (1994) dosed 10 healthy female volunteers with 5 different doses of vitamin A and studied the effects on plasma vitamin A and its metabolites. The single supplements were provided as either retinyl palmitate (15000 and 45000 μg RE) or an equivalent dose in fried calf liver. Blood was collected at intervals within 12 hours of dosing and thereafter for 6 days. The results showed substantial increases in plasma retinyl palmitate, 13-cis- and all-trans-retinoic acid, and 13-cis and all-trans-4-oxoretinoic acid. Women who received the supplement had significantly higher concentrations of retinoids than did those who received the liver, possibly because the food matrix may have ameliorated the absorption rate or altered the circulating forms of vitamin A. However, plasma retinol changed only slightly, which supported the view that this method was not an appropriate means by which to evaluate a vitamin A supplementation trial. Based on the formation of all-*trans*-retinoic acid, it

was concluded that liver and supplements were not of equivalent teratogenic potential.

47 Hartmann et al (2005) evaluated plasma concentration-time curves of retinyl esters, retinol and their metabolites at increasing doses of vitamin A in 3 groups (12 per group) of non-pregnant women aged 18-40 years. The women received once daily oral doses of vitamin A palmitate up to 30,000 IU (9000 μg RE) /day over 21 days. The area under the plasma concentration-time curve (AUC (24h)) served as indicator for exposure. The AUC (24h) of retinyl esters increased linearly with dose. Retinol concentrations were unaffected. All-trans RA exhibited a diurnal-like concentration-time profile (Maximum blood concentration (Cmax) at 3 h; minimum blood concentration (Cmin) at 8 h), concentrations decreasing below pre-dose levels at 5 h and regaining pre-dose levels at 16 h. The maximum temporary increase in exposure was 33% (single dose) and 19% (repeated doses) above baseline, but the AUC (24h) remained unaltered. The AUC (24h) increased linearly with dose for 13-cis RA and 13-cis-4-oxo RA. Repeated doses caused a 25% increase in exposure with the highest vitamin A intake. Accumulation of 13-cis-4-oxo RA at 30,000 IU (9000 μg RE)/day doubled compared to the 4,000 IU (1200 μg RE)/day intake.

48 Nohynek et al (2005) investigated the effect of topical vitamin A on human endogenous plasma levels of Vitamin A and its metabolites. Two groups of 14 female volunteers of child-bearing age were kept on a vitamin A-poor diet and treated topically for 21 days with creams containing 0.30% retinol or 0.55% retinyl palmitate on approximately 3000 cm^2 of their body surface area. This gave a total dose of approximately 30,000 IU (9000 μg RE) vitamin A/subject/day. After a 12-day wash-out period, the study groups received single oral doses of 5600 and 16800 μg RE retinyl palmitate (RP), corresponding to the maximal EU allowance during pregnancy or three-times higher, respectively. Blood samples were collected over 24h on study days -3 (pre-study), 1, 21 (first and last days of topical treatment) and 34 (oral administration) at 0, 1, 2, 4, 6, 8, 12, 14, 16 h and 24 h after treatment. Plasma concentrations of retinol (REL), retinyl palmitate (RP), retimol oleate (RO) and retinol stearate (RS), 9-cis-, 13-cis-, all-trans- (AT), 13-cis-4-oxo- or AT-4-oxo-retinoic acids (RAs) were analysed. With the exception of transient mild (RP-group) to moderate (REL-group) local irritation on the treatment sites, no adverse local or systemic effects were noted. On days 1 or 21 of topical treatment, no changes were measured in individual or group mean plasma Cmax, AUC (0-24 h) or other pharmacokinetic parameters of REL, retinyl esters or RAs relative to pre-study data. In contrast, single oral doses of RP at 3000 or 9000 μg RE produced dose-related and sustained increases in Cmax and AUC (0-24 h) values of plasma RP, RO, RS, 13-cis- and 13-cis-4-oxo-RAs, as well as a transient increase in AT-RA. Topical exposure to retinol- or retinyl ester-containing cosmetic creams at 9000 μg RE /day and maximal use concentrations were therefore found to not affect plasma levels of retinol, retinyl esters or RAs, whereas single oral doses at 3000 or 9000 μg RE produced significant increases in plasma retinyl esters and RAs.

Teratogenicity- oral and topical medications

49 The potential teratogenicity of topically applied retinoic acid prescribed clinically for treatment of acne has been a subject of debate in the past (Wilkinson 1975, Danby, 1978).

50 Lammer et al (1985, from abstract) investigated 154 human pregnancies with fetal exposure to isotretinoin. The outcomes were 95 elective abortions, 26 infants without major malformations, 12 spontaneous abortions, and 21 malformed infants. A subset of 36 of the 154 pregnancies was observed prospectively. The outcomes in this cohort were 8 spontaneous abortions, 23 normal infants, and 5 malformed infants. Exposure to isotretinoin was associated with a relative risk of 25.6; (95%confidence interval, 11.4 to 57.5). Among the 21 malformed infants there was a characteristic pattern of malformation involving craniofacial, cardiac, thymic, and central nervous system structures. These included microtia/anotia (15 infants), micrognathia (6), cleft palate (3), conotruncal heart defects and aortic-arch abnormalities (8), thymic defects (7), retinal or optic-nerve abnormalities (4), and central nervous system malformations (18). The pattern of malformation closely resembled that produced in animal studies of retinoid teratogenesis. The authors deemed it possible for a major mechanism of isotretinoin teratogenesis leading to these effects to be a deleterious effect on cephalic neural-crest cells in the fetus.

51 Kizer et al (1990) reviewed the teratogenic effects of isotretinoin. Willhite et al (1986) reported that isotretinoin administered orally to 64 women during the first trimester of pregnancy resulted in 34 cases of fetal defects and 30 cases of spontaneous abortion. Defects involved pathological changes in the central nervous system, facial malformations with small or absent external ears, and cardiovascular impairments. The total number of infants with birth defects consistent with those associated with isotretinoin use was unclear, with the estimates ranging from 62 to 1,300.

52 Kizer et al (1990) stated further that human birth defects and spontaneous abortions had been associated with the use of etretinate, the trimethylmethoxyphenyl ethyl ester of all-trans-retinoic acid. Etretinate is currently approved for use in the treatment of psoriasis. In Europe, seven cases of fetal malformations due to etretinate exposure during pregnancy had been reported: these included meningomyeloceles, craniofacial and skeletal abnormalities, severe brain defects with anophthalmia, and low-set ears. A case of congenital malformation was reported in a child born to a woman from Brazil who had discontinued etretinate therapy almost a year before she conceived (Lammer et al, 1988). There had been no reports of birth defects associated with its use in the United States, but it was approved only in late 1986. The lowest human teratogenic doses for the two retinoids, isotretinoin and etretinate, are estimated to be 0.4 and 0.2 mg per kg per day, respectively (Ross, 1983). Vitamin A is metabolized to the all-trans-retinoic acid, which differs from isotretinoin only in the conformation of the isoprenoid side chain.

53 AT-RA also produces 13-cis-retinoic acid as a metabolite. Whether vitamin A itself (as retinol or retinyl esters) is a human teratogen is unclear. There have been several isolated case reports of human birth defects with high levels of maternal vitamin A exposure. Some of the malformations are consistent with the pattern of defects produced by isotretinoin, but a causal relationship has not been established. Although poor exposure surveillance confounded by the simultaneous intake of other

nutritional supplements makes the available data inconclusive, the evidence certainly raises concerns about possible human teratogenicity of high doses of vitamin A. Isotretinoin, etretinate, and vitamin A as retinol or retinyl esters have been confirmed as embryotoxic and teratogenic in studies of animals. These findings contrast with those related to naturally occurring carotenoids, which are metabolised in mammals to vitamin A and have not been identified as teratogenic or embryotoxic in studies of either animals or humans (Howard et al. 1986). The existing guidelines from the manufacturer (Hoffmann- LaRoche Inc) for the use of Accutane (isotretinoin) by women stress the necessity of obtaining a negative pregnancy test two weeks before initiating therapy with Accutane and the importance of using an effective form of contraception a month before, during, and for a month after taking Accutane. Women who become pregnant while using isotretinoin are advised to discuss with their physicians the advisability of continuing the pregnancy. Despite these restrictions and warnings to physicians and consumers, women taking Accutane continue to become pregnant and produce malformed infants (Kizer, 1990).

54 Zomerdijk *et al* (2014) estimated isotretinoin exposure in 203,962 Dutch pregnant women and analysed the occurrence of adverse fetal or neonatal outcomes in these pregnancies. Proportions of adverse fetal or neonatal outcomes, defined as intrauterine deaths ≥16 week of gestation and neonates with major congenital anomalies were measured in relation to isotretinoin exposure in the 30 days before or during pregnancy. ORs with 95% CIs adjusted for maternal age were calculated to estimate the risk of adverse fetal or neonatal outcome of 51 pregnancies, 2.5 (95% CI 1.9 to 3.3) per 10,000 pregnancies, were exposed to isotretinoin despite a pregnancy prevention programme being in place in the EU since 1988. Forty-five of these pregnancies were exposed to isotretinoin and six women became pregnant within 30 days of discontinuing treatment. In five out of the 51 isotretinoin exposed pregnancies (53 fetuses), 9.4% (95% CI 1.3% to 17.6%), had an adverse fetal or neonatal outcome (OR 2.3 95% CI 0.9 to 5.7 after adjustment for maternal age). The authors concluded that isotretinoin exposed pregnancies and adverse fetal and neonatal events potentially related to the exposure still occur and that in the Netherlands at least there was no full compliance to the isotretinoin prevention programme.

