Reproductive effects of vitamin A

In this guide

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48 It is now generally believed that all-trans RA (ATRA) supports both male and female reproduction as well as embryonic development. (Zile, 1998; Clagett-Dame and DeLuca, 2002; and 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 RAR null mutants. The differential activity of CYP26 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.

49 The teratogenic effects of RA have been documented both in animals and in humans (Zile, 1998). RA induces differential patterns of malformations in mammalian embryos based on the different stages of embryonic development. Children exposed in utero to isotretinoin ingested by their mother 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

50 Within embryos of experimental animals, both 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 RA on the development of rodent embryonic kidneys. Both vitamin A excess and deficiency lead to lack of kidney development (bilateral renal agenesis) in the hamster and the mouse

before any morphologically identifiable precursor of the organ is present. 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 enzymes. 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.

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

Oral exposure

52 Piersma et al (1996) tested the teratogenicity of a single dose of retinyl palmitate in rats. Pregnant rats were treated at gestation day 10 by gavage with 100, 300 or 1000 mg/kg body weight retinyl palmitate on a dietary background level of 5 mg/kg feed. By gestation day 11 the number of embryos with an open cranial neural tube had increased with the dose. At gestation day 21, the high dose group showed an increase in late resorptions, whereas both the high and the medium dose groups had a high incidence of fetuses with malformations typical of retinoid embryopathy. The data suggested that delayed neural tube closure had occurred in a large proportion of the embryos. In a second experiment, the high oral dose was applied on gestation day 10 in pregnant rats receiving retinyl palmitate at 1.5, 5, 15, or 50 mg/kg feed for 6 weeks. Delayed neural tube closure, post-implantation loss and the nature and incidence of malformations were similar between diet groups, as well as being reminiscent of the high dose group in the first experiment. Thus, the dietary status of the animals did not seem to influence the teratogenic potential of a single high dose of retinyl palmitate.

53 As reviewed by EFSA (2006), Ritchie et al (1998) quantified the teratogenic potencies of retinoids on cultured rat embryos, and compared them with circulating concentrations of the same metabolites in vivo after administration of a teratogenic dose of vitamin A. Their conclusion was that plasma retinol was the best predictor of teratogenicity, and that an intake of 7,500 µg RE/day of vitamin A would be unlikely to generate teratogenic plasma concentrations of retinoids. However, species differences, protein binding and transfer to the embryo were not taken into account, preventing the recommendation of this method to predict the teratogenicity of vitamin A in

humans. Work by Wiegand et al, (1998) on Cynomolgus monkeys indicated that a dose of 2,250 µg RE/kg bw/day (as retinyl palmitate) from the 16th to the 27th day of gestation did not produce any malformations of the offspring compared with controls fed a diet providing 300 µg RE/kg bw/day. Extrapolating these data to humans on the basis that the dose-responses for the teratogenicity of isotretinoin (13 cis RA) and the conversion of cis RA to trans RA appeared similar in monkeys and humans, led to the conclusion that a daily intake of 9,000 µg RE should be considered non-teratogenic in humans.

54 Schnorr et al. (2011) dosed rats with vitamin A at 750, 3750 and 7,500 mg 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 affected 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.

Dermal exposure

55 Willhite et al. (1990) found that topical administration to intact skin of up to three consecutive doses of 10.5 mg/kg/d all-trans-RA 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 did not induce a significant teratogenic response. Topical application of 0.01-1.0 mg/kg of the synthetic carotenoid arotinoid (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 difference in systemic effects of the retinoids was attributed to differences in their toxicokinetics and biological potencies. 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.

56 A technical report by the US National Toxicology Program (NTP, 2012) quotes a study by Seegmiller et al. (1990) in which, time-mated Sprague-Dawley rats were administered RA 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 RA 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.

Human studies

Teratogenicity- Food and food supplements

57 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 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. Relative risk estimates and (95 % confidence intervals) were 2.5 (1.0 - 6.2) for month 1, 2.3 (0.9 - 5.8) for month 2, and 1.6 (0.6 - 4.5) for month 3. 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.

