

EXPERT GROUP ON VITAMINS AND MINERALS

REVISED REVIEW OF VITAMIN C

The attached review of vitamin C is a slightly amended version of the paper presented to the Expert Group on Vitamins and Minerals at the meetings on 7 July 1999 and in October 2001.

The following annexes are also included with this paper:

- Annex 1 Tables referred to throughout the review
- Annex 2 Intakes of vitamin C from food and supplements in the UK
- Annex 3 Summary table of selected nutrition information and existing guidance

Expert Group on Vitamins and Minerals Secretariat
August 2002

Vitamin C

Chemistry

1. Vitamin C is a six carbon compound structurally related to glucose. It comprises two compounds; L-ascorbic acid (mw 176), which is a strong reducing agent and its oxidised derivative, L-dehydroascorbic acid. Both forms have biological activity and are interconvertible by an oxidation/reduction reaction (Basu and Dickerson, 1996). Most vitamin C in body fluids and tissues exists in the reduced form, with the oxidised form accounting for less than 10%.

Natural Occurrence

2. Most plants and animals have the ability to synthesise vitamin C from D-glucose or D-galactose via the glucuronic acid pathway. The only mammals that are unable to synthesise vitamin C are primates, including man, and guinea pigs. This is due to a deficiency in the hepatic enzyme L-glulono- γ -lactone oxidase, necessary for the conversion of 2-keto-L-gulonolactone to L-ascorbate (Basu and Dickerson 1996). These species are dependent upon exogenous sources of the vitamin.

Occurrence in foods and medicines

Food

3. Vitamin C is found almost exclusively in foods of plant origin. Kidney is the only food of animal origin considered to be a significant source of vitamin C. Particularly rich sources of vitamin C are citrus and soft fruits, with most leafy green vegetables being moderately rich sources. In the UK, potatoes are an important source of vitamin C in the human diet because of the relatively large amounts eaten.

4. The amount of vitamin C present in plant foods is determined by various factors, such as the part of the plant eaten, the maturity of the plant and the duration of storage once harvested, with levels of vitamin C depleting significantly in fresh foods upon storage. The degree to which vitamin C is depleted depends on the type of vegetable since low pH (as in citrus fruits) can stabilise vitamin C (Klein and Kurilich, 2000.) Methods of food preparation or production, such as peeling and chopping, cooking or canning, can increase the loss of vitamin C from foods. Vitamin C is readily lost in cooking due to its solubility in water, thus when plant foods are eaten raw the availability of the vitamin is generally higher (Basu and Dickerson 1996, Henshall 1981). Vitamin C rapidly decomposes in water, due to rapid oxidation by atmospheric oxygen, but there is no evidence of heat degradation (Hornig and Moser 1981). Cooking methods where less water is used (eg microwaving) preserve vitamin C content (Klein and Kurilich, 2000.)

5. In addition to its natural occurrence in foods, vitamin C is used as a chemical preservative in numerous food processing procedures. It is added to soft drinks as an antioxidant, to meat and meat products for curing and to flour to improve its baking quality. Consequently, foods such as cereals, cakes, confectionery, fish and meat

products and soft drinks can all become important sources of vitamin C. There are no specified limits on the amount of ascorbic acid that can be added to a food as an additive (see paragraph 95).

6. In most European countries, the US and Canada, vitamin C is consumed as a dietary supplement. It is available in a large number of preparations, in tablets that contain from 25 to 1500 mg and in solutions containing various concentrations. Vitamin C is usually administered orally but in conditions that prevent its uptake via the gastrointestinal tract, sodium salt solutions may be given by intramuscular or intravenous injection (Miller and Hayes 1982). No purity issues have been identified with regard to vitamin C in food supplements.

Licensed medicinal products for oral use.

7. Forty-five products contain ascorbic acid (and/or its salts) as their sole active constituent. All may be sold in supermarkets and other retail outlets without the supervision of a pharmacist.

8. Ascorbic acid is also included as an active ingredient in 110 multi-constituent products. Twenty-six of these may be sold in supermarkets and other retail outlets, whilst thirty-six are only available in a pharmacy. Their licensed uses include the prevention and treatment of nutrient deficiencies, debility, supplementation of special diets and malabsorption. Thirty-eight products contain ascorbic acid in combination with an analgesic such as paracetamol (and in some cases other constituents such as a decongestant), and are licensed to relieve the symptoms of colds and flu. Some are for general sale, others are only available in pharmacies. Ten products contain ascorbic acid in combination with a range of other co-constituents.

9. The maximum daily doses specified in the licences for these medicinal products are up to 3000 mg.

Intake/Exposure

10. Intakes of vitamin C from food and supplements in the UK have been provided (see Annex 1). Median intakes of vitamin C from food and supplements in young people and in adults range from 50 to 68.5 mg/day. Higher level (97.5%ile) range from 151 to 285 mg/day; vitamin C intakes are slightly lower in older people. When calculated on a body weight basis, intakes are highest in children.

Recommended amounts

11. The optimal vitamin C requirements for humans are unknown (Levine *et al*, 1995). A daily intake of 40-60 mg per day is known to maintain the saturated state with a 1500 mg or greater body pool size of vitamin C. In the UK the Reference Nutrient Intake for adults is 40 mg/day (COMA, 1991).

12. In conditions such as pregnancy and lactation, the demands for ascorbic acid are higher. During the third trimester it is recommended that intake is increased by 10 mg/day (COMA, 1991). There is a moderate extra drain on body stores throughout

pregnancy and during the latter stage, the foetus concentrates the vitamin at the expense of maternal stores and circulating vitamin levels. Vitamin C crosses the placental barrier against a concentration gradient, resulting in foetal levels 50% greater than the maternal levels at term (Schorah, 1981). During lactation, an intake of 70 mg/day will ensure that maternal stores are maintained and that breast milk ascorbic acid levels are maintained in the upper half of the physiological range for human milk (COMA, 1991).

13. Using radio-labelled doses of vitamin C, Kallner *et al* (1981) have shown smoking to be associated with a reduced absorption rate (from a 180 mg dose, average absorption was found to be 84% in non-smokers, compared to 76% in smokers) and reduced half-life of the vitamin. This group of the population has a higher turnover of vitamin C and therefore, an increased requirement compared to non-smokers. According to a recent recommendation in Canada, the intakes for heavy smokers should be increased by as much as 50%. In 1989 in the US, the Recommended Dietary Allowance (RDA) of vitamin C was increased from 60 mg to 100 mg/day for smokers (Basu and Dickerson, 1996).

14. It is recognised that plasma and leukocyte concentrations of vitamin C decline with increasing age in both males and females. However, most elderly individuals with low vitamin C status, have persistently low intakes and there is no compelling evidence of an increased requirement for the vitamin in old age (Schorah, 1981; Newton *et al* 1985).

15. The Food and Nutrition Board of the National Academy of Sciences in the US recently reviewed the recommendations for vitamin C intake. They proposed a Recommended Dietary Allowance of 120 mg/day compared to the 1989 figure of 60 mg. This is because extensive biochemical, molecular, epidemiologic and clinical data have become available since 1989 (Levine *et al* 1999).

Measurement of tissue levels and Vitamin C status

16. Vitamin C status is most commonly measured in plasma (Basu and Dickerson, 1996). However these levels are at best an indication of vitamin C status since they reflect preceding intake or can be depleted while tissue stores are adequate. Urinary vitamin C can also be measured but this too may reflect preceding intake. Leukocyte vitamin C is a useful measure as the concentrations are more stable, however, it is technically more difficult to prepare the leukocytes and a larger blood sample is required. A leukocyte vitamin C level of less than 10 µg per 10⁸ cells is generally regarded as deficient (Levine *et al* 1995).

17. Tissues such as the adrenal glands, pituitary gland and the retina, contain higher vitamin C concentrations, approximately 1-2 mg/g; tissues, such as the liver, lung, pancreas and leukocytes, have intermediate levels, 0.1-1 mg/g. Others, such as kidney, muscles and red blood cells have still lower levels, 0.02-0.1 mg/g (Kallner 1981, Olson and Hodges 1987). Up to an intake of approximately 90 mg/day, concentrations of ascorbic acid in plasma and tissues are directly related, though not linearly, both to each other and to the vitamin intake (Olson and Hodges 1987).

Bioavailability

18. The type of food consumed does not affect the absorption of intrinsic or supplemental vitamin C. The bioavailability of vitamin C in foods does not differ significantly from that of synthetic vitamin C (reviewed FNB, 2000).

Interactions*Metal Ions*

19. By virtue of its reducing and chelating properties, vitamin C exerts a wide variety of effects on metal ions, both during their absorption from the diet and during their metabolism and distribution throughout the body. Ascorbic acid is known to interact with absorption of trace minerals, particularly iron and copper.

Drugs and diagnostic tests

20. Ascorbic acid has been reported to decrease the effects of the drug warfarin. A patient discharged on a regimen of 7.5 mg warfarin sodium/day with a prothrombin time of 23 seconds, remained in that range for 4 weeks. His prothrombin time then dropped to 19, 17 then 14 seconds and no response was seen to an increase in warfarin dose to 10, 15 then 20 mg/day. It was discovered that the patient was self-administering ascorbic acid with the warfarin, within 2 days of the cessation of the vitamin treatment, the prothrombin time rose to 28 seconds (Rosenthal, 1971).

21. Ascorbic acid has been reported to have a synergistic relationship with aspirin, increasing the severity of gastric ulcers in rats compared to rats treated with aspirin alone (Lo and Konishi, 1978) (see paragraph 80).

22. Since ascorbic acid is a powerful reducing agent, large quantities may affect certain urine glucose tests. It produces a positive reaction with the copper reduction test, which can be recorded as a positive test for sugar, it also inhibits the enzymic test for glucose, based on the glucose-oxidase-peroxidase-chromogen system (Free and Free 1973). Such interference can complicate the diagnosis and management of diabetes. High circulating ascorbic acid levels may also interfere with estimations of serum transaminase and lactate (Singh *et al* 1972).

Amino acids

23. In healthy human volunteers given “megadoses” of ascorbic acid (3 g/day for 5 weeks) urinary excretion levels of cysteine were decreased from mean pre-treatment levels of 276-284 $\mu\text{M}/24$ h urine to mean levels of 182, 142 and 133 $\mu\text{M}/24$ h urine following 1, 3 and 5 weeks of treatment (Basu 1976). The author suggests that this may be due to the fact that, the amino acid is utilised to metabolise the vitamin and that depletion of cysteine in this way, could lead to over-exposure to drugs that are normally detoxified through conjugation with sulphate. Although other workers (see paragraph 26) have reported that sulphates are minor metabolites, this route of metabolism may become more important at high levels of exposure.