55 MacDonald *et* al (2019) used the 2011–2015 Truven Health MarketScan® Database to identify pregnancies, including losses and terminations, in a cohort of non-pregnant women filling a prescription for isotretinoin or tretinoin (all-*trans*-retinoic acid) and a second group of women without either prescription. The group identified 86,834 isotretinoin and 973,587 tretinoin treatment episodes in 76,053 and 606,966 non-pregnant women respectively These episodes were matched to an unexposed group of 5,302,105 non-pregnant women. The women were followed for 365-days or until conception, medication discontinuation, or enrolment discontinuation (“prescription episode”). Rates of pregnancy, risks of pregnancy losses, and prevalence of infant malformations at birth were assessed by exposure. The authors identified 2,179,192 livebirths, 8,434 stillbirths, 2,521 mixed births, 415,110 spontaneous abortions, 124,556 elective terminations, and 8,974 unspecified abortions. There were 86,834 isotretinoin and 973,587 tretinoin episodes, matched to 5,302,105 unexposed women. Pregnancy rates were 3 (isotretinoin), 19 (tretinoin), and 34 (unexposed) per 1,000 person-years. Risk of pregnancy losses were similar, but terminations were more common in the women exposed to isotretinoin (28%

[95% CI: 21–36%]), than those exposed to tretinoin(10% [95% CI: 9– 11%]) or unexposed (6%). Malformations occurred in 4.5% (95% CI:3.5–5.6%) of the tretinoin-exposed pregnancies and 4.2% of the unexposed pregnancies (adjusted OR: 1.16 [95% CI: 0.85–1.58]); isotretinoin-exposed births were too few to assess malformations.

56 Robson et al (2020) state: "It is estimated that annually 1 in 500 pregnant women are exposed to oral isotretinoin. Although the UK Teratology Information Service maintains a list of teratogenic medicines, an agreed list of common teratogens with similar interventions to reduce pregnancy exposure in general practice remains an outstanding task for regulatory and professional bodies.

57 The UK Teratology Information Service (UKTIS) states that: "Acitretin (a metabolite of etretinate) is a second-generation oral retinoid, licensed for the treatment of severe psoriasis, congenital ichthyosis and keratosis follicularis (Darier's disease). Concurrent exposure to alcohol may induce reverse metabolism to etretinate, which is stored in the liver and has a much longer half-life. Effective contraception (ideally two complimentary forms) is therefore recommended for four weeks prior to commencing treatment, during and for three years after treatment with acitretin. Multiple malformations, including facial dysmorphia, cleft palate, cardiovascular malformations, and limb and skeletal defects have been reported following *in utero* exposure to acitretin. The available data are, however, limited and the risk of malformation following acitretin exposure *in utero* remains unquantified, although experience from other retinoids suggests that it is likely to be high. An increased risk of spontaneous abortion and impaired neurodevelopment in the absence of malformation have been observed following *in utero* exposure to isotretinoin and exposure to acitretin may carry similar risks**."**

58 Regarding tretinoin, the UKTIS states: "Although sporadic case reports have described malformations, including cardiovascular defects, limb defects, ear defects and CNS defects following maternal use of topical tretinoin during the first trimester of pregnancy, no increased risk of congenital malformation has been shown in subsequent larger cohort studies of topical first trimester tretinoin exposure. These data are, however, too limited to definitively exclude a fetal risk and use during pregnancy is therefore not generally recommended. An individual risk assessment is advised where exposure to supratherapeutic doses of topical tretinoin has occurred, or risk factors which increase absorption of the drug are present in association with pregnancy. There are insufficient data (particularly relating to first trimester exposure) to quantify the risks posed to a developing fetus following oral exposure to tretinoin. The risk-benefit balance of maternal vs. fetal wellbeing must be addressed on an individual basis. Other retinoids are known to be teratogenic at therapeutic doses and the likelihood of an increased risk of structural malformation and neurodevelopmental impairment with tretinoin use in the first trimester should therefore be considered and discussed with the patient. The manufacturer advises that there is a high risk of severe malformations and that effective contraception (progesterone-only pills are not considered to be an effective measure of contraception during treatment with tretinoin) must be used for the duration of oral treatment and for one month afterwards**."**

59 Nau (1995) investigated the influence of toxicokinetic parameters, including

metabolism and placental transfer, on the teratogenic potency of retinoids. Activation (oxidation of retinoic acids: hydrolysis of glycoconjugates) and deactivation reactions (isomerisation from *trans*- into *cis*- configuration; β-glucuronidation) appeared to relate to teratogenesis. The β-glucuronides of retinoic acids showed poor placental transfer and prolonged presence in the maternal animals. The chemical structure of retinoids had a major influence on placental transfer. All-trans-retinol and all-trans-retinoic acid showed extensive placental transfer in the mouse, rat and rabbit, while the *cis*-isomers of retinoic acid *(9-cis,* 13-cis, 9,13-di-cis-retinoic acid) showed a much more limited placental transfer. The reasons behind these structure-specific differences are not clear and may be due to differential binding affinities of these retinoic acids to binding proteins. The placental transfer of 13-cis-retinoic acid was much greater in the monkey than in the rodent species, and the author speculated that the different placental structure between the rodents the monkey could explain this. Extensive transfer of 13-cis-retinoic acid to the monkey embryo was held to be an important factor in its teratogenicity in monkeys, which may also be applicable to humans. Moreover, the primary metabolic pathway proceeded to the 13-cis-4-oxo-retinoic acid in the monkey, which may serve as an activation pathway of teratogenesis. In contrast, in rats and mice the main plasma metabolite was 13-cis-retinoyl-β-glucuronide which shows very poor placental transfer. Plasma clearance of 13-cis-retinoic acid was also much greater in rats and mice than in monkeys. The low teratogenic potency of 13-cis-retinoic acid in the rat and mouse may have been explained by limited placental transfer, rapid plasma clearance and extensive metabolic detoxification; conversely, the high teratogenic activity of this retinoid in the monkey (and possibly) could be the result of more extensive placental transfer, slower plasma clearance and extensive metabolism to the active 4-oxo-metabolite. The author also showed evidence that non-retinoid compounds such as antiepileptic agents appeared to exert some of their teratogenicity via alteration of endogenous retinoid levels.

Other reproductive and developmental endpoints

60 Cox et al (2006) performed a double-blind, randomised, placebo-controlled trial of weekly vitamin A supplementation in pregnant and lactating women and found that supplementation was significantly associated with an increased ratio of mitogen-induced proinflammatory cytokine (IFN-γ) to anti-inflammatory cytokine (IL-10) levels during pregnancy and in the postpartum period. A group of 89 pregnant Ghanaian women were assigned randomly to receive 3000 μg RE weekly of vitamin A, as retinyl palmitate, or placebo (groundnut oil plus tocopherol) until 6 weeks postpartum. While this appeared to be beneficial with regard to the maternal response to infections, the authors recognised that potentiation of pro-inflammatory responses during pregnancy could potentially be a double-edged sword. While stronger Th-1 responses might reduce the risk of infection during pregnancy, providing protection against opportunistic viral, bacterial and protozoal infections that target the placenta and/or the developing fetus, inappropriate overproduction of type 1 cytokines may cause placental damage and threaten the viability of the fetus and the mother, potentially leading to spontaneous abortion (Raghupathy et al 1999, Raghupathy et al 2000, Kwak-Kim *et al*, 2003).

61 Cohen et al (2015) performed a systemic review and meta-analysis searching PubMed, Embase, and several other databases from 1970–2013 for observational studies that measured maternal blood levels of non-enzymatic antioxidants (vitamins A, C, E, and carotenoids) during pregnancy or within 72 hours of delivery. The aim was to explore any association between maternal antioxidant levels during pregnancy and preeclampsia or small-for-gestational-age (SGA) offspring. Retinol was measured in the first trimester in one study, second trimester in two studies, and third trimester in thirteen studies in relation to pre-eclampsia. The meta-analysis of twelve third trimester studies showed a significantly negative standardised mean difference (SMD) but substantial heterogeneity. One additional study reported that blood retinol was non-significantly lower for pre-eclampsia cases versus controls. For mild and severe preeclampsia where there were fewer studies, the pooled SMDs were null with substantial heterogeneity. Three studies found that retinol levels measured in the second trimester were similar among pregnancies resulting in SGA versus appropriate for gestational age (AGA) birth. Three other studies measured retinol in the third trimester, all of which provided raw data for meta-analysis. Results were very heterogeneous. Only one study measured levels before delivery and found significantly higher retinol in mothers who delivered SGA babies. The study in question (Ortega-Senovilla et al, 2010, from abstract) suggests that intrauterine growth restriction pregnancies may be partially due to reduced placental transfer of vitamin A, leading to higher-than-expected maternal blood levels. Two of the studies measured retinol levels shortly after delivery and found no significant differences for mothers who delivered SGA compared to AGA babies.

62 Mawson and Croft (2019) considered the hypothesis that the signs and symptoms of rubella may be due to virus-induced alterations in vitamin A metabolism and its accumulation in the liver, leading to mild hepatic inflammation and dysfunction and to the spillage of stored vitamin A compounds into the circulation in correspondingly low concentrations and hence mild toxicity. The authors' hypothesis suggests that autism due to rubella infection in the early weeks of pregnancy may similarly result from maternal liver dysfunction and exposure of the fetus to excess endogenous vitamin A, resulting in embryopathy and long-term metabolic and neurodevelopmental disorders. However, while this may be a feasible hypothesis and open to testing, the authors also go on to speculate about the effects of vaccines and diet on the development of autism in infants, a hypothesis which has been discredited. Therefore, while this work is noted for completeness, the COT do not endorse any such link

Vitamin A and bone

63 Yee et al (2021) reviewed the effects of vitamin A on bone health. While the majority of the papers they cited related to effects in males and post-menopausal women, they referred to the paper of Händel *et al* (2016), which documents the associations between maternal serum retinol and β-carotene concentrations during late pregnancy and offspring bone mineralization assessed at birth observed in the Southampton Womens' Survey. In this survey, the maternal health, lifestyle, and diet

of a mother-offspring birth cohort were assessed pre-pregnancy and at 11 and 34 weeks of gestation. In late pregnancy, maternal serum retinol and β-carotene concentrations were measured. In total, 520 and 446 mother-offspring pairs had measurements of maternal serum retinol and β-carotene, respectively. Offspring total body bone mineral density (BMD), bone mineral content (BMC), and bone area (BA) were measured within 2 weeks after birth. All outcome and exposure variables were standardised to obtain normally distributed variables with a mean of 0 and SD of 1. Associations were presented as standardised β-coefficients (SD/SD), which compared the strength of the effect of the independent variables with the dependent variables. The higher the value of the calculated beta coefficient, the stronger is the association. Link here

64 The results of the Southampton Womens' Survey (Händel et al, 2016), showed that higher maternal serum retinol in late pregnancy was associated with lower offspring total body BMC (β = −0.10 SD/SD; 95% CI: −0.19, −0.02; p = 0.020) and BA (β = −0.12 SD/SD; 95% CI: −0.20, −0.03; p = 0.009) but not BMD. Conversely, higher maternal serum β-carotene concentrations in late pregnancy were associated with greater total body BMC (β = 0.12 SD/SD; 95% CI: 0.02, 0.21; p = 0.016) and BA (β = 0.12 SD/SD; 95% CI: 0.03, 0.22; p = 0.010) but not BMD. Maternal serum retinol and β-carotene concentrations had differing associations with offspring bone size and growth at birth: retinol was negatively associated with these measurements, whereas β-carotene was positively associated. These findings highlighted the need for further investigation of the effects of maternal retinol and carotenoid status on offspring bone development.