58 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 these 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 4,500 mg RE of preformed vitamin A per day from food and supplements to the prevalence among the babies whose mothers consumed 1,500 mg RE or less per day was 3.5 (95 % 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 3,000 mg RE per day to that among the babies whose mothers consumed 4,500 mg 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 3,000 mg 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 3,000 mg RE of preformed vitamin A per day in the form of supplements, about 1 infant in 57 had a malformation attributable to the supplement.

59 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, these were 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. Five case-control studies since 1990 retrospectively estimated the intake of vitamin A in control subjects and mothers of malformed babies (see Table 1), but these 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 (~3,000 mg RE) and fetal malformations. Moreover, few women consumed high amounts of vitamin A, markedly limiting the power of the study. 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 3,000 mg RE was associated with increased risk of malformations (prevalence ratio: 4.8; 95 % CI: 2.2,10.5). However, the latter paper had been largely criticised because of 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 1,800 mg 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.

Table 1. Case-controlled studies comparing the intake of vitamin A in control subjects and in mothers of malformed babies, as identified by Azaïs-Braesco and Pascal (2000).

Cases	Controls	Exposure (mg RE/day)	Odds ratio for defects (95 % confidence interval)	Reference
11,193	11,293	> 3,000 > 13,000	2.7 (0.8, 11.7) 1.1 (0.5, 2.5)	Biesalski HK, 1989
2,658	2,609	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	3,026	Multivitamin supplements	0.57 (0.33, 1.00) Conotruncal defects	Werler et al., (1990)
548 (NTDs)	573	> 2,400 >3,000	NTDs: 0.91 (0.31, 3.68) Other defects: 1.05 (0.51, 2.18) NTDs: 0.73 (0.40, 1.53) Other defects: 0.92 (0.40, 2.11)	Botto et al., 1996

426	432	0 – 3,333	1.0	
16	12	3,000 – 4,500	1.4 (0.6, 2.8)	Mills et al., 1997
6	7	>4,500	NTDs only	

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

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

61 Buss et al. (1994) found that, based on the formation of all-trans-RA, consuming liver and taking supplements were not of equivalent teratogenic potential, due to differences in systemic exposure to all-trans-RA. The authors suggested that advice to pregnant women on the consumption of liver based on the reported teratogenicity of vitamin A supplements should be reconsidered.

62 Hartmann et al. (2005) reported that repeated oral doses of up to 30,000 IU (9,000 mg RE) of vitamin A in addition to dietary vitamin A were without safety concern. Safe doses were probably higher, since plasma concentrations and exposure to RA remained at levels earlier shown to be without increased risk of teratogenicity in pregnant women.

63 Piersma et al. (2017) reviewed the central role of RA in embryo development and how the biomarkers of its actions may be used in developmental toxicity testing. This included the enzymes of RA anabolism and catabolism, as well as related morphogenetic factors and their genes, the expression of which may be affected by changes in RA balance. The authors noted that a preliminary adverse outcome pathway for RA-mediated malformations had been published and that expansion of this framework and its application in developmental toxicity testing may allow the detection of a large variety of embryotoxicants with diverse modes of action. RA homeostasis could provide a set of molecular tools to be used in mode of action driven animal-free developmental toxicity testing.

Teratogenicity- oral medications

64 The effects of exposure of pregnant women to oral isotretinoin (also known by the Hoffman-La Roche trade name of Accutane) was investigated by a number of groups (for example Lammer et al. (1985, from abstract), Willhite et al. (1986) Howard et al. (1986), reviewed by Kizer et al. (1990)). Various outcomes were observed and their frequencies (shown in brackets) reported: spontaneous abortions (8 %), elective abortions (47 – 62 %), malformed infants (14 – 53 %), normal infants (17 %). Women who became pregnant while using isotretinoin were advised to discuss with their physicians the advisability of continuing the pregnancy. The guidelines from the manufacturer for the use of Accutane by women stressed the necessity of obtaining a negative pregnancy test two weeks before initiating therapy and the importance of using an effective form of contraception a month before, during, and for a month after taking it. Despite these restrictions and warnings to physicians and consumers, women taking Accutane continued to become pregnant, resulting in a number of malformed infants. Overall, the risk for serious birth defects in infants of pregnant women with exposure to isotretinoin was about 25 %. (Kizer et al., 1990).