Absorption and Distribution

24. Ascorbic acid is absorbed from the intestine by a sodium-dependent, active transport process (Hornig and Moser 1981). When low doses (4-64 mg) are ingested by humans, the absorption efficiency may be as high as 98%, i.e. less than 2% of an ingested radioactive dose appears in the faeces (Baker *et al* 1969). When larger doses (30-180 mg) are consumed the absorption efficiency falls to 80-90% (Kallner *et al* 1979), to 75 % at 1 g, 50% at 1.5 g, 26% at 1.6 g and 16% at 12 g (Kallner *et al* 1977, Hornig and Moser 1981). L-ascorbic acid is transported in the plasma as the free anion. It is freely transported into cells, including red blood cells and leukocytes. Leukocytes contain higher concentrations of vitamin C than those in plasma, whole blood or erythrocytes.

25. Ascorbic acid is widely distributed in all tissues of the body with higher levels being found in tissues such as the adrenal glands, pituitary gland and retina compared to lower levels in kidney and muscle tissue. Tissue concentrations are normally 3-10 times higher than those in the plasma, but no controlled storage mechanism or specific binding proteins have been identified for vitamin C. Brubacher *et al* (2000) have carried out a recent meta-analysis of the relationship between vitamin C intakes and plasma concentrations for different subgroups i.e. adult, elderly, smokers, non-smokers, so as to identify the intake necessary to achieve plasma concentrations of 50 $\mu\text{M/l}$. They estimate that 50% of the general population can achieve plasma concentrations of 50 $\mu\text{M/l}$ at an intake of 83.4 mg. The elderly and smokers, however, would need higher intakes: 150.2 mg and 206.6 mg respectively.

Metabolism & Excretion

26. Metabolism of ascorbic acid in humans occurs via the irreversible hydrolysis of dehydroascorbic acid to diketogulonic acid, followed by oxidation to oxalic and threonic acids. These metabolites, together with some ascorbate-2-sulphate, are excreted in the urine, along with un-metabolised ascorbic acid.

27. In humans the principle route for the elimination of the metabolic products of ascorbic acid is urinary excretion. Faecal excretion of ascorbic acid or its metabolites has been found to be approximately 3% of the oral dose, with a dose in the region of 60 mg. The daily urinary excretion, from a 60 mg intake, consists of approximately 20-25% as unchanged ascorbic acid (of which a small proportion will be in the reduced form, dehydroascorbic acid), about 20% is excreted as 2,3- diketogulonic acid and about 40% is excreted as oxalate (Kallner, 1981). Ascorbate-2-sulphate and saccharoascorbic acid are minor metabolites. At increased doses of ascorbic acid more of the vitamin is excreted unchanged. Approximately 60% of a daily dose of 180 mg is excreted as ascorbic acid, approximately 80% of a 1 g dose and 90 % of a 3 g dose (Baker *et al* 1969, Kallner *et al* 1979).

28. Oxidation of vitamin C to carbon dioxide is not normally a major route of elimination in humans, but at high doses elimination by this route is increased. Oral administration of ^{14}C labelled ascorbic acid showed that at doses above 180 mg, CO_2

derived from the ascorbic acid was recovered from the breath (Kallner *et al* 1985). At doses between 180 mg to 1 g of ascorbic acid, between 1 and 30% of dose was recovered as carbon dioxide, in a dose-dependant manner. It is suggested that the formation of carbon dioxide is due to a pre-systemic effect, as a result of microbiological or chemical degradation of ascorbic acid in the intestine. High iron levels are another factor, which are thought to accelerate oxidation of vitamin C to CO₂ (Basu and Dickerson 1996).

Function

29. The functions of vitamin C include synthesis of collagen, neurotransmitters and carnitine, absorption of non-haem iron and as an enzyme cofactor. Vitamin C plays a major role as an antioxidant and free-radical scavenger and is involved in the detoxification of many foreign compounds. It is essential in the metabolism of folic acid.

30. Vitamin C is a strong reducing agent and hence has a general importance as an antioxidant, affecting the body's 'redox potential'. It has many diverse biochemical functions that are a consequence of its ability to donate one or two electrons. Ascorbic acid is oxidised to dehydroascorbic acid through a short-lived intermediate, the ascorbate free radical called monodehydroascorbic acid, which is generally regarded as innocuous. It in fact forms part of the body's antioxidant defences against reactive oxygen species and free radicals, thereby preventing tissue damage (Stadtman 1991). Regeneration of ascorbic acid from its oxidation products, by reducing agents such as glutathione and NADH, potentiate its antioxidant activity.

31. Vitamin C plays an important role in many reactions involving oxygenases. These reactions also require molecular oxygen and Fe²⁺ or Cu²⁺ as a cofactor. Ascorbic acid plays one of two roles; it can act either as a direct source of electrons for reducing molecular oxygen or as a protective agent for maintaining iron and copper in their reducing states.

32. Non-haem iron (from plant food) normally constitutes more than 90% of the dietary iron. However, the absorption of non-haem iron is considerably less than that of haem iron (from animal food). Ascorbic acid is a potent enhancer of non-haem iron absorption. Ascorbic acid in the intestine is thought to keep iron in its reduced form, preventing the formation of insoluble ferric hydroxide and hence aids absorption. Ascorbic acid may also be involved in the transfer of iron into the blood, as well as mobilising it from its stores. In the circulation iron is generally in its oxidised form bound to transferrin, whereas the reduced form of iron is bound to ferritin in the liver (Roesser *et al* 1980).

Deficiency

33. The involvement of vitamin C in numerous biological systems means that the early signs of deficiency, that is, scurvy, are relatively non-specific (Basu and Dickerson, 1996). They often include fatigue, weakness, shortness of breath, aching bones, joints and muscles and loss of appetite. These are followed by more specific signs, such as swollen, bleeding and sensitive gums, hardening and roughness around

hair follicles (hyperkeratosis), petechial haemorrhages under the skin and delayed wound healing. These clinical signs of scurvy are due to the inhibition of collagen synthesis, this leads to failure to maintain the cellular structure of supporting tissues of mesenchymal origin, such as bone, dentine, cartilage and connective tissues. As the dentine becomes porous, alveolar bone becomes osteoporotic, and the teeth loosen and fall out. Such conditions are associated with severe pain and immobility. Infants with scurvy adopt a characteristic 'frog leg' position, it is possible that this is the position least painful to them. Wound healing is impaired, since in deficiency, although fibroblasts proliferate they remain immature and fail to synthesise collagen. The cartilage matrix of the epiphyseal plate builds up between long bones and can become calcified; this results in compressed and brittle bone. Additional clinical manifestations observed in vitamin C deficiency can include behavioural changes, often apathy, depression and emotional disturbances. Such observations are thought to be the consequence of a depression in catecholamine synthesis. A loss of blood may also be observed due to petechiae, perifollicular haemorrhages and bleeding gums. Vitamin C plays an important role in iron absorption and utilisation, thus, in patients with vitamin C deficiency, hyperchromic anaemia may be seen.

34. If vitamin C is withdrawn from the diet, it takes from 100-160 days for the advanced clinical signs of scurvy to develop. With the vitamin C pool being depleted at a rate of approximately 2.6% per day, mild signs of deficiency become evident when the body pool is less than 300 mg (Basu and Dickerson, 1996). Crandon *et al* (1940) were able to induce signs of scurvy in a volunteer described as a "normal active adult". They found that vitamin C levels in white cells decreased and reached an undetectable level in approximately 90 days, at which time the plasma ascorbate level had already been zero for about 50 days. The first clinical signs of scurvy appeared after 132 days as hyperkeratotic papules. Wounds were intentionally made twice during the study. The first wound healed normally although the plasma ascorbate level was zero. The second wound, made when plasma ascorbate had been zero for 140 days and white blood cell levels zero for 61 days, showed poor healing and the experiment was terminated. Petechiae had been evident over the lower limbs for 21 days.

Intakes associated with other beneficial effects

35. There are many reports of vitamin C having beneficial effects in the healthy population at intakes and tissue levels considerably greater than those needed to prevent or treat scurvy (COMA, 1991). There has been a lot of interest in the role of the antioxidant nutrients, of which vitamin C is one, in the prevention of chronic diseases such as cancer and coronary heart disease (CHD). It is proposed that vitamin C may prevent the oxidation of low density lipoprotein (LDL) which in turn may decrease vascular damage and CHD (COMA 1994). Studies to date of vitamin C supplementation in both smokers and non-smokers, however, have tended to be small, use different biomarkers of lipid oxidation and produce mixed results (Carr and Frei, 1999). It has also been suggested that large doses of supplementary vitamin C can reduce blood cholesterol levels, possibly by stimulating the conversion of cholesterol to bile acids (Lazarides 1997). Studies have also reported beneficial effects of vitamin C on the lipoprotein profile, coagulation factors and vasodilation (Carr and Frei, 1999). Diets with 200 mg or more of vitamin C from fruits and vegetables are

associated with lower risks of cancer; however, the evidence that vitamin C supplements are also beneficial is insufficient (COMA 1998).

36. It has been suggested that regular daily doses of vitamin C in the range of 200 mg-2 g/day can act beneficially as a prophylactic against the common cold (reviewed by Hemila, 1997) though Levine *et al* (1999) noted that patients who may gain a slight reduction in cold incidence are probably a small subset who are vitamin C deficient.

37. A few studies have suggested that vitamin C supplementation may have beneficial effects on a wide range of conditions. In 12 asthmatics, 500 mg vitamin C supplements reduced asthma symptoms after exercise compared with the placebo (Schachter and Schlesinger 1982). The relationship between diet and asthma was reviewed by Fogarty and Britton, (2000) who noted that low dietary intakes of vitamin C were associated with an increased risk of asthma (both incidence and severity) and pulmonary disease. However certain studies indicate that the relationship may be with fruit consumption rather than vitamin C intake *per se*. The results of intervention trials are noted to be conflicting.

38. It has been suggested that high intakes of vitamin C reduced the rate of cartilage loss by 70% in osteoarthritis sufferers (McAlindon *et al*. 1996). Vitamin C is found in high concentrations in the lens and aqueous humour of the eye. Use of vitamin C supplements may reduce the risk of cataracts by 50% (Robertson *et al* 1989) but there are few prospective studies or long-term intervention trials on which to draw conclusions (Carr and Frei, 1999). Simon and Hudes (1999a) found a relationship between serum ascorbic acid and prevalence of cataract in 60-74 year old Americans in the NHANES-II survey. Every 1 mg/dl increase in ascorbic acid was independently associated with a 26% decrease in the prevalence of cataract. In diabetic patients, high dose vitamin C supplementation was found to have a beneficial effect on blood sugar and blood lipid levels (Eriksson and Kohavakka 1995, Paolisso *et al* 1995).

39. High doses of vitamins C and E taken by patients with early Parkinson's disease delayed the need for medication (levodopa) by an average of 2.5 years compared with control patients (Fahn 1991). In a 30-week double blind trial extremely high levels of supplementation (8 g/70 kg body weight/day) resulted in a decrease in the severity of symptoms in eight autistic children (Dolske *et al*. 1993). Manic-depressives and depressives receiving high dose (3g) vitamin C improved significantly (Naylor and Smith 1981 and Naylor 1984).