Interactions

65 Zachmann and Gummer (2006) reviewed the literature on interactions between ethanol and retinoic acid as a possible mechanism for birth defects described as fetal alcohol syndrome. Different models have been proposed:

- the synthesis of retinoic acid from retinol, catalysed by alcohol dehydrogenase, might be competitively inhibited by ethanol leading to retinoic acid deficiency;

- ethanol consumption might affect maternal retinol, retinyl ester, or retinoic acid levels, RAR binding, and the levels of RAR expression in developing fetal organs, as has been seen in rats, although specific defects resulting from specific RAR changes have not yet been identified;

- ethanol treatment (or exposure) might mimic vitamin A deficiency, since retinoic acid appears to prevent the adverse effects of ethanol in a quail model;

- retinoic acid and ethanol might reverse or block each other's effects, as has been seen in neuroblastoma cells *in vitro.*

66 The authors suggested that these experiments showed definite interactions between ethanol and vitamin A, but further studies would be needed to determine if any of these mechanisms significantly contributed to prenatal ethanol consumption embryopathy.

67 An early paper on the interaction between vitamins A and D (Cruess & Clark, 1964) indicated that an interaction occurred between toxic amounts of vitamins A and D in rats, which prevented, to a large extent, the alterations in bone lipids (increased triglycerides, esterified cholesterol and phospholipids) that were seen to occur in hypervitaminosis D.

68 Rohde et al (1999) investigated the hypothesis that vitamin A intensifies the severity of the bone mineralization disease, rickets, and inhibits the ability of vitamin D to cure this disease. Weanling Holtzman rats were fed a 1.2% calcium, 0.1% phosphorus diet and 15.5 ng ergocalciferol (vitamin D2) every 3 days for 21 days in the presence of increasing amounts of retinyl acetate (0 mg to 8621 mg/day). Increasing exposure to retinyl acetate led to a progressive and significant decrease in total bone ash ($p = 0.001$) and an increase in epiphyseal plate width ($p = 0.001$). Repeating the experiment with increasing amounts of vitamin D2 (0 to 645 ng/day) indicated that retinyl acetate antagonised all vitamin D2 dosages. To further investigate this antagonistic relationship, weanling rats were fed a 0.47% calcium, 0.3% phosphorus diet and 15.5 ng vitamin D2 every 3 days for 33 days in the presence of increasing retinyl acetate (0 to 3448 mg/day). In the absence of retinyl acetate, these rats maintained a normal serum calcium level (2.34 mmol/L). Increasing retinyl acetate, however, eliminated the ability of vitamin D2 to elevate the level of serum calcium (1.35 mmol/L). The authors proposed that the mechanism for the observed antagonistic effects was competition for the RXR receptor by both vitamins

69 Parr et al (2018) investigated the association between maternal intakes of vitamins A and D during pregnancy, infant exposure to dietary supplements containing these nutrients, potential nutrient interaction, and the development of asthma in school age offspring. The authors studied 61,676 school-age children (born during 2002–2007) from the Norwegian Mother and Child Cohort, considering data on maternal total (food and supplement) nutrient intake in pregnancy (food-frequency questionnaire validated against biomarkers) and infant supplement use at age 6 months. Maternal subjects were controlled for age at delivery, parity, pre-pregnancy BMI, education, history of asthma and atopy and smoking in pregnancy. Log-binomial regression was used to calculate adjusted risk ratios. There were interactions between vitamin A and various dietary components and pharmaceuticals, including vitamin D, which appeared to ameliorate vitamin A toxicity to some extent and vice versa. Asthma increased according to maternal intake of total REs. in the highest (≥2031 REs/day) compared with the lowest (≤779 REs/day) quintile (aRR: 1.21; 95% CI: 1.05, 1.40). The authors concluded that a diet naturally high in vitamin A combined with the use of supplements containing retinol during pregnancy placed women at risk of vitamin A excess, which was associated with increased susceptibility to asthma in their children by the time they reached school age. This effect was observed for maternal intakes of ≥2.5 times the recommended dose, below the EFSA TUL for retinol of 3000 µg/day. Vitamin D

intake close to recommendations was associated with a reduced risk of asthma at school age but not when maternal intake of vitamin A was high. The balance of vitamin A and vitamin D intake during pregnancy appeared to be important to asthma susceptibility in the offspring.

70 Dietary conjugated linoleic acid (CLA) has been found to increase tissue levels of retinol (vitamin A alcohol) and its sole specific circulating carrier protein retinol-binding protein (RBP or RBP4). However, the precise mechanism of this action has not been elucidated. Carta *et* al (2014) suggest that retinol and CLA may compete for catabolic pathways modulated by the activity of peroxisome proliferator-activated receptor- α (PPAR-α) and RXR heterodimer. The authors also presented preliminary data that may position PPAR-α at the crossroads between the metabolism of lipids and vitamin A.

71 Christian and West (1998) reviewed how zinc status has been purported to influence several aspects of vitamin A metabolism, including its absorption, transport, and utilization. Postulated mechanisms relate to either the regulatory role of zinc in vitamin A transport mediated through protein synthesis, and /or the oxidative conversion of retinol to retinal by a zinc-dependent retinol dehydrogenase enzyme. A curvilinear relation has been suggested to describe an effect of plasma zinc on vitamin A transport but clear evidence of synergy between these two micronutrients and its public health significance in humans is lacking.

72 The EVM (2003) stated that vitamin A may antagonise the action of vitamin K in blood clotting function and may potentiate the development of intracranial hypertension when taken in combination with tetracycline and minocycline type antibiotics. 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 and may have an anti-thyroid effect.

Beta-carotene

73 The Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered β-carotene in 1974 and concluded that hypercarotenaemia *per se* was harmless and caused no adverse symptoms or hypervitaminosis A; the condition disappears if excess intake of beta-carotene is discontinued. JECFA quoted an older study, (Greenberg *et al,* 1959) where fifteen subjects received 60 mg beta-carotene daily for three months. Serum carotene levels rose from 128 µg to a maximum of 308 µg/100 ml after one month while Vitamin A levels remained unchanged. No clinical signs of hypervitaminosis A were seen. Other subjects ate several pounds of raw carrots daily, resulting in some skin discoloration. Beta-carotene appeared in breast milk. High doses of beta-carotene were found to reduce liver storage of labelled dl-gamma-tocopherol acetate (vitamin E) to 70%. In their evaluation, JECFA (1974) found a NOAEL in rat of 50 mg/kg bw and derived a human ADI of 0 – 5 mg/kg bw based on a four-generation study at dietary levels of 0 ppm and 1000 ppm of beta-carotene for 110 weeks that showed no adverse effects in any of the generations.

74 In 2017, at the 84th meeting of JECFA, the Committee noted that new data showed large differences in absorption of β-carotene between rodents and humans

and therefore considered that rodents were an inappropriate animal models for establishing an ADI for β-carotene, At the 87th meeting in 2019, the Committee considered that "…no adverse health effects were observed in the general population in large, well-conducted human intervention studies in which healthy participants were administered 20–50 mg β-carotene per day for up to 12 years, in addition to background exposure from the diet. …For the general population, the Committee concluded that the estimated high exposure to β-carotene of 9 mg/day for a 30 kg child and 6 mg/day for a 60 kg adult from its current uses as a food additive, in addition to background exposure from the diet, would not be expected to be a safety concern," (JECFA 2019).

75 Allen and Heskell (2002) found no reports of high-carotene intakes from foods ever having caused vitamin A toxicity. it had been assumed that about one-third of a dose of dietary carotenoids was absorbed and half that amount was converted to retinol, resulting in a bioconversion factor of 6:1 for β-carotene to retinol. This bioconversion factor had been used in most food composition tables to convert carotenoids to retinol equivalents. However, in the early 1990s it become apparent that absorption of carotene from plant sources, especially from vegetables, was substantially less than one-third that absorbed from a dose given in oil. More recent estimates of β-carotene absorption from a diet consisting mainly of vegetables showed that absorption was about one half what was previously assumed. Based on such studies, the Institute of Medicine estimated that 1 retinoic acid equivalent was equal to 12 μg of β-carotene instead of the 6 μg of β-carotene estimate used previously.

76 Omenn et al (1996) reported the commencement of the β-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high-risk populations: smokers and asbestos-exposed workers. CARET was a multicentre, two-armed, double-blinded randomized chemoprevention trial in Seattle, Portland, San Francisco, Baltimore, Connecticut, and Irvine, to test whether oral administration of β-carotene (30 mg/day) plus retinyl palmitate (25,000 IU/day) could decrease the incidence of lung cancer in high-risk populations,

77 Goodman et al (2004) reported that CARET was stopped ahead of schedule because participants who were randomly assigned to receive the active treatment were found to have a 28% increase in incidence of lung cancer, a 17% increase in incidence of death and a higher rate of cardiovascular disease mortality compared with participants in the placebo group. With follow-up through December 31, 2001, the post-intervention relative risks of lung cancer and all-cause mortality for the treatment group compared with the placebo group were 1.12 (95% confidence interval [CI] 0.97 to 1.31) and 1.08 (95% CI 0.99 to 1.17), respectively. Relative risks remained above 1.0 throughout the post-intervention follow-up but conversely, the relative risk of cardiovascular disease mortality decreased rapidly to 1.0 after the intervention was stopped. During the post-intervention phase, females had larger relative risks of lung cancer mortality (1.33 versus 1.14; p=.36), cardiovascular disease mortality (1.44 versus 0.93; p=0.03), and all-cause mortality (1.37 versus 0.98; p=.001) than males. The reported adverse effects of β-carotene and retinyl palmitate on lung cancer incidence and all-cause mortality in cigarette smokers and

individuals with occupational exposure to asbestos persisted after drug administration was stopped although they were no longer statistically significant. Subgroup analyses planned at the time suggested that the excess risks of lung cancer were restricted primarily to females, and cardiovascular disease mortality primarily to females and to former smokers.