65 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 at ≥16 weeks of gestation and neonates with major congenital anomalies were measured in relation to isotretinoin exposure in the 30 days before or during pregnancy. Isotretinoin prescriptions dispensed on the same day were assumed to be used simultaneously and therefore these were pooled and considered as one dispensing, so prescriptions of 10 and 20 mg tablets dispensed at the same time were combined to reach a daily dosage of 30 mg. Odds Ratios (ORs) with 95 % confidence intervals (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 (for women of child-bearing age on oral retinoids) 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). At the time, isotretinoin exposed pregnancies and adverse fetal and neonatal events potentially related to the exposure still occurred, and in the Netherlands at least, there was no full compliance with the isotretinoin exposure prevention programme.

66 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-RA) and a second group of women without either prescription. 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.

67 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."

68 Etretinate is currently approved for oral 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 bw per day, respectively (Ross, 1983). Vitamin A is metabolized to all-trans-RA, which differs from isotretinoin only in the conformation of the isoprenoid side chain.

69 The UK Teratology Information Service (UKTIS) states that: “Acitretin (etretin, 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.”

70 Choi et al. (2021) performed a systematic review and meta-analysis on the rates of major malformations after gestational exposure to isotretinoin covering the period from 1982 – 2011, in the USA, Germany, the Netherlands, Canada, Italy and Israel. The review covered 2,783 isotretinoin-exposed women from ten studies. Of the studies that report a dose rate, it ranged from 0.5 to 80 mg/kg bw/day but individual rates of malformations did not relate directly to dose rates. Overall, the rate of major malformations weighted for the sample size was 15 %. Pooled odds ratio of major malformations for isotretinoin-exposed women before 2006 was 3.76. After 2006, the pooled odds ratio of major malformations for isotretinoin exposure was significantly lower at 1.04, probably due to lower doses being prescribed. Of the studies that include a non-exposed group (3), the rate of major malformations varied between 0.7 and 4.3 % of the pregnancies. The authors acknowledged various study limitations: only three studies had both exposed and unexposed groups; the included individual studies had limited sample sizes and inconsistent characteristics; they may have underestimated the malformation rates due to numerous abortions; not all the studies were of high quality, which may have affected the accuracy of the results; the only evaluated fetal outcome was malformation and no longer-term evaluations were carried out.

71 Isotretinoin is contraindicated in those who are pregnant or thinking of becoming pregnant. Women who become pregnant while using isotretinoin are advised to stop taking the drug and to speak to their doctor as soon as possible (NHS, UK).

Teratogenicity – topical medications

72 The potential teratogenicity of topically applied RA prescribed clinically for treatment of acne has long been recognised and has been the subject of debate in the past. For example, Wilkinson, (1975, 1976) and Morrison (1976) debated in letters the efficacy and safety of RA for the treatment of acne in Canada, where its use in women of childbearing age was contraindicated. Wilkinson's opinion was that the treatment was a "new, highly effective modality" and should be available there as it was in other countries. However, the opinion of Morrison and the Health Protection Branch of Health and Welfare Canada was that RA had the potential for deleterious effects on the human fetus. The Branch recommended that contraindication for women of childbearing potential should be written into product monographs and package inserts and that indications should be restricted to two types of acne. No pharmaceutical manufacturer had at that time agreed to market RA under those conditions.

73 Panchard et al. (2012) performed a prospective, controlled, multicentre, observational study that involved 11 teratology information services and pregnant women exposed to topical retinoids during the first trimester. These women or their doctors had contacted an information service to seek advice between 1992 and 2006. Patients were asked for consent to further gather follow-up information. The women were exposed to adapalene, tretinoin, isotretinoin, motretinide, retinol, or tazarotene; if more than 1 topical retinoid was used, exposure was classified as a combination. A control group of women had used drugs considered as nonteratogenic during pregnancy (e.g., paracetamol, labetalol, meclozine, loratadine, salbutamol, ranitidine, amoxicillin, omeprazol, budesonide inhalation). There were no significant differences in infants exposed to topical retinoids compared with controls for any outcome measured, except for elective pregnancy termination. There was no evidence of an increase in anomalies consistent with RA embryopathy. The findings were consistent with the known limited systemic bioavailability of retinoids applied by the transdermal route.