40. Sub-optimal Vitamin C status has been suggested as a risk factor for gallbladder disease as vitamin C affects the rate-limiting step in the catabolism of cholesterol to bile and vitamin C deficient guinea-pigs frequently develop gallstones. Cross-sectional data from NHANES-II and III has been analysed by Simon *et al*, (1998 and 2000). In NHANES-II, gallbladder disease history was ascertained by questionnaire and an inverse U-shaped relationship was found between serum ascorbic acid levels and gallbladder disease and cholecystectomy in women. In NHANES-III, ultrasound was used to assess asymptomatic gallstones. One standard deviation increase in serum ascorbic acid was independently associated with 13%

lower prevalence of clinical gallbladder disease and asymptomatic gallstones in women. Neither study found a relationship with men.

41. Vitamin C has been investigated as a means to protect against lead toxicity. In rats, vitamin C has decreased intestinal absorption and increased renal clearance of lead. Hsu et al (1998) have shown that in lead exposed rats vitamin C can prevent reactive oxygen species generation in sperm and enhance sperm-oocyte penetration. Vitamin C supplements of 1000 mg caused whole blood lead levels in male smokers to fall from 1.8 to 0.4 $\mu\text{mol/l}$ within a week but a supplement of 200 mg had no effect (Dawson et al, 1999). The author suggests that vitamin C could be an effective prophylactic for subclinical chronic lead exposure. Analysis of data from over 19,000 Americans in the NHANES III survey found a significant inverse relationship between blood lead levels and serum ascorbic acid concentration but not dietary ascorbic acid (Simon and Hudes, 1999b).

Toxicity

Human Toxicity – see Table 1

42. Toxic effects of vitamin C that have been reported include metabolic acidosis, oxaluria, renal stones, renal tubular disease, gastrointestinal disturbances, sensitivity reactions, conditioned scurvy, coagulation and cholesterol disturbances, vitamin B₁₂ destruction, fatigue and sterility. However, the majority of the data indicate that vitamin C is associated with few adverse effects. Cases of acute toxicity seldom occur and there is no substantial evidence that in normal healthy individuals daily doses of up to 1 g, taken over periods of week, e.g. for the prophylaxis of colds, will lead to toxic symptoms. However, higher doses taken over long periods of time, in certain predisposed individuals may cause serious toxic effects (Miller and Hayes 1982). For example patients with pre-existing hyperoxaluria may have an increased risk of nephrolithiasis at vitamin C doses of 1 g or more (Auer *et al* 1998).

Gastrointestinal effects

43. The most common adverse reactions to high vitamin C intakes are gastrointestinal disturbances, such as diarrhoea, nausea and abdominal cramps. The precise level at which this occurs is variable and few data are available from controlled studies. In a stepped study by Cameron and Campbell (1974) human volunteers were given doses of vitamin C which increased by 1g per week. When the dose reached 3-4 g/day flatulent distension, diarrhoea and transient colic were described as “fairly frequent”. In a brief letter, Hoyt (1980) reported that diarrhoea in runners occurred at doses of 1 g day vitamin C and above. In a study in which volunteers were given 8 g/day vitamin C (presumably in divided doses) Stein *et al* (1976) reported that mild diarrhoea was observed in 1 of 3 volunteers given a dose of 4 g/day vitamin C. Hanck (1982) reported 10-15 g/day vitamin C had been given to cancer patients with the only adverse effect being an initial laxative effect which eased after 3-4 days of treatment. In contrast, Hornig and Moser (1981) cite a study in which gastrointestinal symptoms were equally distributed between control and treatment groups receiving 10 g/day vitamin C or placebo. The gastro intestinal

symptoms associated with vitamin C appear to be due to the direct ionic effect of ascorbic acid increasing peristalsis, since they can be avoided by taking the vitamin as a buffered salt, rather than as the free acid (Barness 1975).

44. In some cases the gastrointestinal symptoms may arise in association with sensitisation reactions, such as hives, angioneurotic oedema and skin rashes, which have also been reported in these patients (Barness 1975). These symptoms disappear within a week and no further adverse consequences are reported. Whether or not ascorbic acid is responsible for such reactions is not clear, since such reports often originate from uncontrolled and often purely anecdotal observations.

Effects on iron metabolism

45. The presence of excessive vitamin C in the gut however can lead to other problems. The ability of ascorbic acid to enhance iron absorption may not be desirable in those people with more than adequate iron stores. Cook and Monsen (1977) report that iron absorption from food was directly proportional to the amount of ascorbic acid added over a range of 25 to 1000 mg. The ratio of iron absorption with/without ascorbic acid reported at these two extremes was 1.65 and 9.57 respectively. It is likely that in normal individuals the amount of iron absorbed would still be regulated by the intestinal mucosa in accordance with body needs and that no long term effect on body iron balance would occur (Cook *et al* 1984). However, in individuals who are not able to regulate iron absorption (those with idiopathic haemochromatosis, thalassemia major and sideroblastic anaemia) ascorbic acid may substantially increase the already excessive absorption of iron. However, Gerster (1999) noted that whether iron accumulation in subjects with haemochromatosis is further enhanced by vitamin C has not yet been studied systematically.

46. Free iron can be lethal, however, two types of protein, one with high affinity (the transferrins) and the other with high capacity (ferritin) for iron keep iron bound whilst it is being transported and stored. These proteins thus prevent iron producing toxic quantities of free radicals. Iron is trapped within the ferritin protein shell as harmless Fe^{3+} . In addition to enhancing intestinal iron absorption vitamin C releases iron from these body stores (Herbert 1963a and 1963b). It is reported that the intravenous vitamin C given to one megaloblastic anaemia patient suffering from scurvy, released so much iron from body stores into the blood that it saturated the transferrin iron-binding protein. This occurs because the bolus of ascorbic acid in the blood stream goes through the pores of the ferritin protein shell; converting harmless ferritin protein-bound Fe^{3+} to harmful Fe^{2+} . Vitamin C drives repetitive generation of destructive free radicals in the presence of high iron levels and catalytic iron free radical generation has the potential to mutate DNA and promote cancer (Ames *et al* 1973).

47. A study by Salonen *et al* (1992) reports that, when patients with high LDL cholesterol are divided into two groups, those with elevated serum ferritins due to the moderately increased body iron stores associated with heterozygous haemochromatosis, have more than twice the coronary artery disease risk of those with serum ferritins within the normal range. Iron catalysed lipid peroxidation (which oxidises harmless LDL cholesterol to its coronary artery damaging oxidised form) is

enhanced *in vitro* more than two-fold by 10 μ M vitamin C, (the equivalent to an adult consuming a 100 mg dose) and more than 100-fold at a concentration of 100 μ M. Over 10% of American whites and 30% of American blacks are affected by heterozygous haemochromatosis and for them excessive vitamin C intake has the potential to “do more harm than good” (Herbert *et al* 1994 and 1996). However, in normal healthy subjects clinical studies have shown that an intake of 2 g of ascorbic acid daily for 16 weeks had no effect on serum ferritin levels (Cook *et al*, 1984). When vitamin C supplementation was continued for a further 20 months in iron replete subjects, serum ferritin determinations again failed to indicate any significant effect of the vitamin on iron reserves. These observations are consistent with the established view that body iron is maintained within narrow limits in normal individuals despite wide variations in the type and nature of dietary iron.

48. In patients with thalassaemia or sickle cell disease, vitamin C can mobilise such huge amounts of iron from their high body iron stores that the iron-binding capacity of proteins is overwhelmed. The resultant free iron causes death within minutes to hours, from iron-induced cardiac failure. As a result, the authors considered that the daily dose of vitamin C should not exceed 200 mg in such patients (Herbert *et al* 1996).

Effects on cholesterol

49. An observed rise in serum cholesterol in patients with atherosclerosis ingesting vitamin C at doses of 1 g/day over 5 weeks was attributed by Spittle (1971) to mobilisation of arterial cholesterol. Calculations on the available data concerning cholesterol content and turnover in human arteries and veins (Morin 1972) show that it is in fact unlikely that all of this cholesterol is mobilised from the arteries. Some or all of the increased serum level must result from other mechanisms, i.e. increased synthesis, decreased excretion or mobilisation from body pools. However, this rise in serum cholesterol may aggravate existing atherosclerosis in patients with a previous myocardial infarction. In healthy young patients, vitamin C has been noted to lower the serum cholesterol levels (Spittle 1971), but in later studies, Crawford *et al* (1975) were unable to find any influence of vitamin C (1 g daily for 3 months) on serum cholesterol levels in healthy volunteer subjects. Harats *et al* (1998) reported that 2 months of supplementation with 500 mg vitamin C/day slightly increased plasma cholesterol (this was offset by reduced *in vitro* LDL susceptibility to oxidation).

Effects on vitamin B₁₂

50. Herbert and Jacob (1974) reported that, an *in vitro* concentration of ascorbic acid, equivalent to an ingested dose of 500 mg, will destroy between 50 and 95% of the vitamin B₁₂ content in food. Homogenisation for 5 minutes in a food blender was used to mimic mastication of the food by teeth and the further homogenisation that occurs in the stomach. A 30 minute incubation of the sample at 37°C was the laboratory mimic of the gastric environment. Further investigations however, proved that the extraction method employed by Herbert and Jacob was not suitable and their low results are apparently due to inadequate protection of the vitamin B₁₂ from degradation during the high temperature extraction of samples (Newmark *et al* 1976).

Vitamin B₁₂ values obtained by officially validated methods of extraction were many fold higher than those obtained by Herbert and Jacob and in agreement with those calculated from literature values. Further investigations have shown, using incubations of 30 minutes at 37°C of a variety of homogenised meals, that up to 1 g of added ascorbic acid had no effect on vitamin B₁₂ content (Herbert *et al* 1978, Newmark *et al* 1979).

51. Clinical observations from an uncontrolled study in 90 patients, all ingesting greater than 500 mg vitamin C daily, showed subnormal vitamin B₁₂ concentrations in only three subjects who had been taking a minimum of 1,000 mg for at least three years. Two of these three patients showed morphological signs of vitamin B₁₂ deficiency, though none was anaemic. A substantial increase in serum vitamin B₁₂ levels was observed in these subjects within three months of the cessation of the regimen of taking large doses of vitamin C (Hines, 1975). However, uncertainty over the original report arose with the demonstration by Thenen (1979) that rats fed high doses of vitamin C along with marginal vitamin B₁₂, showed no reduction in vitamin B₁₂ body stores and no biochemical signs of vitamin B₁₂ deficiency. The authors concluded that any detrimental effect of ascorbic acid on vitamin B₁₂ was not of great concern.