78 Tayyem et al (2019) conducted a case-controlled study on 400 Jordanian women aged 20 - 65. Two hundred women recently diagnosed with breast cancer were matched in age, income, and marital status to 200 breast cancer-free women. A food frequency questionnaire was used to assess nutrient intake patterns. A significant increase in breast cancer risk was associated with high vitamin C and β-carotene intake (the highest for the fourth quartile; odds ratio [OR], 5.42; 95% confidence interval [CI], 2.11 to 13.91; ptrend=0.001). Conversely, a significant inverse trend was detected for the risk of breast cancer and high calcium, phosphorus, and vitamin D intake. A high-fat nutrient intake also showed a significant direct association with breast cancer risk in the third (OR, 3.88; 95% CI, 1.58 to 9.51) and fourth (OR, 3.87; 95% CI, 1.53 to 9.77) quartiles (ptrend=0.001).

79 However, the authors pointed out that there was somewhat weaker evidence for a link between vitamin C and β-carotene with hormone-sensitive breast cancer in the study of Bakker et al (2016). Moreover, Nagel et al (2010) demonstrated no associations and of breast cancer with high dietary intake of vitamin C and β-carotene. For their study, Tayyem et al offered the explanation of Salganik et al (2001), who reported that reactive oxygen species in moderate concentrations act as mediators of apoptosis and phagocytosis, and that in people with a low level of reactive oxygen species, an excess of antioxidants could block these mechanisms and promote cancer. This would also explain how an excess of antioxidants could be cancer-promoting in people who are regularly exposed to the effect of environmental carcinogenic factors (tobacco smoke, industrial pollutants) that result in a high accumulation of pre-cancerous and cancerous cells.

Exposure

Vitamin A levels in pregnant women and newborns

80 Söderlund et al. (2005) measured serum concentrations of all-trans retinoic acid and 13-cis retinoic acid in Swedish newborns and their mothers and in women in the first trimester of pregnancy. Ten newborns from normal deliveries and their mothers as well as 16 healthy women in their first trimester of pregnancy were studied, with 17 healthy women as controls. All-*trans* and 13-*cis* retinoic acid and retinol concentrations were measured by HPLC. The newborns had significantly lower retinol concentrations (1.0 μmol/L) than did their mothers (1.7 μmol/L; p = 0.013). Serum all-*trans* retinoic acid was also significantly lower in the newborns (3.4 nmol/L) than in their mothers (5.8 nmol/L; p = 0.008). Serum concentrations of 13-*cis* retinoic acid were significantly lower in the newborns (2.0 nmol/L) than in their mothers (2.6 nmol/L; p = 0.005). The serum concentrations of all-trans retinoic acid and retinol did not correlate in any group. The authors concluded that retinol

concentrations did not accurately reflect the concentrations of the biologically active derivative all-trans retinoic acid.

81 Pregnant women and parturient mothers had significantly lower serum concentrations of retinol than control subjects. The concentration of all-trans retinoic acid was higher in the parturient mothers than in the control subjects. In contrast, the concentrations of 13-*cis* retinoic acid were lower in parturient mothers than in the control subjects. No difference was observed in the concentrations of all-*trans* and 13-*cis* retinoic acid between pregnant women and control women. Pregnant women had significantly higher concentration of 13-*cis* retinoic acid than did the parturient mothers.

82 Hanson et al (2016) used the National Health and Nutrition Examination Survey (NHANES) to assess the relationship between serum retinol concentrations and socioeconomic factors in women of childbearing age in the United States. Women aged14–45 years *n* = 3170) from NHANES cycles 2003–2004 and 2005–2006 were included. Serum retinol concentrations were divided into categories according to World Health Organization criteria. All statistical procedures accounted for the weighted data and complex design of the NHANES sample. WHO poverty score and race were significantly associated with vitamin A status after adjustment for confounders. Odds of retinol concentrations of <1.05 μmol/L were 1.85 times higher for those of lower socioeconomic status when compared to those of higher status (95% CI: 1.12–3.03, $p = 0.02$), and 3.1 times higher for non-Hispanic blacks when compared to non-Hispanic whites (95% CI: 1.50–6.41, $p = 0.002$). Dietary intakes of retinol activity equivalents were significantly lower in groups with higher poverty scores ($p = 0.004$).

83 Mactier and Weaver (2005) found that most relatively healthy preterm infants in a neonatal unit in Glasgow had plasma retinol concentrations <0.7 μmol/l and 20% of extremely low birthweight babies who had not received intramuscular vitamin A had plasma retinol concentrations <0.35 μmol/l at 28 days after birth. The significance for preterm infants of low plasma concentrations of vitamin A, in terms of functional vitamin status was not clear.

Population estimates

84 In many regions of the world, for example, regions of Africa and south-west Asia (Harika et al, 2017) the issue with vitamin A is deficiency and the deleterious effects this has upon the health of unborn children. However, in developed countries, many people regularly have an intake that exceeds EFSA's dietary reference value, with a range of values across European countries being reported, although values did not exceed the tolerable upper limit of 3000 μg RE/day (Jenab et al, 2009). Allen and Haskell (2002) found that in the United States, the 95th percentile of RE intake from foods and supplements for non-pregnant, non-lactating women aged 19–30 years exceeded the EFSA UL but was below the NOAEL for women of reproductive age. Specifically, for non-pregnant, non-lactating women aged 19–30 years, the median intake of vitamin A (retinol and provitamin A carotenoids) from food was 530 μg of RE/day, and the 95th percentile was1112 μg of RE/day. Reportedly, 17% of

this group took supplements. For these women, the median level of vitamin A in the supplements was about 1422 μg of RE/day with a 95th percentile of 2543 μg/day. A woman who consumed the 95th percentile of vitamin A from both diet and supplements would consume about 3655 μg of RE/day, which exceeds the UL of 3000 μg/day. However, a daily intake of 4500 μg of RE/day (the NOAEL) among women of reproductive age is not associated with any adverse effects.

85 EFSA (2015) estimated the average dietary intake in adults as being between 816 and 1,498 μg RE/day (retinol and provitamin A carotenoids). Average daily intakes were in most cases slightly higher in males than in females, mainly owing to the larger quantities of food consumed per day.

86 UK Government dietary advice, as communicated via the NHS.uk website recommends a daily vitamin A intake for adults aged 19 to 64 of 700 μg for men and 600 μg a day for women and that the diet should provide this. Pregnant women are warned about eating liver or liver products such as pate, or supplements that contain vitamin A to avoid potential harm to the unborn baby.

UK retinoid intake

87 The FSA Exposure Assessment Team have sourced information on vitamin A/ pro-vitamin A carotenoid intake in women of childbearing age from years 1 – 11 of the rolling National Diet and Nutrition Study (NDNS). Full details are given in Appendix A. The following data were extracted from the Appendix. Table 2 gives the daily intake of RE in women aged 16 – 49.

Table 2. Chronic exposure of Vitamin A (retinol equivalents) in women from food sources only (Bates et al., 2014, 2016; 2018)**.

	(μg/person/day)*	(μg/person/day)*	(μg/kg bw/day)*	(μg/kg bw/day)*
Age group	Mean	97.5th percentile	Mean	97.5th percentile
16 – 49 yrs	760	2600	11	39
19 – 64 yrs	830	2800	12	43

*Rounded to 2 significant figures

**Based on total population

88 Liver and liver products in the diet constitute a major source of dietary pre-formed RE. As shown in Table 3, only a small number of consumers was recorded in the NDNS. The small number of liver consumers creates uncertainty surrounding the data, However, the Exposure Assessment Team cross referenced the data from NDNS with online sources of intake (from supermarkets and recipes) and found that the number of consumers from these sources were similar to those in the Survey

Table 3. Chronic exposure of Vitamin A from Liver (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018)^.

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
25	3500	7500	50	97

*Rounded to 2 significant figures

**Consumption or exposure estimates made with a small number of consumers may not be accurate. The number of consumers is less than 60, this should be treated with caution and may not be representative for a large number of consumers.

^Based on food consumers on all types of liver

Ghee

89 Heat-clarified butter, known as ghee, forms a staple part in the cuisine of some Asian cultures and thus contributes to vitamin A intake in these population groups. The FSA Analytics Team investigated whether this potentially ethnicity-based dietary habit might lead to a small “hot spot” of the population being exposed to a disproportionately high intake of vitamin A. Although Asian and Asian British women of childbearing age are more likely to consume ghee than those in other ethnic groups, the majority of ghee consumers in this age/sex group were found to be White (77%). This results mainly from White people accounting for the majority of the population and the lower consumption of ghee by ethnic groups other than White or Asian people.

Table 4. Chronic consumption of ghee (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; Roberts et a*l.,* 2018).

Number of consumers	Mean Intake (g/person/day)	97.5th Percentile Intake (g/person/day)	Mean Intake (g/kg bw/day)	97.5th Percentile Intake (g/kg bw/day)	Number of respondents
123	3.0	12	0.043	0.18	1874

*Rounded to 2 significant figures

Table 4a. Chronic exposure of Vitamin A from ghee (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; Roberts et al., 2018).

Number of consumers	Mean Intake (g/person/day)	97.5th Percentile Intake (g/person/day)	Mean Intake (g/kg bw/day)	97.5th Percentile Intake (g/kg bw/day)
123	9.6	120	0.14	1.8

*Rounded to 2 significant figures

90 Appendix A, table 16 gives a list of food products fortified with vitamin A. On the basis of one of these products being consumed once daily, the highest contribution that any one of them would make would be an extra 432 µg RE/day (Dr Witt Multivitamin drink).