74 More recently, Williams et al. (2020) stated: "…rational drug design has been applied to create today's third-generation retinoids (adapalene, tazarotene, and bexarotene). These compounds include aromatic rings within their molecular cores to provide structural rigidity that contrasts with the flexible aliphatic backbone of vitamin A and the earlier generations of retinoids, and thus limits their off-target activity. As a result of these design features, the teratogenic potential in animals of the third-generation retinoids and those reformulated for

topical use is generally less than seen with oral administration of earlier generations of retinoids. The available, but limited, epidemiologic data further show little-to-no teratogenic potential associated with real-life use of these compounds in humans. Given the paucity of epidemiologic data available at this time, however, it is recommended that the use of topical retinoids during pregnancy be avoided. However, in circumstances when inadvertent exposure in pregnancy may occur, the available data provide some reassurance that adverse pregnancy outcomes are unlikely."

75 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**."**

Other reproductive and developmental endpoints

76 The pleiotropic nature of the actions of retinoids leads to the possibility that doses of vitamin A given to counter deficiency in pregnancy, at a level that would not be expected to carry a significant risk of fetal malformation, could still lead to negative effects. Cox et al. (2006) dosed a group of 89 pregnant Ghanaian women receiving either 3,000 mg RE weekly of retinyl palmitate, or placebo (groundnut oil plus tocopherol) until 6 weeks postpartum. While this appeared to improve maternal response to opportunistic viral, bacterial and protozoal

infections, it also potentiated Th1-mediated pro-inflammatory responses which carried the risk of placental damage and could threaten the mother and the viability of the fetus, potentially leading to spontaneous abortion (Raghupathy et al. 1999, Raghupathy et al. 2000, Kwak-Kim et al., 2003).

77 Cohen et al. (2015) performed a systematic review and meta-analysis on observational studies that measured maternal blood levels of vitamins A, C, E, and carotenoids during pregnancy or within 72 hours of delivery, and related maternal antioxidant levels during pregnancy with preeclampsia or small-for-gestational-age (SGA) offspring. The studies were heterogeneous with regard to the trimester in which blood retinol was measured, the presence and severity of preeclampsia and the levels of retinol that were correlated with SGA versus appropriate for gestational age (AGA) birth. One study suggested that intrauterine growth restriction pregnancies may be partially due to reduced placental transfer of vitamin A, leading to higher-than-expected maternal blood levels, but two other studies measured retinol levels shortly after delivery and found no significant differences for mothers who delivered SGA compared to AGA babies.

78 A paper by Mawson and Croft (2019) is included in this statement as it explores the possibility that alterations in the hepatic metabolism of vitamin A may underlie signs and symptoms seen in rubella infection. However, this paper also discusses a discredited relationship between vaccines and autism, a hypothesis which the Committee wishes to emphasise it does not in any way support. The authors provide evidence that rubella can induce alterations in the metabolism of vitamin A and its accumulation in the liver. It is proposed that this would lead to mild toxicity due to hepatic inflammation and dysfunction and to the release of stored vitamin A compounds into the circulation in low concentrations. The authors hypothesise that these effects in the early weeks of pregnancy with maternal liver dysfunction could lead to exposure of the fetus to excess endogenous vitamin A, leading to predisposition to long-term metabolic and neurodevelopmental disorders.

Vitamin A and bone

79 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.

80 The results of the Southampton Womens' Survey were that higher maternal serum retinol in late pregnancy was associated with lower offspring total body BMC (β = −0.10 SD/SD [standardised beta coefficients]; 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. The authors concluded that maternal serum retinol and β-carotene concentrations had different 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.