Effects on coagulation

52. Hanck (1982) reported that vitamin C supplementation of 4 g/day for 3 weeks had no significant influence on coagulation of venous blood. It has been claimed that vitamin C interferes with warfarin controlled anticoagulation (see paragraph 20); however, Feetham *et al* (1975) reported no clinically significant decrease in prothrombin times in warfarin stabilised patients administered up to 10 g/day vitamin C for 1 week. The authors speculated that vitamin C induced diarrhoea may have accounted for the interactions reported in the 2 previous case reports, thus decreasing the bioavailability of orally administered warfarin.

Effects on kidney and bladder

53. Concern has been expressed over the potential toxicity of excessive ascorbic acid excretion and its effects on the kidney and bladder. For example, Nakamoto *et al* (1998) report a case of a 70 year old woman who had consumed 3 g vitamin C/day for 10 years. The patient developed chronic, irreversible tubulointerstitial nephritis. The interstitium was found to be loaded with oxalate granules. Ascorbic acid is partially converted to oxalic acid prior to excretion and accounts for between one third to a half of the oxalate present in the urine of normal subjects (Barness 1975). Large doses of vitamin C are reported to cause a modest but significant increase in urinary oxalate production (Lambden and Chrytowski 1954, Atkins *et al* 1964). Lambden and Chrytowski report that a daily ingestion of 4 g or more ascorbic acid led to increased excretion of oxalic acid; whereas a daily ingestion of less than 4 g produced no significant change in oxalate excretion. Some individuals, however, exhibit accelerated conversion of ascorbic acid to oxalate. Briggs *et al* (1973) and Briggs (1976) suggest greater inducibility of enzymes in the ascorbate-oxalate pathway in such affected individuals. In a 7 day ascorbate test for hyperoxaluria in 67 healthy volunteers, 3 were found to have this metabolic defect. Two of these three subjects

were related, suggesting that the defect may be hereditary. The hyperoxaluria of these individuals, following large doses of vitamin C, increases the risk of urinary stone formation. There are brief reports of men who have passed urinary stones after taking 2 g vitamin C daily for 2 weeks (Briggs *et al* 1973) and 1 g daily for several months (Roth and Breitenfeld 1973). Poser (1973) concluded that the connection between vitamin C and formation of oxalate stones in these cases was tenuous, though oxalate excretion decreased considerably on withdrawal of the vitamin supplements. It is likely that only a small percentage of the population has this genetic predisposing abnormality, so the magnitude of risk to humans is uncertain (Poser, 1973). Studies in guinea pigs (see para ??) also suggest that high intakes of vitamin C may increase the risk of renal calcification and stone formation in individuals with pre-existing hypercalcuria or hyperoxaluria (Singh *et al*, 1993).

54. Daily ingestion of 4 g ascorbic acid (in two 2 g doses) was reported to have no significant effect on oxalate excretion by Schmidt *et al*, 1981. Oxalate excretion in these subjects remained within the normal range. Less than 25% of the ingested dose and 2% of the absorbed ascorbic acid was metabolised to oxalate. Only daily ingestion of 9 g ascorbic acid elevated the oxalate excretion significantly above the pre-treatment range, from 50 mg to about 87 mg/day. The average increase in oxalate formation is reported to be small in relation to the large amount of ascorbic acid available for possible conversion. This increase in excretion of oxalate is comparable to the change in urinary oxalate content that is seen with change in diet (Hagler and Herman 1973). Similarly, Auer *et al* (1998a) reported that in 10 healthy males administered 4 g ascorbic acid/day for 5 days there was no statistically significant increase in urinary oxalate excretion.

55. Urine was collected from calcium oxalate stone patients (presumed by the authors to be at greatest risk from the effects of ascorbic acid), administered 100, 500, 1000, or 2000 mg ascorbic acid on days 2 and 3 postoperatively and analysed enzymatically. Specimens were collected directly into preservative to stabilise ascorbic acid and oxalate; oxalate was measured following the removal of ascorbic acid with sodium nitrite. The measured increase in urinary oxalate was statistically significantly at doses of 500 mg or more; the authors estimated that there is a 6-13 mg increase in urinary oxalate excretion per 1 g ascorbic acid which they conclude would increase the risk of calcium oxalate kidney stones (Urivetzky *et al* 1992). Auer *et al* (1998b) described a male subject who consumed 8 g ascorbic acid/day for 8 days (the protocol was initially 9 days but the study was stopped on day 8 due to haematuria). Urinary oxalate excretion increased by 350%; large aggregates of calcium oxalate dihydrate crystals were observed by scanning electromicroscopy immediately after detection of haematuria. The authors emphasised that “this individual’s response to vitamin C is probably rare...but highlights the potential dangers of long term megadose ingestion of this vitamin...”.

56. In their recent review, Levine *et al* (1999) concluded that the safe upper limit for vitamin C should be 1 g/day because patients with pre-existing hyperoxaluria may increase the risk of nephrolithiasis at vitamin C doses of 1 g or more, and because this dose might increase oxalate excretion in some healthy people, though the consequences of any such increase are unclear. The authors acknowledge that this latter conclusion is controversial (due to methodological problems [see below] and

conflicting results). They cite their earlier study (Levine *et al*, 1996) to support their recommendation. Steady state plasma ascorbic acid concentrations, urinary ascorbic acid and urinary oxalate concentrations (the latter determined by an enzymatic method reported to be free from interference by ascorbic acid) were measured in 7 young healthy non-smoking subjects. At vitamin C intakes of 1 g/day there were statistically significant increases in urinary oxalate excretion (though still within physiological limits) (Levine *et al*, 1996).

57. Wandzilak *et al* (1994) discussed the conflicting and somewhat confusing evidence reported on the effect of vitamin C on urinary oxalate excretion in the previous forty years. They conclude that the primary reason for the discrepancies in these reports appear to be due to methodological problems in urinary oxalate determination. The controversy has arisen due to the lack of a reliable and accurate method of measuring urinary oxalate. They report non-enzymatic conversion of ascorbic acid to oxalate *in vitro* and interference of ascorbic acid with several assays for urinary oxalate. A re-evaluation of the effects of high dose ascorbic acid on urinary oxalate levels was performed using ion chromatography modified to minimise ascorbic acid interference. Ascorbic acid added directly to urine *in vitro* resulted in a statistically significant but modest increase in measured oxalate. Measurement of oxalate levels in the urine of subjects following doses of 5 and 10 g ascorbic acid showed similar, modest increases, which could be accounted for entirely by oxalate production during analytical procedures. Thus, under these conditions no increase in urinary oxalate was demonstrable despite a greatly increased ascorbate intake.

58. Gerster (1997) reviewed both the clinical and epidemiological studies in relation to vitamin C and renal stones. With regards to whether administration of vitamin C increases urinary oxalate, she concluded that the question is controversial but questioned whether the reported increase (whether real or artefactual) is of clinical significance. Gerster noted that none of the vitamin C supplementation studies had documented an increase in kidney stones. Furthermore, as part of a large scale prospective study, 45251 male health professionals were followed up for 6 years to investigate the development of renal stones and assess vitamin C intake from the diet and supplements (Curhan *et al*, 1996). A non-significant reduced relative risk of kidney stones (0.78) was found for individuals in the highest intake group [>1.5 g vitamin C/day] compared to those in the lowest intake group [<250 mg vitamin C/day]; 95% confidence intervals 0.54-1.11) for kidney stone formation. In a similar study in female health professionals vitamin C intake was not associated with an increased risk of kidney stone formation (Curhan *et al*, 1999). However, while concluding that high vitamin C intakes in the general population is not of concern regarding the formation of kidney stones, Gerster (1997) recommended that in patients with a history of renal stones intakes should be limited to 100-200 mg/day so as to avoid any increase in urinary oxalate.

59. Several studies have investigated vitamin C intakes and oxalate metabolism in end-stage renal disease patients (*inter alia* Pru *et al*, 1985). Gerster (1997) reviewed such studies concluding that intakes should be restricted to 50-100 mg/day so as not to significantly raise plasma oxalate concentrations.

60. Another concern related to elevated ascorbic acid excretion is its effect on the clearance of uric acid. Stein *et al* (1976) studied the effect of ascorbic acid on serum and urinary uric acid in 14 subjects. It is reported that 2-6 hours after ingestion of 4 g ascorbic acid, the fractional clearance of uric acid increased by $202\% \pm 41\%$, compared to pre-dose control values. This increase is completely inhibited by acetylsalicylic acid and pyrazinamide, suggesting that urinary excretion of uric acid increases as a result of altered tubular function. The vitamin C induced uricosuria, along with the acidification of urine by vitamin C, may promote the development of renal stones or nephrocalcinosis, particularly in people with a predisposition (Barnes 1975). Chronic administration of ascorbic acid (8 g/day) resulted in sustained uricosuria and a substantial reduction of serum uric acid. It is suggested that reduced serum uric acid by increased renal clearance could obscure the diagnosis of gout and could conceivably precipitate acute gouty arthritis in predisposed individuals, although no evidence for these effects is presented (Stein *et al* 1976).

Urinary acidosis

61. Hanck (1982) investigated the potential toxicity of vitamin C in relation to urinary acidification, citing a number of earlier studies reporting decreased urinary pH following vitamin C supplementation. In two separate studies both administering 4 g ascorbic acid per day for 2 and 3 weeks respectively to 12 healthy volunteers, vitamin C had no significant effect on urinary pH, with the diurnal variation unaffected.

Oxidant effects

62. Vitamin C is a redox active compound and can not only act as an antioxidant but also as a pro-oxidant in the presence of redox active transition metal ions. Reduction of metal ions e.g. copper and iron, by vitamin C *in vitro* can result in formation of highly reactive hydroxyl radicals via reaction of the reduced metal ion with hydrogen peroxide by the Fenton reaction (Carr and Frei, 1999). Furthermore, ascorbic acid is oxidised to dehydroascorbic acid and this reaction is reversible, as a result of which reactive oxygen species (ROS) are formed by redox cycling (Ballin *et al*, 1988).

63. It is reported that vitamin C can cause oxidative damage to erythrocytes (Ballin *et al*, 1988). Mengel and Greene (1976) reported an increase in the *in vitro* lytic sensitivity of erythrocytes from normal subjects to hydrogen peroxide following 3 days of 5 g/day vitamin C administration, though none of the patients showed any signs of haemolysis (Mengel and Greene 1976). The authors speculated that an increased risk of haemolysis may occur with vitamin C, particularly in those with a genetic predisposition, e.g. in individuals with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. Fatalities have reportedly occurred as a result of haemolysis in patients with G-6-PD deficiency (Campbell *et al* 1975) and this may be related to vitamin C intake. Iwamoto *et al* (1994) reported on a case of ascorbic acid induced haemolysis in a patient with paroxysmal nocturnal haemoglobinuria (PNH). The patient had rapidly progressing anaemia and drank several bottles of an ascorbic acid enriched (1 g/100 ml) soft drink. Marked intravascular haemolysis was apparent, with exacerbation of jaundice and haemoglobinuria. No signs of infection or other factors which accelerate haemolysis were found. The authors concluded that abrupt

increases in serum ascorbic acid should be avoided. It is not stated whether this applies to the general population as well as PNH patients.