91 Appendix A Table 17 gives a list of food supplements containing β-carotene or vitamin A. The supplements containing 1 – 7 mg of β-carotene do not have warnings against their use by pregnant women because of the accepted low risk of this provitamin, but the supplements containing various esters of preformed vitamin A (300 – 906 µg RE/serving) are not recommended in pregnancy.

92 An internet search reveals that in Norway at least, the processing of raw cod liver A to produce the refined product for sale to the public leads to a reduction in the vitamin A and D content and these vitamins are then added back to the product so that the recommended dose to the consumer is 1100 to 4600 IU (330 – 1380 µg RE) vitamin A per teaspoon. At the maximum level, 2 teaspoons of the oil would result in an intake of 2760 µg RE per day, which is below the maximum level set by EFSA but exceeds the “appropriate” level set by EVM. Link here

Risk Characterisation

93 As noted in paragraph 18 (above) EFSA (2006) derived a tolerable upper limit for vitamin A of 3000 µg of RE per day for women of childbearing age. This was based upon a study by Rothman *et al* (1995). In Paragraph 46 (above) Azaïs-Braesco and Pascal (2000) pointed out that this study was inconsistent with previous retrospective studies and had been widely criticised on the grounds of possible misclassification of deformities but could not be ruled out in the consideration of the teratogenic effects of the vitamin. On the other hand, the EVM (2003) was unable to reach a firm conclusion on an upper intake limit, but considered that an intake greater that 1500 µg/day was “inappropriate”.

94 Based upon the EFSA TUL and taking women of childbearing age as a whole, the intake of retinol equivalents from food at the 97.5th percentile of consumption is close to, but still below, the TUL and therefore would not be a concern for their health or for the development of a fetus borne by these women. However, the small group in the food surveys who consume liver at the mean level have an intake that marginally exceeds the TUL (117%) and those who are in the highest consumer group have an intake of 250% of the TUL. If the latter level of consumption were continued into a pregnancy, then this may lead to an increased risk of the fetus suffering a neural tube defect or other developmental lesion that may lead to deformity. Despite the caveat that the data on liver consumption is only recorded in a small number of women of childbearing age, this underlines the rationale behind the UK Government's advice for pregnant women to abstaining from consuming liver during pregnancy.

95 Likewise, fortified food products if eaten to excess, may contribute to an exceedance of the TUL, although this would only be marginal.

96 Moreover, although the consumption of vitamin A rich food supplements on their own do not provide enough RE to exceed the TUL, the nature of their consumption, in addition to a normal diet, especially in the case of cod liver oil, could push RE intake over the TUL. This is consistent with the current Government advice that supplements containing vitamin A are not recommended for pregnant women.

97 Conversely, taking the EVM maximum "appropriate " consumption level of 1500 μg RE per day, then although the mean consumption in the diet is within the acceptable range, it is exceeded by the 97.5th percentile consumption. Even the mean consumption of liver would result in exceedance of the suggested appropriate intake. On this basis, current Government advice for pregnant women to limit their consumption of liver and other foodstuffs containing high concentrations of preformed vitamin A is still valid.

Discussion and conclusions

98 Vitamin A in the diet, either as pro-vitamin A carotenoids from plants or as preformed retinol from animal sources is essential for health in general and for fetal development and vision in particular.

99 The functions of vitamin A are mediated by various isomeric forms of retinaldehyde and retinoic acid. Retinaldehyde has a central function in vision that is not specific to any section of the population. Retinoic acid is involved in multiple aspects of embryogenesis and is a known teratogen in excess.

100 Retinol and β-carotene may have differential effects on fetal bone development, the former being detrimental and the latter beneficial, but further data are required to confirm this.

101 Teratogenicity and embryotoxicity have been observed in animals exposed to high doses of vitamin A as retinol or retinyl esters and to isotretinoin and etretinate. Oral Isotretinoin exposure has been observed in human case control studies to be correlated with an increased risk of spontaneous abortions and birth defects. However, in general, findings in humans, although suggestive of neural crest defects in development, have been mixed and in some cases, their aetiology uncertain. Despite this ambiguity, pregnant women or those considering becoming pregnant are not recommended to consume foods, such as liver, or take supplements that are rich in pre-formed vitamin A.

102 Intake of vitamin A in developed countries often exceeds the intake deemed acceptable by the EVM and the TUL as set by EFSA.

103 Topical application of retinoids does not appear to contribute markedly to overall plasma levels and hence are of low risk to pregnant women and their fetuses.

104 Oral supplements of vitamin A or synthetic analogues may lead to retinoic acid levels that could exert teratogenic or other effects in humans, although dietary levels generally do not.

105 Excess intake of β-carotene does not lead to increased plasma retinol concentrations because of its low conversion rate but may, for example in heavy smokers, increase the risk of cancer in specific circumstances. However, the Committee considers that the risks posed by smoking in pregnancy are in themselves unacceptable to mother and fetus, irrespective of any increase caused by concurrent consumption of beta carotene. Therefore, the smoking habit should continue to be discouraged since that in itself is a major health risk.

106 The current UK Government advice for pregnant women and those planning pregnancy to limit their consumption of preformed vitamin A remains valid.

Abbreviations

AGA	Adequate (size) for Gestational Age
AUC	Area under the dose-response curve
BA	Bone area
BMC	Bone mineral content
BMD	Bone mineral density
Bw	Body weight
CARET	Beta Carotene and Retinol Efficacy Trial
CI	Confidence Interval
Cmax	Maximum concentration in plasma
COT	Committee on the Toxicity of Chemicals in Food, the Environment and Consumer Products
CYP	Cytochrome P_{450}
DNA	Deoxyribonucleic Acid
DRV	Dietary Reference Value
EFSA	European Food Safety Authority
EVM	Expert committee on Vitamins and Minerals
FSA	Food Standards Agency
HIV	Human Immunodeficiency Virus
IFN-γ	Interferon-gamma
IU	International Units
JECFA	Joint FAO/WHO Committee on Food Additives
LPL	Lipoprotein Lipase
LRAT	Lecithin: Retinoic acid Acyltransferase
mg	milligram
NOAEL	No-Observed Adverse Effect Level
NTD	Neural tube defect
OR	Odds Ratio
PPAR	Peroxisomal Proliferator Activated Receptor
PRI	Population Reference Index
RA	Retinoic acid
RAL	Retinaldehyde
RAR	Retinoic Acid Receptor
RBP	Retinol Binding Protein
RDA	Recommended Daily Allowance
RE	Retinol equivalents
RP	Retinyl palmitate
RXR	Retinoid-X-Receptor
SACN	Scientific advisory Committee on Nutrition
SGA	Small for Gestational Age
TUL	Tolerable Upper Limit
VAD	Vitamin A Deficiency
μg	microgram

References

Allen LH, Haskell M. Estimating the Potential for Vitamin A Toxicity in Women and Young Children. Journal of Nutrition 2002; **132**: 2907S–2919S,.

Azaïs-Braesco V, Pascal G. Vitamin A in pregnancy: requirements and safety limits The American Journal of Clinical Nutrition, 2000, **71**(5): 1325S–1333S, Link here

Bakker MF, Peeters PHM, Klaasen VM, *et al.* Plasma carotenoids, vitamin C, tocopherols, and retinol and the risk of breast cancer in the European Prospective Investigation into Cancer and Nutrition cohort. American Journal of Clinical Nutrition 2016;**103**:454–64.

Barnard JH, Collings JC,[, Whiting, A, Przyborski SA, Marder TB. Synthetic Retinoids: Structure–Activity Relationships. Chemistry: a European. Journal. 2009, 15, 11430 – 11442

Biesalski HK. Comparative assessment of the toxicology of vitamin A and retinoids in man. Toxicology 1989;**57**:117–61.

Botto LD, Khoury MJ, Miulinare J, Erickson JD. Periconceptional multivitamin use and the occurrence of conotruncal heart defects: results from a population-based, case-control study. Pediatrics 1996;**98**:911–7.

Bowman WC, Rand MJ Textbook of Pharmacology, second edition, 1982. Blackwell Scientific Publications, Oxford. ISBN 0-632 09990 9.

Buss NE, Tembe EA, Prendergast BD, Renwick AG, George CF. The teratogenic metabolites of vitamin A in women following supplements and liver Human and Experimental Toxicology 1994 **13**(1):33-43. doi: 10.1177/096032719401300106. Link here

Carta G, Murru E, Cordeddu L , Ortiz B , Giordano E, Belury MA, Quadro L, Banni S. Metabolic Interactions between Vitamin A and Conjugated Linoleic Acid Nutrients 2014, 6, 1262-1272; doi:10.3390/nu6031262 nutrients ISSN 2072-6643 Nutrients.

Christian P, West KP Jr. Interactions between zinc and vitamin A: an update. American Journal of Clinical Nutrition 1998,**68**(suppl):435S–41S.

Clagett-Dame M and DeLuca HF The Role of Vitamin A in Mammalian Reproduction and Embryonic Development. Annual Review of Nutrition 2002 **22:**347-381

Clagett-Dame M, Knutson D. Vitamin A in Reproduction and Development. Nutrients 2011, **3,** 385-428; doi:10.3390/nu3040385 Link here

Cohen JM, Beddaoui M, Kramer MS, Platt RW, Basso O, Kahn SR Maternal Antioxidant Levels in Pregnancy and Risk of Preeclampsia and Small for Gestational Age Birth: A Systematic Review and Meta-Analysis. PLoS ONE 2015 **10**(8): e0135192. doi:10.1371/journal.pone.0135192 Link here

Collins MD, Mao GE. Teratology of retinoids. Annual Review of. Pharmacology and Toxicology 1999. **39**, 399–430.