Isotretinoin and depression

81 Masgin et al. (2005) reviewed the evidence for a link between isotretinoin use and depression and suicide in acne patients. There had been case reports linking isotretinoin to depression or suicide in the medical and psychological literature since 1982. Between 1982 and 2000 the FDA had received reports of 394 cases of depression, and 37 suicides occurring in patients exposed to isotretinoin. Isotretinoin was recorded as the fifth most common drug reported to the US Adverse Event Reporting System (AERS) in association with depression, and the tenth most common (and the only non-psychotropic drug) in suicide reports. In Canada, fifty-six events of psychiatric adverse effects in patients taking isotretinoin had been reported to Health Canada between 1983 and 2003 and forty-two psychiatric reactions, including a small number of suicides, had been reported to the British Medicines Control Agency between 1982 and 1998. In Australia from 1995 to 1998 the Adverse Drug Reactions Advisory Committee received 12 reports of depression in patients taking isotretinoin. Two cases were described as severe, in four there were psychotic features, in three there were suicidal thoughts and there were three suicide

attempts (with one completed suicide). The authors also found that many of the studies relating isotretinoin and depression were subject to confounders such as other drug use and methodological problems, such as inappropriate controls. They concluded that GPs should be aware of the possibility of such problems when prescribing but that actual cases are probably rare.

82 Huang and Cheng (2017) conducted a systematic review and meta-analysis of the literature published up to September 30, 2016, including controlled or prospective non-controlled trials on ≥15 acne patients receiving isotretinoin treatment. The prevalence of depression and change in depression scores were calculated. Thirty-one studies met the inclusion criteria. In the controlled studies, the change in depression scores from baseline was not significantly different between patients receiving isotretinoin treatment and those receiving an alternative treatment (standardized mean difference -0.334, 95% CI -0.680 to 0.011). The prevalence of depression after isotretinoin treatment significantly declined (relative risk [RR] 0.588, 95% CI 0.382-0.904). The mean depression scores significantly decreased from baseline (SMD -0.335, 95% CI -0.498 to -0.172). However, the main limitations were that no randomized controlled trials were reviewed and large inter-study variation was observed. Overall, isotretinoin treatment for acne did not appear to be associated with an increased risk for depression and the treatment of acne appeared to ameliorate depressive symptoms.

83 Li et al. (2019) performed a systematic review and meta-analysis on the use of isotretinoin and risk of depression in patients with acne. Twenty studies were identified via electronic searches of PubMed, Embase and the Cochrane Library up to 28 December 2017, comparing isotretinoin with other interventions in patients with acne. Seventeen studies showed a significant association of the use of isotretinoin with improvement in depressive symptoms compared with the baseline before treatment (SMD = −0.33, 95% CI −0.51 to −0.15, p_2=76.6%, p

84 Luvizetto and Schmitt (2020) performed a prospective study on patients treated with isotretinoin. Patients were evaluated before the start of isotretinoin and in months 1, 3, 6, 9, and 12, until the final date of use of the medication, for demographic data; severity of acne, severity of scars, and depression. The majority of patients (6/7) who presented clinically significant depression did so at the start of treatment and most of these patients showed improvement at subsequent evaluations. There was no correlation with the clinical severity of acne at the outset, indicating that underlying factors, such as socioeconomic level may have had a psychological impact. In the first months there was significant

reduction in depression scores, suggesting that the expectation and perception of continued improvement may have had a psychological effect. There appeared to be an association between the intensity of mucocutaneous adverse effects and depressive symptoms, so the importance of being attentive to these factors, applying measures to effectively mitigate them and guiding the patient in advance, especially at the beginning of treatment, was highlighted for physicians.

85 In a review of isotretinoin use in acne treatment (Bagatin and Costa, 2020), PubMed was searched for systematic reviews, clinical trials and observational studies, using the terms isotretinoin and acne, up to March 2020. Only one study was found in which a negative association between treatment with isotretinoin and depression had been found. In the majority of studies conducted on this endpoint, the psychological state of adolescents improved with reduction of the symptoms of acne brought about by the treatment. The authors concluded that: “This drug is effective, despite common, controllable, and reversible mucocutaneous side effects. Serious adverse events are rare and represent individual reactions. Teratogenicity is the most severe, requiring rigorous control. We believe that no other therapeutic option, even topicals combined to oral antibiotics accomplish same results. Recurrence after treatments other than isotretinoin is the rule, prolonging risk of scars, compromising skin appearance, and causing emotional distress in teenagers. If there is no absolute contraindication, isotretinoin should be the first line treatment for moderate to severe inflammatory acne.”