64. Ballin *et al* (1988) reported on a case of Heinz body haemolytic anaemia in a premature infant which appeared to be related to administration of multivitamins, including 30 mg/day vitamin C for 2 days at 3 weeks of age. On re-challenge with the same preparation on days 37-42 haemoglobin levels dropped and Heinz bodies reappeared but haemolytic did not occur. Subsequently the authors incubated erythrocytes from premature infants, full term infants and adults with 0.1 mg/ml sodium ascorbate. The erythrocytes from premature infants were significantly more sensitive to Heinz body development than those from full term infant and adults (which were of similar sensitivity). In the cells from premature infants, Heinz body development occurred with ascorbic acid concentrations as low as 0.1 mg/ml whereas in full term infants/adult erythrocytes Heinz body formation occurred at concentrations of 0.5 mg/ml. The authors noted that the recommended amount of vitamin C for infants of 30 mg/day (in the UK the RNI is 25 mg/day for infants) will result in high plasma concentrations of vitamin C, potentiated by lower glomerular filtration in premature infants. It was noted that the average plasma vitamin C concentration in new-born infants was 2.87 mg/dl, compared to 2.01 mg/dl in adult controls. However, the authors concluded that the significance of their finding to the routine management of premature infants remains to be determined. The increased production of Heinz bodies has also been demonstrated in young animals (Ballin *et al* (1988) (see paragraph ??).

65. Doses of 500 mg vitamin C per day given to volunteers (16 females and 14 males aged between 17 and 49) exhibited a pro-oxidative effect. The levels of the potentially mutagenic lesions, 8-oxoadenine and 8-oxoguanine, markers for DNA damage mediated by oxygen radicals, were measured by GC-MS. Supplementation of diets with 500 mg vitamin C for 6 weeks gave a statistically significant increase in 8-oxoadenine levels in DNA harvested from lymphocytes. No significant increase was observed in those subjects receiving placebo. In the 6 week period following treatment 8-oxoadenine levels returned to those observed at baseline or during placebo. In contrast, 8-oxoguanine levels were significantly reduced. (Podmore *et al* 1998). Supplementation of 100 mg vitamin C/day for 20 weeks to both smokers and non-smokers resulted in a significant decrease in oxidative base damage to lymphocyte DNA as measured by a modified comet assay. In addition the lymphocytes showed an increased resistance to H₂O₂ induced oxidative damage *in vitro* (Duthie *et al*, 1996).

66. Yang *et al* (1999) investigated the effects of iron and vitamin C co-supplementation (either 14 mg Fe/60 mg vit C or 14 mg Fe/260 mg vit C per day for 12 weeks) on platelet function and LDL oxidation in healthy adult volunteers. At 6 and 12 weeks, platelet aggregation in response to ADP was significantly reduced in the high dose vitamin C group and non-significantly reduced in the low dose group. The platelet response to prostaglandin E (PGE) was unaffected. Furthermore, there was a decreased susceptibility of LDL to oxidation which was significant in the high dose group. Similar results were reported by Harats *et al* (1998) following 2 months of 500 mg/day vitamin C supplementation. Yang *et al* (1999) concluded that their

observations are highly suggestive of an antioxidant effect of the supplementation i.e. no evidence of pro-oxidant effects were observed.

67. As part of the study described above, Rehman *et al* (1998) measured base damage in leukocyte DNA, reporting an increase in damage after 6 weeks which returned to baseline after 12 weeks, which the authors speculate may be due to up-regulation of DNA repair enzymes. A later study by the same group found no evidence of a pro-oxidant effect of 260 mg/day vitamin C supplementation alone or in combination with 14 mg/day iron on DNA after 6 weeks treatment (Proteggente *et al*, 2000). Brennan *et al* (2000) assessed lymphocyte DNA damage in healthy subjects administered 1000 mg vitamin C/day for 42 days. There was no effect on supplementation on endogenous DNA damage. Furthermore, there was no effect on 10 μM H_2O_2 induced DNA damage *in vitro*. However, at *in vitro* H_2O_2 concentrations of 200 μM , vitamin C supplementation significantly reduced lymphocyte DNA damage (when compared to H_2O_2 induced DNA damage prior to supplementation) both during the supplementation period and the subsequent 6 week washout period.

68. Lee *et al* (2001) investigated the *in vitro* vitamin C mediated formation of genotoxins from lipid hydroperoxides in the absence of transition metal ions. The authors noted that transition metal ion mediated decomposition of lipid hydroperoxides to DNA reactive metabolites is initiated by a one-electron reduction to an alkoyl radical. The authors proposed that such an alkoxy radical would also be formed by the one-electron reduction of lipid hydroperoxides by vitamin C. When one-electron reduction of hydrogen peroxide occurs, the resulting hydroxyl radical is scavenged by vitamin C. The authors proposed that when the alkoxy radical is attached to lipids, radical propagation would proceed before vitamin C mediated termination. Hydroperoxide was allowed to decompose in the presence of varying concentrations of vitamin C (0-12 mM) in the absence of transition metal ions and the concentrations of various specific electrophilic metabolites determined. The authors reported that vitamin C induced the decomposition of lipid hydroperoxides to electrophiles at concentrations equivalent to those found *in vivo* (intracellularly). The authors speculated that the efficiency of vitamin C in inducing decomposition of lipid hydroperoxides suggests that this process could cause substantial DNA damage *in vivo* and may explain why vitamin C has not demonstrated substantial efficacy in cancer chemoprevention trials.

69. Reviewing the various studies on the pro-oxidant effects of vitamin C supplementation, Proteggente *et al* (2000) commented on the wide analytical variations in detecting any such oxidative damage (which is being addressed by the European Standards Committee on Oxidative DNA damage), concluding "at this stage it is difficult to identify in humans the effects of [vitamin C] supplementation...on oxidative DNA damage..."

Reproductive Toxicity

70. A number of anecdotal reports suggest excess vitamin C may affect fertility. However, data from properly conducted epidemiology studies are not available. Briggs (1973) reports that two healthy young women failed to conceive whilst taking

2-5 g vitamin C (variable) daily for 6 months, each conceived during their first treatment free cycle. Further communications report that 9 women taking 0.4-1 g vitamin C daily all conceived without difficulty. One woman (26 years) failed to conceive in 14 months whilst taking 2 g vitamin C daily, extensive investigations for fertility revealed no abnormality in her or her husband. Another is reported to have taken 1-5 g ascorbic acid daily for 2 months. She conceived once, had an early spontaneous abortion, with reabsorption of the foetus and has failed to conceive again, despite there being no detectable reasons for infertility. A further 4 women, all of whom had previous children, taking daily doses of 2-4 g for 6 to 17 months, failed to conceive during this treatment. Two of the four conceived within 3 months of the cessation of the supplementary vitamins. In contrast, other studies have reported no negative effects on human fertility and pregnancy; doses of up to 10 g of ascorbic acid were well tolerated during pregnancy. In 3,000 women who were administered 2-10 g vitamin C daily for several years, neither a reduction in fertility nor an effect on the new-born was observed (reviewed Hornig and Moser 1981).

Conditioned Scurvy

71. In two observed cases of infantile scurvy it was concluded that both infants were receiving adequate amounts of vitamin C, approximately 60 mg of ascorbic acid daily. The possibility of "conditioned scurvy" arose when detailed questioning revealed that both mothers had consumed large supplements of vitamin C during pregnancy; in excess of 400 mg/day (Cochrane 1965). It was suggested that ingestion of excessive amounts of certain vitamins by the mother during pregnancy may condition the offspring to require greater than the expected or recommended daily intakes. However, there is no further evidence in the literature to support these clinical observations which are not from accurately controlled studies and which involve moderately high rather than excessive quantities of vitamin C. Cochrane (1965) reported that "conditioned scurvy" could also be demonstrated in guinea pigs (see paragraph 86).

72. Siegel *et al* (1981) report a case of conditioned or "rebound" scurvy due to withdrawal of megavitamin C. A 49 year old male consumed 1 g ascorbic acid/day for over a year in addition to other vitamins and a health food diet. One to one and a half weeks after stopping the vitamin C dosage, the patient presented with sore gums, petechial haemorrhages, ulceration and oral capillary fragility. On resumption of the megadoses, the symptoms disappeared. However, Clark (1981) noted the rapid onset of the condition which was considered to have occurred in a shorter time than would be needed to deplete body stores and pointed out that no attempt was made to determine vitamin C levels in the patient.

Supplementation studies

73. Relatively few supplementation studies involving vitamin C alone have been identified. A number of investigations have been considered earlier in this review and are arranged by endpoint. The remaining studies are discussed below.

74. Greenberg *et al* (1994) administered 1 g vitamin C/day (plus 400 mg vitamin E with and without 25 mg β -carotene for 4 years to 380 patients with colorectal

adenomas. The incidence of new adenomas at 1 year and 4 years of the trial was investigated by colonoscopic examination. No adverse events were reported and it was noted that patients adhered well to the therapy. Treatment with vitamins C, E and β -carotene did not affect the rate of occurrence of new adenomas.

75. Blot *et al* (1993) reported the results of a 5 year, randomised, placebo-controlled, primary prevention trial involving 29, 584 adults (male and female, aged 40-69) living in Linxian county, China, a region with a very high incidence of oesophageal and stomach cancers. The aim of the study was to assess the value of daily supplementation with four different combinations of dietary factors:- Factor A] 5000 IU retinol + 22.5 mg zinc, Factor B] 3.2 mg riboflavin + 40 mg niacin, Factor C] 120 mg ascorbic acid + 30 μ g molybdenum, Factor D] 15 mg β -carotene + 50 μ g selenium + 30 mg α -tocopherol. The study design was a fractional ($2 \times 2 \times 2 \times 2$) factorial style, with 9 supplementation groups taking combinations of 2 factors, all 4 factors, or placebo. The measured endpoints were 1) cancer incidence (oesophageal, gastric cardia, other stomach, others) and 2) total mortality. Compliance, assessed by measuring nutrient concentrations in blood every 3 months and counting unused pills, was reported to be excellent. Ascorbic acid and molybdenum in combination had no effect on cancer incidence and mortality, though no adverse effects of supplementation were reported.

76. No adverse effects were reported in 20 elderly subjects receiving 120 mg vitamin C for 6 months in combination with β -carotene and α -tocopherol or 20 subjects receiving the same vitamins plus trace elements compared to those receiving placebo or trace elements only (Girodon *et al*, 1997).

Adverse Drug Reactions

77. Suspected adverse reactions to medicinal products are reported to the Committee on Safety of Medicines/Medicines Control Agency. Many factors influence the number of reports received, and in most situations there is considerable "under-reporting" of reactions. Most of the adverse reactions reported for oral products containing vitamin C relate to multi-constituent products, and may not, therefore, be directly attributable to the vitamin. Single constituent products are associated with a low number of suspected adverse reactions, with no trends suggesting an association with treatment.