Carazo A, Macáková K, Matoušová K, Krˇcmová LK, Protti, M, Mladˇenka, P. Vitamin A update: forms, sources, kinetics, detection, function, deficiency, therapeutic use and toxicity. Nutrients 2021, **13**, 1703. Link here

Cox SE, Arthur P, Kirkwood BR, Yeboah-Antwi K, Riley EM.Vitamin A supplementation increases ratios of proinflammatory to anti-inflammatory cytokine responses in pregnancy and lactation. Clinical and Experimental Immunology,2006 **144**: 392–400

Cruess RL, Clark I. Alterations in the lipids of bone caused by hypervitaminosis A and D. Biochemical Journal 1965 **96**(1):262-265

Danby FW. Retinoic acid in acne therapy. Canadian Medical Association Journal 1978 **119** 854.

Das BC, Thapaa P, Karkia R, Dasa S, Mahapatraa S, Liuc T-C , Torregrozac I, Wallaced DP, Kambhampatia S, Van Veldhuizena P, Vermae A, Rayf SK, Evans T. Retinoic Acid Signaling Pathways in Development and Diseases. Bioorganic Medicinal Chemistry. 2014: **22**(2): 673–683. doi:10.1016/j.bmc.2013.11.025. Link here

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2006 Tolerable upper intake levels of vitamins and minerals. ISBN: 92-9199-014-0

EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015. Scientific opinion on dietary reference values for vitamin A. EFSA Journal 2015;13(3):4028, 84 pp. doi:10.2903/j.efsa.2015.4028. Link here

Goodman GE, Thornquist MD, BalmesJ ,. Cullen MR, Meyskens FL Jr., Omenn GS, Valanis B, Williams JH Jr. The beta-carotene and retinol efficacy trial: incidence of lung cancer and cardiovascular disease mortality during 6-year follow-up after stopping β-carotene and retinol supplements. Journal of the National Cancer Institute 2004;**96**:1743–1750.

Greenberg R, Cornbleet T, Joffay A I. (1959) Accumulating and excretion of vitamin a-like fluorescent material by sebaceous glands after the oral feeding of various carotenoids. Journal of Investigational Dermatol., **32**, 599.

Händel, MN, Moon RJ, Titcombe P, Abrahamsen B, Heitmann BL, Calder PC, Dennison EM, Robinson SM, Godfrey KM, Inskip HM, Cooper C, Harvey NC· Maternal serum retinol and β-carotene concentrations and neonatal bone mineralization: results from the Southampton Women's Survey cohort. American Journal of Clinical Nutrition. 2016 **104**(4): 1183–1188.

Harika R, Faber M, Samuel F, Kimiywe J, Mulugeta A, Eilander A micronutrient status and dietary intake of iron, vitamin A, iodine, folate and zinc in women of reproductive age and pregnant women in Ethiopia, Kenya, Nigeria and South Africa:

a systematic review of data from 2005 to 2015. Nutrients 2017, **9**: 1096; doi:10.3390/nu9101096. Link here

Hanson C, Lyden E, Abresch C Anderson-Berry A. Serum retinol concentrations, race, and socioeconomic status in of women of childbearing age in the United States Nutrients 2016, ***8***(8), 508;

Hartmann S, Brørs O, Bock J, Blomhoff R, Bausch J, Wiegand U-W, Hartmann D, Hornig DH. Exposure to retinyl esters, retinol, and retinoic acids in non-pregnant women following increasing single and repeated oral doses of vitamin A Annals of Nutrition and Metabolism. 2005; **49**(3):155-64 doi: 10.1159/000086879. Link here

Howard WB, Willhite CC: Toxicity of retinoids in humans and animals. Journal of Toxicology Toxin Reviews 1986; **5**:55-94

Huang P, Chandra V, Rastinejad F. Retinoic Acid Actions Through Mammalian Nuclear Receptors. Chemical Reviews 2014 **114**(1): 233–254. doi:10.1021/cr400161b. Link here

Jenab M, Salvini S, van Gils CH, Brustad M, Shakya-Shrestha S, Buijsse B, Verhagen H, Touvier M, Biessy C, Wallstro¨m P, Bouckaert K, Lund E, Waaseth M, Roswall N, Joensen AM, Linseisen J, Boeing H, Vasilopoulou E, Dilis V, Sieri S, Sacerdote C, Ferrari P, Manjer J, Nilsson S, Welch AA, Travis R, Boutron-Ruault MC, Niravong M, Bueno-de-Mesquita HB, van der Schouw YT, TormoMJ, Barricarte A, Riboli E, Bingham S, Slimani N. Dietary intakes of retinol, β-carotene, vitamin D and vitamin E in the European Prospective Investigation into Cancer and Nutrition cohort. European Journal of Clinical Nutrition 2009 **63**, S150–S178

Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1975) Revision of the Directive on colouring matters authorized for use in foodstuffs intended for human consumption Reports of the Scientific Committee for Food. First Series p17 – 30,

JECFA (1974) Eighteenth Report of the Joint FAO/WHO Expert Committee on Food Additives, Wld Hlth Org. techn. Rep. Ser., 1974, No. 557.FAO Nutrition Meetings Report Series, 1974, No. 54.

JECFA (2019) Eighty- seventh meeting Rome, 4–13 June 2019

Kamm JJ, Toxicology, carcinogenicity, teratogenicity of some orally administered retinoids. Journal of the American Academy of Dermatology 19826(4), Part 2, 652-659.

Kizer KW, Fan AM, Bankowska J, Jackson RJ, Lyman DO. Vitamin A- a pregnancy hazard alert. Western Journal of Medicine 1990; **152**:78-81.

Kwak-Kim JY, Chung-Bang HS, Ng SC, Ntrivalas EI, Mangubat CP, Beaman KD, Beer AE, Gilman-Scahs A., et al. Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. Human Reproduction 2003; **18**:767–773.

Lammer EJ, Chen DT, Hoar RM, Agnish ND, Benke PJ, Braun JY, Curry CJ, Fernhoff PM, Grix Jr AW, Lott IT, et al. Retinoic acid embryopathy New England Journal of Medicine1985 **313**(14):837-41. doi: 10.1056/NEJM198510033131401 Link here

Lammer EJ: Embryopathy in infant conceived one year after termination of maternal etretinate (Letter). Lancet 1988; **2**:1080-1081

Lee LMY, Leunga C-Y, Tanga WWC, Choia HL, Leung YC, McCaffery PJ, Wanga C-C, Woolfe AS, Shuma ASW. A paradoxical teratogenic mechanism for retinoic acid. PNAS 2012 **109**(3), 13668–13673

Loughrill E, Govinden P, Zand N. Vitamins A and E content of commercial infant foods in the UK: A cause for concern? Food Chemistry 2016 **210**:56-62. doi: 10.1016/j.foodchem.2016.04.014 Link here

MacDonald SC, Cohen JM, Panchaud A, McElrath TF, Huybrechts KF, Hernández-Díaz S. Identifying Pregnancies in Insurance Claims Data: Methods and Application to Retinoid Teratogenic Surveillance. Pharmacoepidemiological Drug Safety. 2019 **28**(9): 1211–1221. doi:10.1002/pds.4794. Link here

Mactier H, Weaver LT Vitamin A and preterm infants: what we know, what we don't know, and what we need to know
Archives of Disease in Childhood - Fetal and Neonatal Edition 2005;**90:**F103-F108.

Maden M. Vitamin A and the developing embryo. Postgraduate Medical Journal 2001;**77**:489-491.

Martines-Frias ML, Salvador J. Epidemiological aspects of prenatal exposure to high doses of vitamin A in Spain. European Journal of Epidemiology 1990;**6**:118–23.

Mawson AR, Croft AM. Rubella Virus Infection, the Congenital Rubella Syndrome, and the Link to Autism. International Journal of Environmental Research and Public Health 2019, **16**: 3543; doi:10.3390/ijerph16193543 Link here

Merck Index, 12th edition Merck & Co. Inc. Whitehouse Station NJ 1996 ISBN 0911910-12-3

Mills JL, Simpson JL, Cunningham GC, Conley MR, Rhoads GG. Vitamin A and birth defects. Am J Obstet Gynecol 1996;177:31–6

Nagel G, Linseisen J, van Gils CH *et al*. Dietary β-carotene, vitamin C and E intake and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). Breast Cancer Research and Treatment 2010 **119**, 753–765 Link here..

Nau H. Chemical structure - teratogenicity relationships, toxicokinetics and metabolism in risk assessment of retinoids. Toxicology Letters 1995 ;**82/83** : 975-979

NHS advice on vitamins and minerals: Vitamin A. Last reviewed, August 2021
Vitamin A

NHS Foods to avoid during pregnancy. Last reviewed August 2021

Foods to avoid in pregnancy

Nohynek GJ, Meuling WJA, Vaes WHJ, Lawrence RS, Shapiro S, Schulte S, Steiling W, Bausch J, Gerber E, Sasa H, Nau H. Repeated topical treatment, in contrast to single oral doses, with Vitamin A-containing preparations does not affect plasma concentrations of retinol, retinyl esters or retinoic acids in female subjects of child-bearing age Toxicology Letters 2006;**163**(1):65-76 doi: 10.1016/j.toxlet.2005.09.029. Link here

Omenn GS, Goodman G, Thornquist M, Grizzle J, Rosenstock L, Barnhart S, Balmes J, Cherniack MG, Cullen MR, Glass A, Keogh J, Meyskens FJr, Valanis B, Williams J Jr. The β-Carotene and Retinol Efficacy Trial (CARET) for Chemoprevention of Lung Cancer in High Risk Populations: Smokers and Asbestos-exposed Workers. Cancer Research Suppd 54. 2(B8s-2ll43s, April 1. IW4]

Ortega-Senovilla H, Alvino G, Taricco E, Cetin I, Herrera E. Enhanced circulating retinol and non-esterified fatty acids in pregnancies complicated with intrauterine growth restriction. Clin Sci. 2010; **118**(5):351–8.

Ozaki R, Kuroda K, Ikemoto Y, Ochiai A, Matsumoto A, Kumakiri J, et al. Reprogramming of the retinoic acid pathway in decidualizing human endometrial stromal cells. PLoS ONE 2017; **12**(3): e0173035. doi:10.1371/journal. pone.0173035.