Interactions

Ethanol

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

- the synthesis of RA from retinol, catalysed by alcohol dehydrogenase, might be competitively inhibited by ethanol leading to RA deficiency;
- ethanol consumption might affect maternal retinol, retinyl ester, or RA 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 exposure might mimic vitamin A deficiency, since RA appears to prevent the adverse effects of ethanol in a quail model;

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

87 The authors suggested that these findings 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.

Vitamin D

88 An early paper on the interaction between vitamins A and D (Cruess & Clark, 1964) indicated that an interaction occurred between excess 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.

89 Metz et al. (1985) investigated the effect of vitamins A and D individually and in combination on the bone growth of turkey poults. Excessive levels of vitamin A in the diet resulted in a rachitic condition characterized by lower growth rate, greater thickness of the proximal tibial epiphyseal plates and marked reduction in bone mineral mass compared to birds fed a diet containing the estimated required level of vitamin A. In addition, high dietary levels of vitamin A were effective in preventing the renal tubular mineralization and growth depression associated with hypervitaminosis D.

90 Rohde et al. (1999) investigated the hypothesis that vitamin A intensifies the severity of rickets, and inhibits the ability of vitamin D to cure this disease. Increasing exposure of weanling rats to retinyl acetate in the presence of dietary calcium, phosphorus and ergocalciferol (vitamin D2) 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/d) indicated that retinyl acetate antagonised all vitamin D2 dosages. Increasing the dose of retinyl acetate eliminated the ability of vitamin D2 to elevate the level of serum calcium. The mechanism proposed to explain the observed antagonistic effects was competition for the RXR receptor by the vitamins.

91 Parr et al. (2018) studied 61,676 school-age Norwegian children, considering data on maternal food and supplement intake in pregnancy 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. Interactions were observed 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 in offspring increased with maternal intake of total RE. 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 UL for retinol of 3,000 µg/day.

Conjugated linoleic acid

92 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) suggested that retinol and CLA may compete for catabolic pathways modulated by the activity of peroxisome proliferator-activated receptor- α (PPAR-α) and RXR heterodimer and may position PPAR-α at the crossroads between the metabolism of lipids and vitamin A.

Zinc

93 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. A curvilinear relationship appeared to describe an effect of plasma zinc on vitamin A transport but clear evidence of synergy and its public health significance in humans was lacking.

Vitamin K

94 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 CYP enzymes, can significantly increase the half-life of RA. Hypervitaminosis A may decrease vitamin C tissue storage and may have an anti-thyroid effect.

Folate and folic acid

95 Folate is a naturally occurring vitamin, found in vegetables such as beans, peanuts and whole grains, and animal products such as liver and eggs. Folic acid is the unionised form of the vitamin that is used in supplements.

96 Qi and Ratnam (2006) found that folate receptor FR-b selectively mediated growth inhibition in human acute myelogenous leukaemia (AML) cells by dideazatetrahydrofolate, and this was greatly potentiated by all-trans RA (ATRA) and the enzyme inducers of RA metabolism trichostatin A (TSA), valproic acid (VPA), and FK228. This effect was also observed by Blaser et al. (2007) and Lynn et al. (2015).

97 Treatment of pregnant women with excess 13-cis-RA can induce craniofacial malformation in their offspring. Therefore Kriangkrai et al. (2017) investigated the effects of pre-treatment with folic acid (FA) on 13-cis-RA-induced cellular damage in developing midfacial processes in rat embryos. Rat embryos in vitro were exposed to 13-cis-RA (20 µM) with or without pre-treatment of FA (100 µM). Midfacial morphogenesis, cell proliferation and apoptosis of the midfacial processes were evaluated. The 13-cis-RA-treated embryos at 24 hours showed atrophy of midfacial processes and significantly decreased morphogenesis and cell proliferation, with increased apoptotic cell death. In contrast, embryos pre-treated with FA for 18 hours, followed by 13-cis-RA treatment for 24 hours showed significantly greater morphogenesis, increased cell proliferation and lower apoptotic cell death in comparison with the 13-cis-RA-treated group. Thus, the FA reduced the teratogenic effects of 13-cis-RA on midfacial process tissue. However, it is important to note that the concentrations of FA selected in this study were particularly high. Future investigations into the anti-teratogenic mechanism of FA on midface malformations induced by 13-cis-RA in pregnant woman were recommended.