Toxicity in Laboratory Animals – see Table 2*Acute/short term toxicity*

78. Toxicity differs from species to species and depends greatly upon the mode of administration. The data are summarised in Hanck, 1982. In the mouse an LD₅₀ dose of 1058 mg/kg bodyweight (bw) vitamin C administered intravenously is quoted. Whereas, when given intraperitoneally or subcutaneously the LD₅₀s for vitamin C are 2000 mg/kg bw and 5000 mg/kg bw, respectively. Vitamin C has low oral toxicity with an LD₅₀ of 8021 mg/kg bw is quoted for mice. (Hanck 1982). Similar figures are quoted for the rat.

79. Ascorbic acid affects the metabolism of several trace elements, including copper (Evans, 1973). Dietary supplements of ascorbic acid have been shown to increase the severity of copper deficiency in chicks and rabbits (Hunt *et al*, 1970). Hunt *et al* observed a significant decrease in hepatic copper concentration in chicks administered ascorbic acid and fed a controlled diet, supplemented with copper (basal diet consistently contained <1 µg/g, experimental diet contained 6 µg/g), suggesting that the vitamin affects either copper absorption or utilisation, or both. Van Campen and Gross (1968) observed that ascorbic acid significantly decreased the absorption of ⁶⁴Cu when it was introduced into a ligated section of rat intestine, along with the radiolabelled copper. Conversely, ascorbic acid had no effect on the excretion of intraperitoneally administered ⁶⁴Cu, indicating that the vitamin affects copper metabolism by depressing its intestinal absorption. Evans (1973) demonstrated that ascorbic acid decreases binding of copper by metallothionein from both intestine and liver. Spectral analysis of the protein with ascorbic acid indicates that the vitamin interacts with metallothionein. This protein mediates copper absorption, thus the interaction of ascorbic acid with metallothionein will affect copper homeostasis.

80. Numerous experiments have been conducted in animals to investigate the relationship between cholesterol metabolism and ascorbic acid ingestion. Most experiments have been done in guinea pigs, which are either deficient or marginally insufficient in ascorbic acid, as they require an exogenous source of vitamin C. In guinea pigs the extra ascorbic acid has increased the destruction or decreased the accumulation of cholesterol (Bannerjee and Singh 1958). Rats fed 150 mg/kg bw ascorbic acid for 150 days, equivalent to a 70 kg man consuming 80 mg in addition to his dietary intake, had a plasma cholesterol concentration (129 mg/dl) significantly higher than those fed no ascorbic acid (109 mg/dl). The mean weight of the animals was not significantly different in the two groups (Klevay 1976). High ratios of zinc to copper are associated with hypercholesterolemia and high risk of heart disease. A decrease in absorption of copper may have resulted in an increase in this ratio, manifesting as hypercholesterolemia.

81. Guinea pigs (fed a stock diet) and administered doses of up to 250 mg/day ascorbic acid by gavage for up to 20 weeks were reported to develop normally compared to controls, who had a background intake of 1.02-1.20 mg/day. (Nandi *et al*, 1973). Urinary excretion of ascorbic acid was high compared to controls, but at the highest dose still only accounted for 2.5% of the administered dose, indicating that most of the ascorbic acid was metabolised. Urinary oxalate excretion remained

similar. There was a marked increase in tissue ascorbic acid concentrations though plasma concentrations were unchanged. Large doses of ascorbic acid were neither beneficial or toxic to guinea pigs fed a normal diet, however, when the basal diet consisted of an unfortified wheat diet, comparable doses of ascorbic acid were toxic. Administration of 50 mg ascorbic acid /day by oral gavage resulted in a significant decrease in growth, compared with doses of 5, 10 and 20 mg. By day 16 there was 50% mortality in the 50 mg/day group and by day 25, 100% mortality. . At a dose of 100 mg ascorbic acid daily all animals died within 16 days. The authors suggested that the effects observed with an unfortified wheat diet may have been due to a deficiency in other nutrients such as B vitamins, minerals or protein. The authors investigated this hypothesis in further experiments by supplementing the unfortified wheat diet with a vitamin or salt mixture, which had no beneficial effect (100% mortality by day 16) and casein, which gave a partial protective effect (50% mortality by day 16; 60% mortality by day 25), against the toxicity encountered with 100 mg ascorbic acid. Ascorbic acid administered at doses up to 100 mg/day by gavage for 25 days was not considered toxic to guinea pigs on a fortified wheat diet. The authors concluded that in geographic areas where the diet consists mainly of cereals, intake of large doses of ascorbic acid could be harmful.

82. Two groups of rats were totally deprived of food for 96 hours, one group was given drinking water containing 30 g/l ascorbic acid, whilst the other group was allowed free access to untreated water. A third group fed *ad libitum* served as a control. After 96 hours all the rats were killed and their stomachs examined for ulceration. The incidence of intestinal ulcers was higher in the group that received ascorbic acid in their drinking water and the ulcers were significantly larger (Glavin *et al* 1978). This dosing also caused an increase in number and severity of glandular stress ulcers caused by activity-stress and restraint –cold procedure.

83. Rats fed diets containing 20, 40 or 60 g ascorbic acid/kg for 4 days did not show an increased incidence of gastric lesions (Lo and Konishi 1978). Administration of a single oral dose of aspirin (30 mg/100 g bw) at the end of the 4th day to rats fed control diet produced gastric ulcers. When rats were given aspirin plus ascorbic acid in the diet (40 and 60 g/g diet) there was a significant increase in the number of lesions compared with controls, suggesting a synergistic action of aspirin and ascorbic acid in producing gastric ulcers.

84. Dehydroascorbic acid is the reduced form of ascorbic acid and in a non-hydrated form its structure is similar to the chemical alloxan which is used to induce diabetes experimentally in rats (Patterson 1950). Repeated intravenous injections of rats with dehydroascorbic acid (80 mg/day for three or four days) can damage Islet cells in the pancreas and produce permanent diabetes.

85. Ballin *et al* (1988) administered i.p. doses of 0, 148, 200, and 500 mg/kg bw/day ascorbic acid for 7 days (background intake was 70 mg/kg bw/day) to groups of adult guinea pigs (4 animals per dose group). One of the animals in the mid dose group and 3 animals in the high dose group died during the study period. On day 8 the number of erythrocytes containing Heinz bodies was significantly increased in the mid and high dose groups; there was also an associated reduction in erythrocyte glutathione concentrations.

Chronic Toxicity

86. In a study by Sorensen *et al* (1974) guinea pigs were fed diets containing 2 g (control) and 86 g (experimental) of ascorbic acid per kg diet for 275 days. The growth rate of the treated guinea pigs was significantly retarded compared to controls during the first 150 days. Intraperitoneal injection of radiolabelled ascorbic acid into experimental and control guinea pigs showed increased catabolism of vitamin C to expired $^{14}\text{CO}_2$ in the experimental animals. This was evident in experimental animals even after subjection to diet devoid of, or containing minimal (3 g/kg) ascorbic acid, for 44 days. This is in agreement with the hypothesis that intake of massive doses of ascorbic acid can lead to accelerated ascorbic acid catabolism, i.e. systemic conditioning. The accelerated rate of catabolism was not reversible by subnormal intakes of the vitamin. Experimental animals maintained higher body pool of ascorbic acid than controls, however, the only tissue in which this difference was statistically significant, were the testes (Sorensen *et al* 1974).

87. Singh *et al* (1993) administered 0, 100, 400, and 600 mg ascorbic acid/kg bw/day for 105 days to normal guinea pigs and 100 mg/kg bw/day for 45 days to guinea pigs made either hypercalciuric or hyperoxaluric through calcium or sodium oxalate feeding. In the normal guinea pigs, the top 2 doses significantly increased oxalic acid excretion; however, on examination there was no crystal deposition, stone formation or calcification in the kidneys, ureters or bladder. In contrast, in those animals rendered hypercalciuric or hyperoxaluric, ascorbic acid supplementation did not alter urine chemistry but histological examination revealed that ascorbic acid intensified the renal and bladder tissue calcification in both groups. The authors concluded that the data suggests the high intakes of vitamin C increases the risk of renal calcification and stone formation in pre-existing hypercalciuric and hyperoxaluric conditions.

88. There were no differences in body weight gain in baboons, given 80 or 1000 mg/kg bw ascorbic acid for 20 months. Serum ascorbic acid levels were significantly higher in the 1000 mg/kg bw dose group, being 2.2 mg/dl compared with 1.76 mg/dl. Serum ascorbic acid levels in free-living baboons are approximately 1.4 mg/dl. Upon sacrifice histological examination of kidneys and bladders revealed no signs of oxalate crystals (Du Bruyn *et al* 1977).

Reproductive and developmental toxicity

89. In a US FDA study, rats and hamsters were given oral doses of 50, 150 or 450 mg/kg/day ascorbic acid and guinea pigs administered an oral dose of 400 mg/kg/day throughout pregnancy. No increases were observed in spontaneous abortion, or mortality in offspring, (Alleva *et al* 1976). These findings are consistent with those of Froberg *et al* (1973) who reported that ascorbic acid had no effects on pregnancy in rats given oral doses of 150-1000 mg/kg/day and mice given 250-1000 mg/kg/day.

90. In similar experiments in rats fed a fortified wheat diet, gavage doses of ascorbic acid of 100 mg/day were administered to male and female rats from 2 weeks prior to mating, through gestation to weaning - a total of 6 weeks. In male rats there was no effect on growth. These doses also had no effect on weight gain and condition

during pregnancy or on the growth of the litters. The average number of pups born per litter and body weight of the litters from ascorbic acid-fed parents was similar to those of controls (Nandi *et al* 1973). In contrast to the studies in guinea pigs, large doses (doses not specified) of ascorbic acid were not toxic to rats fed a whole grain wheat flour diet; no mortality was observed in a 30 day period. Similar observations were made when the animals were fed a diet of whole grain rice flour.

91. Cochrane (1965) studied the *in utero* effect of ascorbic acid in guinea pigs. Twelve female guinea pigs were divided into 4 groups receiving 3, 20, 200 or 1000 mg vitamin C daily. After weaning, the young were maintained on a normal diet and given daily intraperitoneal injections of 1.5 mg ascorbic acid, sufficient to meet normal requirements. Two out of ten of the young died in the ensuing two to five weeks, both offspring of mothers that received 1 g of vitamin C daily. These findings suggested that an ascorbic acid dependency was induced in the young by exposure to the vitamin *in utero*. It was considered possible that ingestion of excessive amounts of the vitamin during pregnancy may “condition” the offspring to requirements that are greater than normal, leading to deficiency if these requirements are not met.