Parr CL, Magnus MC, Karlstad Ø, Holvik K, Lund-Blix NA, Haugen M, Page CM, Nafstad P, Ueland PM, London SJ, Håberg SE, Nystad W. Vitamin A and D intake in pregnancy, infant supplementation, and asthma development: the Norwegian Mother and Child Cohort. American Journal of Clinical Nutrition 2018;**107**:789–798.

Penniston KL, Tanumihardjo SA The acute and chronic toxic effects of vitamin A. American Journal of Clinical Nutrition 2006;**83**:191–201.

Raghupathy R, Makhseed M, Azizieh F, Hassan N, Al-Azemi M, Al-Shamali E. Maternal Th1- and Th2-Type Reactivity to Placental Antigens in NormalHuman Pregnancy and Unexplained Recurrent Spontaneous Abortions Cellular Immunology1999 **196**:122–130.

Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. Human Reproduction 2000; **15**:713–718.

Robson J, Moss N, McGettigan P, Beardsley SJ, Lovegrove E, Dezateux C. First do no harm: valproate and medicines safety in pregnancy British Journal of General Practice 2020; **70** (699): 477-478

.

Rohde CM, Manatt M, Clagett-Dame M, DeLuca H-F. Vitamin A antagonizes the action of vitamin D in rats. Journal of Nutrition 1999 **129**: 2246–2250,

Rosa FW: Teratogenicity of isotretinoin (Letter). Lancet 1983; **2**:513

Rothman KJ, Moore LL,. Singer MR, Nguyen U-SDT, Mannino S, Milunsky A Teratogenicity of high vitamin a intake. New England Journal of Mediciine 1995; **333**:1369-73.

Russell RM The vitamin A spectrum from deficiency to toxicity American Journal of Clinical Nutrition 2000;**71**:878–84.

Rutkowski M, and Grzegorczyk K adverse effects of antioxidative vitamins International Journal of Occupational Medicine and Environmental Health 2012;**25**(2):105 – 121 DOI 10.2478/S13382-012-0022-x Link here

Salganik RI The benefits and hazards of antioxidants: controlling apoptosis and other protective mechanisms in cancer patients and the human population Journal of the American College of Nutrition. 2001;**20**(5 Suppl):464S-472S.

SCF (Scientific Committee on Food), 2002. Opinion on the Tolerable Upper Intake Level of preformed vitamin A (retinol and retinyl esters). Link here

Schnorr CE, Da Silva Morrone M, Weber MH, Lorenzi R, Behr GA, Claudio J, Moreira F. The effects of vitamin A supplementation to rats during gestation and lactation upon redox parameters: Increased oxidative stress and redox modulation in mothers and their offspring. Food and Chemical Toxicology 2011; **49**: 2645–2654

Söderlund MB, Fex GA, Nilsson-Ehle P. Concentrations of retinoids in early pregnancy and in newborns and their mothers. American Journal of Clinical Nutrition 2005;**81**:633– 6.

Spiegler E, Kim Y-K, Wassef L, Shete V, Quadro L. Maternal-fetal transfer and metabolism of vitamin A and its precursor β-carotene in the developing tissues Biochimica et Biophysica Acta. 2012; **1821**(1): 88–98..

Tayyem RF, Mahmoud RI, Shareef MH, Marei LS. Nutrient intake patterns and breast cancer risk among Jordanian women: a case-control study. Epidemiology and Health Volume: 4**1**,.Article ID: e2019010, 7 pages Link here

Van den Berg H, Hulshof KF, Deslypere JP. Evaluation of the effect of the use of vitamin supplements on vitamin A intake among (potentially) pregnant women in relation to the consumption of liver and liver products European Journal of Obstetrics, Gynecology and Reproductive Biology 1996 **66**(1):17-21.

Werler MW, Lammer EJ, Rosenberg L, Mitchell AA Maternal vitamin A supplementation in relation to selected birth defects Teratology 1990 Nov;42(5):497-503 doi: 10.1002/tera.1420420506 Link here

Wilkinson RD. Safety of retinoic acid in acne therapy. Canadian Medical Association Journal 1975 **113** 606

Willhite CC, Hill RM, Irving DW, Isotretinoin-induced craniofacial malformations in humans and hamsters. J Craniofacial Genetics and Developmental Biology 1986;**2**(suppl): 193-209 Link here

Willhite CC, Sharma RP, Allen PV, Berry DL. Percutaneous retinoid absorption and embryotoxicity. Journal of Investigational Dermatology. 1990; **95**(5):523-9. doi: 10.1111/1523-1747.ep12504873. Link here

WHO (2021) Vitamin A supplementation during pregnancy

Yee MMF, Chin K-Y, Ima-Nirwana S, Wong SK. Vitamin A and Bone Health: A Review on Current Evidence. Molecules 2021, **26**,1757.

Zachman RD, Grummer MA. The Interaction of Ethanol and Vitamin A as a Potential Mechanism for the Pathogenesis of Fetal Alcohol Syndrome. Alcoholism: Clinical and Experimental Research 1998; **22**(2): 1544 – 1556

Zile MH Vitamin A and Embryonic Development: An Overview The Journal of Nutrition, 1998 128(2), 455S–458S. Link here

Zomerdijk IM, Ruiter R, Houweling LMA, Herings RMC, Sturkenboom MCJM, S Straus SMJM, Stricker BH. Isotretinoin exposure during pregnancy: a population-based study in The Netherlands. BMJ Open 2014; **4**:e005602. doi:10.1136/ bmjopen-2014-005602 Link here

Literature search

The following terms were input into PubMed and the relevant papers found as well as references therein were cited in this paper:

Vitamin A AND	maternal health
	prerconception
	conception
	pregnancy
	postnatal
	fetus OR foetus
	teratogen*
	abortion
	absorption
	distribution
	metabolism
	excretion
	toxicity
	repro*
	interactions
	beta carotene
	preeclampsia
	cancer

Appendix 1: Vitamin A content of foods, fortified food products and supplements

Table 1. Approximate Vitamin A concentrations in foods (FSA, 2021)

Food type	Retinol Equivalent (µg /100g)	Type of Vitamin A
Liver calf (fried in corn oil)	25217	Pre formed retinol
Liver, chicken, fried in corn oil	10500	Pre formed retinol
Giblets, turkey, boiled	3100	Pre formed retinol
Eel, yellow, raw	1200	Pre formed retinol
Ghee, butter	1233	Pre formed retinol
Fat spread, low fat (26-39%), polyunsaturated	962	Pre formed retinol
Carrots. raw	1961	Carotenoids
Carrots, boiled	1850	Carotenoids
Spinach, boiled	1101	Carotenoids
Sweet potato, flesh only, boiled in unsalted water	927	Carotenoids
Curly Kale, raw	525	Carotenoids
Melon, Canteloupe-type, flesh only, weighed with skin	194	Carotenoids
Mangoes, ripe, flesh only, raw	116	Carotenoids
Apricots, dried	105	Carotenoids
Peaches, raw, flesh and skin	19	Carotenoids

Consumption and exposure assessments for vitamin A in various food sources

The following tables (Tables 2 to 13a) details consumption of selected foods containing vitamin A and indicate estimated exposure to vitamin A. The exposure estimates are derived from individual consumption of these foods and take into account various forms of the foods as well as recipes. For example, liver from different animal sources contain varying amounts of vitamin A (Table 1). As such, exposure estimates take account of only some of the concentrations shown in Table 1. All variations of foods available within the NDNS database were used to obtain the consumption and exposure estimates.

Table 2. Chronic exposure of Vitamin A (retinol equivalents) in women from food sources only (Bates et al., 2014, 2016; 2018)**.

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Age group	Mean	97.5th percentile	Mean	97.5th percentile
16 – 49 yrs	760	2600	11	39
19 – 64 yrs	830	2800	12	43

*Rounded to 2 significant figures

**Based on total population

Liver

Table 3. Chronic consumption of all types of liver (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018)^.

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
25	22	38	0.33	0.56

*Rounded to 2 significant figures

**Consumption or exposure estimates made with a small number of consumers may not be accurate. The number of consumers is less than 60, this should be treated with caution and may not be representative for a large number of consumers.

^Based on food consumers of all types of liver

Table 3a. Chronic exposure of Vitamin A from all types of liver (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018)^.

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
25	3500	7500	50	97

*Rounded to 2 significant figures

**Consumption or exposure estimates made with a small number of consumers may not be accurate. The number of consumers is less than 60, this should be treated with caution and may not be representative for a large number of consumers.

^Based on food consumers on all types of liver

Butter

Table 4. Chronic consumption of butter (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018).

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
1474	5.9	25	0.09	0.37

*Rounded to 2 significant figures

Table 4a. Chronic exposure of Vitamin A from butter / ghee (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018).

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
1474	40	230	0.60	3.5

*Rounded to 2 significant figures

Milk

Table 5. Chronic consumption of cow's milk (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018).

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
1814	150	460	2.2	7.1

*Rounded to 2 significant figures

Table 5a. Chronic exposure of Vitamin A from cow's milk (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018).

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
1814	36	120	0.54	1.8

*Rounded to 2 significant figures

Egg yolk

Table 6. Chronic consumption of egg yolk (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018)**.

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
903	8.5	25	0.15	0.38

*Rounded to 2 significant figures

**Assumption – average egg contains 29% yolk.

Table 6a. Chronic exposure of Vitamin A from egg yolk (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018)**.

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
903	12	34	0.17	0.52

*Rounded to 2 significant figures

**Assumption – average egg contains 29% yolk.

Carrots

Table 7. Chronic consumption of carrots (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018).

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
1327	21	74	0.31	1.1

*Rounded to 2 significant figures

Table 7a. Chronic exposure of Vitamin A from carrots (with recipes) in women aged 16-49 (Bates et al., 2014, 2016; 2018)*.

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
1327	330	1300	4.9	20

*Rounded to 2 significant figures

Peppers

Table 8. Chronic consumption of peppers (with recipes) (Bates et al., 2014, 2016; 2018).

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
1049	14	60	0.21	0.91

*Rounded to 2 significant figures

Table 8a. Chronic exposure of Vitamin A from peppers (with recipes) (Bates et al., 2014, 2016; 2018).