98 Piersma et al. (2017) also highlighted interactions of diverse classes of xenobiotics on RA disposition that might play a role in their dysmorphogenic effects. These included anticonvulsants (valproic acid, an HDAC inhibitor that also down regulates expression of CYP26A1, alone and in combination with the CYP inducers phenytoin, phenobarbital, carbamazepine, or ethosuximide); triazole antifungals, through interaction with CYP26 expression, transforming growth factor beta (TGFb) and cellular RA binding protein (CRABP); methylmercury, through alterations in RA-related gene expression; tributyl tin chloride causing a decrease in RARa and sonic hedgehog expression (in fish embryos); flame retardants (monosubstituted isopropylated triaryl phosphate (mITP) and

triphenylphosphate) affecting RA homeostasis and Hox family gene expression. Dioxin-induced cleft palate had also been shown to depend on RA signalling that controls AhR expression and polybrominated biphenyl ethers (PBDEs) to affect RA homeostasis and lead to embryotoxicity under marginal vitamin A status in rats.

Beta-carotene

99 The Joint FAO/WHO Expert Committee on Food Additives (JECFA) considered b-carotene in 1974 and concluded that hypercarotenaemia per se was not toxicologically significant and caused no adverse symptoms or hypervitaminosis A; the condition disappears if excess intake of beta-carotene is discontinued. JECFA quoted a study from 1959, (Greenberg et al., 1959) where fifteen subjects received 60 mg beta-carotene daily for three months. Serum carotene levels rose from 128 µg/100 ml 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 (FAO/WHO,1974) identified a NOAEL of 50 mg/kg bw per day (1,000 ppm), the highest (and only) dose tested, in a four-generation study of beta-carotene in rats for 110 weeks and established an acceptable daily intake (ADI) of 0 – 5 mg/kg bw. A reduced safety factor of 10 was considered appropriate, based on its generally low toxicity and the fact that it was a normal constituent of the human diet.

100 In 2017, at its 84th meeting, JECFA 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 model 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,” (FAO/WHO, 2019).

101 Omenn et al. (1996) reported the commencement and study design of the b-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 b-carotene (30 mg/day) plus retinyl palmitate (25,000 ID/day) could decrease the incidence of lung cancer in high-risk populations. The outcomes of this trial are discussed below.

102 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 (RR =1.28, 95% CI =1.04 to 1.57; P=0.02), a 17 % increase in incidence of death (RR = 1.17, 95% CI = 1.03 to 1.33; P=0.02) 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 % [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 cardiovascular disease mortality (1.44 versus 0.93; p=0.03), and all-cause mortality (1.37 versus 0.98; p=.001) than males.

103 Despite b-carotene being widely considered as protective against cancer and cardiovascular diseases, researchers of the CARET concluded that “supplements containing β-carotene were harmful to cigarette smokers, causing increases in the incidence of lung cancer and in overall mortality”. The reported adverse effects of b-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. b-carotene was considered to be responsible for the effects on lung cancer incidence as similar effects were seen in the treatment group of the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Trial, and that of a skin cancer trial. This trial administered higher doses of retinol than that administered in the CARET study and reported no adverse effects (although the trial was not powered to study lung cancer incidence). Additionally, 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. However, the low statistical power of this study led to wide confidence intervals, leaving the results on lung cancer difficult to interpret clearly (Goodman et al., 2004).

104 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 % 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).

105 Evidence was somewhat weaker 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) found no associations 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 ROS in moderate concentrations act as mediators of apoptosis and phagocytosis, and that in people with a low level of ROS, an excess of antioxidants could block these mechanisms and promote cancer.