92. Supplementary ascorbic acid, at levels of 220 mg/kg of diet, resulted in the increased mobilisation of calcium and phosphate from the skeleton in chicks (Thornton 1970, Brown 1973). Increased excretion of calcium and phosphate was also observed in these animals. It is suggested that the observed changes in the mineral dynamics of the bone is as a result of a distant reaction, not as a direct action on the bone itself. However, this effect suggests that supplementary ascorbic acid may have untoward effects on the metabolism of the bone, especially in young, rapidly growing animals (Brown 1973). This effect has not been observed in humans but could be a consideration in individuals who are susceptible to bone fractures, such as the elderly, osteoporosis sufferers and those with musculoskeletal abnormalities (Chalmers 1975).

Genotoxicity

In vitro

93. Stich *et al* (1976) suggested that ascorbic acid, particularly in the presence of copper, has a mutagenic effect based on findings from several experimental systems. They report that ascorbic acid in the presence of oxygen converts covalently-closed circular DNA to open circular DNA molecules, causes single strand breaks in DNA, inactivates transforming DNA of *Pneumococcus*, degrades bacteriophage R17, induces reverse mutations in *S. typhimurium*, triggers DNA repair synthesis and elicits chromosome aberrations including chromatid breaks and exchanges. It was considered likely, that as these effects are only observed in the presence of copper, they are the effects of hydroxyl radicals generated in the interaction of Cu^{2+} with H_2O_2 , which is produced by the ascorbic acid. This view is confirmed by Omura *et al* (1978) who studied the mutagenic action of ascorbate with and without cupric ions using *S. typhimurium* TA 100 strain. Ascorbic acid itself had no mutagenic action. The mutagenic effect seen in the presence of copper can be blocked completely by EDTA. The observed mutagenic action of ascorbic acid may be purely an *in vitro* effect as, *in vivo* there is a complete lack of free cupric ions and the intact cell has very effective mechanisms for removing H_2O_2 to prevent cell damage.

94. Exposure to ascorbic acid at concentrations of 10^{-3} to 10^{-6} M resulted in a dose related increase in sister chromatid exchange in V79 Chinese hamster cells (Speit *et al*, 1980).

In vivo

95. In Chinese Hamster Ovary (CHO) cells, mutations were induced only at high ascorbic acid concentrations which were also cytotoxic. If catalase was added, both mutagenesis and toxicity were prevented, suggesting that the mutagenic properties of ascorbate involve peroxide radicals (Shamberger 1984).

96. Contrary to their results *in vitro*, ascorbic acid did not lead to an induction of sister-chromatid exchanges (SCE) in the bone marrow of Chinese hamsters in the SCE test *in vivo*. The range of test doses was between 200 and 10,000 mg/kg bw and was given orally as well as by intraperitoneal injection (Speit *et al* 1980).

97. A dominant lethal test in male Wistar rats showed no *in vivo* mutagenic effects on germ cells of male Wistar rats when fed with daily intakes of 0.54 or 2.6 g/kg daily for two weeks (Chauhan *et al* 1978).

Mechanisms of Toxicity

98. The adverse effects of ascorbic acid result from a number of different mechanisms. These include interference with other physiological components such as copper and iron stores. High doses may cause gastro-intestinal disturbance through osmotic effects on the gut. It has also been suggested that vitamin C may have pro-oxidant effects.

Regulatory Considerations

99. Under the terms of the Miscellaneous Food Additive Regulations, ascorbic acid can be used *quantum satis*. This means that no maximum level is specified but that the amount used should be in accordance with good manufacturing practice and should not exceed the amount needed for the additive's function.

Existing Recommendations on maximum intake levels

100. COMA (1991) noted that adverse effects occurred at intakes of "grams/day" but did not make any specific recommendations.

101. The EU Scientific Committee for Food (1993) recommend a maximum intake of 1000 –10,000 mg/person/day. A world-wide ADI has not been specified for ascorbic acid or its sodium, potassium and calcium salts by the WHO Joint Expert Committee on Food Additives (JECFA).

Existing Recommendations on maximum supplementation levels

102. In 1991, the Department of Health and Ministry of Agriculture, Fisheries and Food Working Group noted that the undesirable chronic dose of vitamin C was 6 g

and suggested that the maximum daily dose for supplementation should not exceed one tenth of that (600 mg).

103. The Council for Responsible Nutrition, a UK Trade Association, recommend a maximum long term supplementation level for vitamin C of 2 g/day and a maximum short term supplementation level of 3 g/day (CRN, 1999). CRN note that the latter is precautionary since no adverse effect has been established.

Summary

104. Vitamin C is necessary for the prevention of scurvy and to aid wound healing in man. It is found in foods of plant origin and as a preservative in processed foods, in addition to its use as a dietary supplement. In the UK the RNI for vitamin C in adults is 40 mg/day.

105. In humans acute toxicity is characterised by gastrointestinal disturbances. Symptoms generally disappear within one week and no further complications are reported. In persons with a genetic predisposition affecting their iron metabolism, excessive vitamin C in the gut may have further consequences since as well as increasing iron absorption from food, vitamin C also releases iron from body stores. Elevated serum ferritins have also been linked to coronary artery disease, due to iron catalysed lipid peroxidation.

106. Concern exists over the effects of vitamin C on the kidney and bladder, due to the possible increased oxalate excretion and ensuing oxalate stone formation. In healthy subjects high vitamin C intakes has little effect on urinary oxalate excretion, though in individuals with previous kidney stones, vitamin C intakes of 500 mg or more are reported to significantly increase oxalate excretion. An increase in urinary uric acid from ingestion of large doses of vitamin C also promotes concerns of development of renal stones or nephrocalcinosis, especially in people with a genetic predisposition. Chronic administration of vitamin C (8 g/day) resulted in sustained uricosuria and a substantial diminution of uric acid. However, epidemiological studies do not indicate that vitamin C is associated with an increased risk of kidney stone formation.

107. Under certain conditions vitamin C is reported to exhibit pro-oxidant activity, though *in vivo* evidence for such effects are conflicting

108. Toxicity in animals varies from species to species and depends greatly upon the mode of administration. Dietary supplements of ascorbic acid have been shown to increase the severity of copper deficiency in chicks and rabbits. It has been demonstrated that vitamin C decreases the binding of copper by metallothionein from both intestine and liver. Studies in rats administered ascorbic acid in their drinking water and deprived of food showed an increase in gastric ulcers. Rats fed ascorbic acid in the diet did not show an increased incidence of gastric lesions, though administration of a single dose of aspirin produced a significant increase in gastric lesions over controls. Calcium and phosphate is mobilised from the skeleton of chicks by supplementary vitamin C (220 mg/kg diet), with an associated increase in urinary excretion of these minerals. No increase in spontaneous abortion or mortality in offspring was noted in rats and hamsters given 50, 150 or 450 mg/kg/day of ascorbic

acid. Consistent with this are the findings that oral doses of 150-1000 mg/kg/day to rats and 250-1000 mg/kg/day to mice had no detrimental effects on pregnancy.

109. Mutagenic effects of ascorbic acid have been reported in *in vitro* experiments. These appear to be observed only in the presence of copper. Ascorbic acid did not show mutagenic properties in *in vivo* tests.

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ANNEX 1 TO EVM/99/21.REVISED SEPT 2001

Table 1. Summary of adverse effects of Vitamin C in humans¹

Endpoint	Dose	Duration	NOAEL/LOAEL	Comment	Reference
Gastrointestinal Upset	Usually >2g/day. Occasionally lower.	Acute	LOAEL	Direct ionic effect	
Sensitisation	Usually >2g/day. Occasionally lower.	Acute	LOAEL	In association with GI effect. Anecdotal reports	Barness (1975)
Increased iron absorption	2g/day – No effect on ferritin in healthy subjects		NOAEL	Of concern if iron metabolism impaired.	Cook <i>et al</i> (1984) Herbert <i>et al</i> (1994, 1996)
Destruction of vitamin B12				<i>In vitro</i> effect, appears to be an artefact. No clear evidence in man, no effect in rats.	Herbert and Jacob (1974) Newmark <i>et al</i> (1976)
Mobilisation of cholesterol in atherosclerosis	1g/day 1g/day 500mg/day – slight increase in plasma cholesterol but LDL cholesterol less susceptible to <i>in vitro</i> oxidation.	? 3 months-in healthy volunteers – negative 2 months	LOAEL	Conflicting reports	Spittle (1971) Crawford <i>et al</i> (1975) Harats <i>et al</i> (1998)

¹ This does not include case reports.

Increased urinary oxalate and oxalate stones	<ul style="list-style-type: none"> ➤ 4g /day- sig increase in urinary oxalate. Some reports of stones at 1-2 g/day ➤ 500mg/day increase in oxalate in patients with existing stones ➤ 4g/day. No increase in urinary oxalate. 	Days 2 and 3 post-operatively 5 days	NOAEL = 100 mg/day NOAEL	May be genetic defect - susceptible sub group.	Lambden and Chrytowski (1975) Poser, (1973) Wandzilak <i>et al</i> (1994) Urivetsky <i>et al</i> (1997) Auer <i>et al</i> (1998)
Increased urinary uric acid.	4g/day 8g/day	Chronic		Could precipitate gouty arthritis or renal stones.	Barness (1975) Stein <i>et al</i> (1976)
Oxidative effects: Increased lytic sensitivity Pro-oxidative effect, increased DNA damage.	- 5g/day in volunteers increased RBC sensitivity <i>in vitro</i> 500 mg/day	2 days 6 weeks	LOAEL LOAEL	Increased risk of haemolysis, especially of genetic pre-disposition eg. G6P deficiency Returned to base line after treatment stopped	Mengel and Greene (1976) Cambell (1975) Podmore <i>et al</i> (1998)
Failure to conceive Fertility and pregnancy	> 2g 2-10g	> 2 months Several years	LOAEL NOAEL	Anecdotal reports No effect found	Briggs (1973) Hornig and Moser (1981)
Conditioned infantile	> 400 mg/day in		LOAEL	Studies of doubtful value	Cochrane (1965)

scurvy in infants receiving RDA.	pregnancy				
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Table 2. Summary of animal toxicity data.