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
1049	12	51	0.18	0.76

*Rounded to 2 significant figures

Spinach

Table 9. Chronic consumption of spinach (with recipes) (Bates et al., 2014, 2016; 2018).

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*

Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
222	19	61	0.24	0.97

*Rounded to 2 significant figures

Table 9a. Chronic exposure of Vitamin A from spinach (with recipes) (Bates et al., 2014, 2016; 2018).

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
222	103	517	1.6	8.4

*Rounded to 2 significant figures

Cantaloupe melon

Table 10. Chronic consumption of Cantaloupe melon (with recipes) (Bates et al., 2014, 2016; 2018).

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
42	46	131	0.78	2.5

*Rounded to 2 significant figures

**Consumption or exposure estimates made with a small number of consumers may not be accurate. The number of consumers is less than 60, this should be treated with caution and may not be representative for a large number of consumers

Table 10a. Chronic exposure of Vitamin A from Cantaloupe melon (with recipes) (Bates et al., 2014, 2016; 2018).

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
42	135	384	2.3	7.5

*Rounded to 2 significant figures

**Consumption or exposure estimates made with a small number of consumers may not be accurate. The number of consumers is less than 60, this should be treated with caution and may not be representative for a large number of consumers

Mango

Table 11. Chronic consumption of mango (with recipes) (Bates et al., 2014, 2016; 2018).

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
235	18	105	0.26	13

*Rounded to 2 significant figures

Table 11a. Chronic exposure of Vitamin A from mango (with recipes) (Bates et al., 2014, 2016; 2018).

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
234	15	94	0.22	1.3

*Rounded to 2 significant figures

Apricot

Table 12. Chronic consumption of apricot (with recipes) (Bates et al., 2014, 2016; 2018).

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
88	5.7	27	0.084	0.40

*Rounded to 2 significant figures

Table 12a. Chronic exposure of Vitamin A from Apricot (with recipes) (Bates et al., 2014, 2016; 2018).

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
88	3.8	20	0.057	0.30

*Rounded to 2 significant figures

Peach

Table 13. Chronic consumption of peaches (with recipes) (Bates et al., 2014, 2016; 2018).

	(g/person/day)*	(g/person/day)*	(g/kg bw/day)*	(g/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
77	24	110	0.34	1.3

*Rounded to 2 significant figures

Table 13a. Chronic exposure of Vitamin A from peaches (with recipes) (Bates et al., 2014, 2016; 2018).

	(µg/person/day)*	(µg/person/day)*	(µg/kg bw/day)*	(µg/kg bw/day)*
Consumers**	Mean	97.5th percentile	Mean	97.5th percentile
77	4.6	21	0.67	0.28

*Rounded to 2 significant figures

Fortified foods

Foods are sometimes fortified with vitamin A such as butter and other fat spreads, milk and nutritional powders and cereal products. Some foods such as spreads and sports drinks are fortified with beta carotenes which are used for colouration of the product.

Table 14. Estimated exposure from fortified food products containing Vitamin A (Tesco, Sainsbury's, Asda, Boots, Holland & Barret, Morrisons, M&S 2021)

Food product	Vitamin A concentration	Vitamin A concentration (µg per serving)	Exposure^
Butters and Spreads	**µg per 100 g**	**µg per 10 g serving**	**µg/kg bw/day***
Flora Original Spread 500G	814	81.4	1.2
Flora Buttery Spread 500g	233	23.3	0.33
Flora Light Spread 500G	839	83.9	1.2
Flora ProActiv Buttery Taste Spread 500G	120	12	0.17
Bertolli Original Spread 500G	800	80	1.1
Bertolli With Butter 400G	800	80	1.1
Benecol Buttery Spread 500G	900	90	1.3
Pure Vegan Dairy Free Olive Spread 500g	800	80	1.1
Pure Vegan Dairy Free Sunflower Spread 500g	800	80	1.1
Nutritional Drink powders	**µg per 100 g**	**µg per serving**	
Complan Nutritional Drink Strawberry 4X55g	551	303 per 55 g	4.3
Complan Nutritional Drink Drink Chocolate 4X55g	522	287 per 55 g	4.1
Complan Nutritional Drink Banana 4X55g	550	303 per 55 g	4.3
Complan Nutritional Drink Original 425G	547	301 per 55 g	4.3
Slimfast Vitality Meal Replacement Shake Chocolate Intensity 400g	81.9 (as prepared)	243 (as prepared) per 40 g	3.5

USN Diet Fuel Ultralean Strawberry Flavoured Meal Replacement Shake	474	256 per 54 g	3.6
Nutritional Drinks	**µg per 100 ml**	**µg per serving**	
Tropicana+ Vitamin Victory Juice 750ml	208	312 per 150 ml	4.4
Benefit Drinks Cleanse Prune Juice	320	800 per 250 ml	11
Oshee Vitamin Cocktail 250ml	160	400 per 250 ml	5.7
Slim-Fast Milkshake Strawberry 6 x 325ml	73.8	240 per 325 ml	3.4
Dr Witt Multivitamin Drink 1 Litre	216	432 per 200 ml	6.1
Nutrient Powder (foods)	**µg per 100g**	**µg per serving**	
Funktional Foods Spirulina Powder 100G	3685	369 per 10 g	5.2
Funktional Foods Wheatgrass Powder 100G	1289	258 per 10 g	3.7
Dried Milk	**µg per 100 ml as prepared**	**µg per serving as prepared (200 ml)**	
Sainsbury's Skimmed Milk Powder 300g	66.7	133	1.9
Tesco Instant Dried Skimmed Milk 340G	71	142	2.0
Marvel Dried Milk Powder 278G	66	132	1.9
Cereal bars	**µg per 100 g**	**µg per serving**	
Oshee Vitamin Muesli Bar Hazlenut & Raisin 40g	300	120 per 40 g	1.7

Oshee Vitamin Muesli Bar Plum & Cranberry 40g	300	120 per 40 g	1.7
Slimfast Meal Replacement Very Berry Bar 4 x 60g	400	240 per 60 g	3.4
Other products	**µg per 100 g**	**µg per serving**	
Blockhead Sugar Free Vitamin D, C, B & A Gum		800 per 2 pieces	11
Tetley Super Fruit Multi Vitamins Berry 20 Tea Bags 40G	30	30 per 100 ml	0.43
Potters Malt Extract with Cod Liver Oil Butterscotch 650g	1720	172 per 10 g	2.4
Boots Malt Extract + Cod Liver Oil - 650g	1400	140 per 10 g	2.0

^Exposure is calculated from the recommended serving size and the average body weight of women aged 16- 49 years (70.3kg)

*Rounded to 2 significant figures

Supplements

Table 15. List of a sample of supplements containing vitamin A (Sources: Lloyds Pharmacy, Boots Pharmacy and Superdrug)

Supplement	**Maternal supplement?^**	**Vitamin A form**	**Recommended daily dose**	**Daily exposure in retinol equivalents (µg/day)**
Vitabiotics pregnacare tablets range	Yes	Beta carotene	1 tablet	1000
Vitabiotics Pregnacare Liquid	Yes	Beta carotene	10ml	500
Vitabiotics pregnacare breastfeeding range	Yes	Beta carotene	1 tablet	1000

Seven Seas all stages during pregnancy	Yes	Beta carotene	1 tablet	500
Seven Seas pregnancy follow on	Yes	Beta carotene	1 tablet	500
Proceive Advanced Fertility Supplement Max Women	Yes	Beta carotene	2 capsules	3500
Seven Seas Adult Complete Multivitamins 28	No	Vitamin A Acetate	1 tablet	800
Healthspan women's multivitamin super fruit 30 gummies	No	Vitamin A Palmitate	1 tablet	800
Pink simply radiant multivitamin for her gummies 60 gummies	No	Vitamin A	2 tablets	750
Superdrug Multivitamin With Iron	No	Vitamin A Acetate	1 tablet	800
Bassets Adult Multivitamin Pastilles	No	Vitamin A	1 tablet	800
Vitabiotics wellwoman original 30 capsules	No	Beta carotene	1 tablet	1000
Boots multivitamins	No	Vitamin A Acetate	1 tablet	800
Centrum Advance multivitamins	No	Vitamin A (RE) (25% as beta-carotene)	1 tablet	800
Centrum Fruity Chewable	No	Vitamin A (RE)	2 tablets	660
Centrum MultiGummies	No	Vitamin A (RE)	2 tablets	660
Centrum Women	No	Vitamin A (RE)	1 tablet	667
SimplySupplements Cod Liver Oil 1000mg	No	Vitamin A	1-3 capsules	300-900
Holland & Barrett Cod Liver Oil Pure Liquid 500ml	No	Vitamin A (RE)	2.5ml	691
Seven Seas Cod Liver Oil One-A-Day Omega-3 Fish Oil & Vitamin D 120 Capsules	No	Vitamin A (RE)	1 capsule	750

Solgar Super Cod Liver Oil Complex - 60 Tablets	No	Retinyl palmitate	1 soft gel	906

*Exposure is calculated from the daily recommended intake and the average body weight of women aged 16- 49 years (70.3kg)

^Indicates whether the supplement is marketed specifically to pregnant or breastfeeding women

References

Bates, B.; Lennox, A.; Prentice, A.; Bates, C.; Page, P.; Nicholson, S.; Swan, G. (2014) National Diet and Nutrition Survey Results from Years 1, 2, 3 and 4 (combined) of the Rolling Programme (2008/2009 – 2011/2012) Available: Here

Bates, B.; Cox, L.; Nicholson, S.; Page, P.; Prentice, A.; Steer, T.; Swan, G. (2016) National Diet and Nutrition Survey Results from Years 5 and 6 (combined) of the Rolling Programme (2012/2013 – 2013/2014) Available at: Here

Food Standards Agency (2021). McCance and Widdowson's The Composition of Foods Integrated Dataset 2021. Link here

Roberts, C.; Steer, T.; Maplethorpe, N.; Cox, L.; Meadows, S.; Page, P.; Nicholson, S.; Swan, G. (2018) National Diet and Nutrition Survey Results from Years 7 and 8 (combined) of the Rolling Programme (2014/2015 – 2015/2016) Available at: Here