Species	Endpoint	Dose	Duration	NOAEL/LOAEL	Comment	Reference
Rats	Increased stomach ulceration	30g/l in drinking water	96 hours	LOAEL	Achieved after rats fasted for 96 hours	Glavin <i>et al</i> (1978)
Rats	No effect on gastric ulcers	Up to 60 g/kg in diet.		NOAEL		Lo and Konishi (1978)
Rats	No effect on reproductive or developmental parameters	Up to 100 mg/kg bw/day	6 weeks	NOAEL		Nandi <i>et al</i> (1973)
Rats	No effects on growth or urinary oxalate levels	Up to 100 mg/kg bw/day	6 weeks	NOAEL		Nandi <i>et al</i> (1973)
Rats	No effect on reproductive or developmental parameters	Up to 450 mg/kg bw	Days 1-19 gestation	NOAEL	Few details provided	Alleva and Ballas (1976)
Guinea pigs	Conditioned increase in requirements. Increased mortality in young from high dose group.	Up to 1000 mg/day, offspring given normal diet.		NOAEL= 200mg/day		Cochrane (1965)
Guinea pigs	Decreased growth, Increased mortality	10-100 mg/kg bw/day by gavage plus wholegrain diet	25 days	NOAEL = 20 mg/kg bw/day	Casein but not vitamin supplementation reduced toxicity.	Nandi <i>et al</i> (1973)

Guinea pigs	Increased cholesterol levels	150 mg/kg bw/day	150 days	LOAEL		Klevay (1976)
Guinea pigs	Decreased growth	Fed diet containing 8600 mg/kg (approximately 344 mg/kg bw)	275 days	LOAEL		Sorensen <i>et al</i> (1976)
Guinea pigs	No effect on reproductive or developmental parameters	Up to 450 mg/kg bw/day Sc injection	During gestation	NOAEL	Few details provided	Alleva and Ballas (1976)
Chicks	Increased severity of copper deficiency	0.5% in diet	12 days	LOAEL		Hunt <i>et al</i> (1970)
Chicks	Mobilisation of Ca and P from skeleton	220 mg/kg diet		LOAEL	Thornton (1970)	
Baboons	No effect on weight gain, or production of oxalate crystals	80 or 1000 mg/kg bw/day	20 months	NOAEL		Du Bruyn <i>et al</i> (1977)

ANNEX 2 to EVM/99/21.REVISED SEPT 2001

INTAKES OF VITAMIN C FROM FOOD AND SUPPLEMENTS IN THE UK

The data presented on vitamin C intakes are obtained from dietary surveys of specific population age groups in Britain carried out over the last 15 years²³⁴⁵⁶. In each survey food consumption data were collected by means of a dietary record (usually weighed) kept for 4 or 7 consecutive days. Nutrient intakes were calculated using a set of nutrient composition data contemporaneous with the time of the survey. Therefore some apparent differences in intakes between population age groups may be due to changes in the nutrient composition data and reflect changes in the nutrient composition of manufactured foods over time.

Total intakes of vitamin C

Table 1 provides information on the median intake, and the upper and lower end of the intake distribution (defined as upper and lower 2.5 percentiles, respectively), of vitamin C by the British population, classified by age and sex.

Median intakes of vitamin C were lowest for pre-school children and highest for infants aged 6-12 months, due to high consumption of vitamin C fortified commercial infant foods and drinks in this group. Intakes increased significantly with age for boys aged 4-18 and for girls aged 4-10 but not for older girls, pre-school children or adults. Intakes for older people (65 years and over) declined with age. Median vitamin C intakes were above the Reference Nutrient Intakes for all groups except the oldest group of women in institutions. Intakes at the 97.5%ile were 3-4 times the median for all groups. Vitamin C intakes adjusted for body weight showed a trend to decrease with age.

Sources of vitamin C in the diet

Table 2 indicates the contribution made by different types of food to average intakes of vitamin C by young people aged 15-18 years. This dataset was collected in 1997 and so most closely reflects current eating habits and fortification practices. The main source of vitamin C in this age group is beverages, that is soft drinks and fruit juice, followed by vegetables and potatoes. Vitamin C is frequently added as a fortificant to soft drinks either for nutritional reasons or as an antioxidant preservative. Soft drinks provided 16% of vitamin C intake in this age group, from both naturally occurring and fortificant vitamin C. Vitamin C is used as a fortificant in some other foods such as children's snacks etc but not on a widespread basis. It is therefore estimated that vitamin C as a fortificant contributes more than about 10% of total intake for older children and adults and less than this for older people who have lower consumption of soft drinks. In infants and young children the contribution of fortificant vitamin C

² Food and nutrient intakes of British infants. 1986

³ National Diet and Nutrition Survey of children aged 1½-4½ years. 1992/3

⁴ National Diet and Nutrition Survey of young people aged 4-18 years. 1997/8

⁵ Dietary and nutritional survey of British adults. 1986/7

⁶ National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

will be higher. Infants obtained two thirds of their vitamin C intake from commercial infant foods and drinks, the majority of this being the fortificant. In young children, soft drinks make a higher contribution to vitamin C intakes and were the major source in 1½-4½ year olds, providing 30% of average intake. In older people vegetables and potatoes are the major source of vitamin C and the contribution from soft drinks and fruit juice is much lower.

Vitamin C intake from supplements

For all age groups dietary supplements provided around 5-10% of population average intakes of vitamin C. Of course, the proportion of intake from supplements is much higher if supplement consumers are considered separately.

Table 3 shows the number of consumers of dietary supplements containing vitamin C in each age group together with the median, range and upper level intakes of vitamin C from supplements for those who consumed them. The highest prevalence of vitamin C supplement use was in the infant, pre-school and primary school age groups. Over 40% of infants took welfare vitamin drops containing vitamin C, obtaining 20% of their total intake from this source. Only a small proportion of older children, adults and older people took vitamin C supplements. It should be borne in mind that the data for adults aged 16-64 years was collected in 1986/87 and use of supplements may have changed since then. The range of intakes from supplements was wide with maximum intakes from this source around one gram a day.

Nutrition Surveys Branch
Food Standards Agency
July 1999

Table 1: Total intakes of vitamin C

Age/sex	Absolute vitamin C intake (mg/day)			Bodyweight adjusted vitamin C intake (mg/kg bwt/day) ⁷		
	<i>intakes from food and supplements</i>					
	2.5%ile	Median	97.5%ile	2.5%ile	Median	97.5%ile
Infants (1986)⁸ 6-12mths/M&F	15.0	81.0	318.0	1.48	8.44	33.90
Pre-school children (1992/3)						
1½-2½ yrs/M&F	10.2	38.4	169.0	0.79	3.16	14.13
2½-3½ yrs/M&F	8.9	39.7	160.0	0.63	2.83	10.91
3½-4½ yrs/M	9.0	41.6	187.0	0.46	2.56	11.30
3½-4½ yrs/F	9.6	41.4	132.0	0.60	2.46	8.09
Young people (1997/8)						
4-6 yrs/M	19.3	63.8	195.9	0.90	2.96	9.65
4-6 yrs/F	19.7	60.6	163.9	1.02	3.07	7.25
7-10 yrs/M	23.9	64.9	235.0	0.76	2.15	7.12
7-10 yrs/F	19.3	68.5	216.2	0.52	2.10	5.92
11-14 yrs/M	24.2	63.0	215.4	0.45	1.40	4.95
11-14 yrs/F	17.4	59.4	230.6	0.36	1.31	5.03
15-18 yrs/M	21.0	66.9	292.3	0.23	1.04	3.91
15-18 yrs/F	20.2	65.0	207.5	0.27	1.07	3.88
Adults (1986/7)						
16-24 yrs/M	19.1	53.1	246.5	0.24	0.80	3.19
16-24 yrs/F	12.0	49.2	188.8	0.21	0.88	3.29
25-34 yrs/M	22.0	60.5	236.0	0.25	0.81	2.85
25-34 yrs/F	11.8	50.0	170.8	0.16	0.80	2.91
35-49 yrs/M	19.4	59.0	235.7	0.24	0.75	3.15
35-49 yrs/F	16.2	56.8	285.5	0.26	0.88	4.54
50-64 yrs/M	17.7	60.2	151.7	0.25	0.77	2.10
50-64 yrs/F	17.9	62.0	191.8	0.20	0.97	3.40
Older people free-living in the community (1994/5)						
65-74 yrs/M	15.6	62.6	199.7	0.21	0.84	2.26
65-74 yrs/F	13.6	56.8	241.8	0.19	0.89	4.17
75-84 yrs/M	8.2	51.4	180.9	0.12	0.71	2.34
75-84 yrs/F	11.1	45.9	180.6	0.17	0.74	2.74
85 and over/M	7.4	46.0	152.2	0.14	0.71	1.81
85 and over/F	8.2	42.2	161.9	0.15	0.70	2.72
Older people living in institutions (1994/5)						
65-84 yrs/M	18.5	45.7	110.2	0.27	0.68	1.80
65-84 yrs/F	23.8	44.0	116.0	0.35	0.78	2.08
85 and over/M	10.9	45.5	104.1	0.16	0.67	1.55
85 and over/F	11.7	36.5	159.6	0.21	0.65	2.76

⁷ Body weights measured for each subject for all age groups except infants aged 6-12 months where reported body weights were used.

⁸ Intakes for infants aged 6-12 months are from food only

Table 2: Sources of vitamin C in the diet⁹

Food Type	Contribution of food types to average daily intake of vitamin C	
	mg/day	% of total
Cereal and cereal products	3.4	4
Milk and milk products	3.8	5
Egg and egg dishes	0	0
Fat spreads	0	0
Meat and meat products	3.3	4
Fish and fish dishes	0.1	0
Vegetables, potatoes and savoury snacks	25.4	32
- of which potatoes	14.7	19
Fruits and nuts	8.3	11
Sugar, confectionery and preserves	0.2	<1
Beverages	33.9	43
- of which fruit juices	20.7	26
<i>soft drinks</i>	12.7	16
Miscellaneous	0.4	1
Total intake from food	78.8	100
<i>Intake from dietary supplements</i>	5.2	6
Total intake from food and supplements	84.0	100

⁹ NDNS: young people aged 4-18 years. 1997/8. 15-18 year group.

Table 3: Vitamin C intakes from supplements

<i>Age/sex</i>	Consumers of vitamin C supplements		Vitamin C intakes from supplements (consumers only) (mg/day)		
	<i>Number</i>	<i>%</i>	<i>Median</i>	<i>Range</i>	<i>97.5%ile¹</i>
<i>Infants (1986)</i> 6-12 mths/M&F	213	44	14	*	
<i>Pre-school children (1992/3)</i> 1½-4½ yrs/M&F	231	14	21.4	1.5 - 90	50.0
<i>Young people (1997/8)</i> 4-10 yrs/M&F	110	13	25.0	3.6 - 575	237
11-18 yrs/M	27	7	15.0	2.9 - 200	
11-18 yrs/F	27	6	27.9	3.6 - 571	
<i>Adults (1986/7)</i> 16-64 yrs/M	54	5	52.5	0.9 - 1000	950
16-64 yrs/F	91	8	40.0	3.6 - 1500	969
<i>Older people free-living in the community (1994/5)</i>					
65 and over/M	38	6	30.0	2.5 - 1000	
65 and over/F	48	7	43.8	0.1 - 560	
<i>Older people living in institutions (1994/5)</i>					
65 and over/M	5	2	24.2	2.5 - 500	
65 and over/F	10	5	17.5	3.8 - 875	

¹97.5%ile only provided for groups with >50 consumers

* No data available

ANNEX 3 TO EVM/99/21.REVISED SEPT 2001-09-21**Vitamin C: Summary table of selected nutrition information and existing guidance on intakes**

Unit of usage	mg /d		mg /100 kcal	mg /100g
	male	female		
<i>UK DRV¹⁰ for adults (19-50+)</i>				
LRNI	10	10		
EAR	25	25		
RNI	40	40		
Mean adult UK dietary intake <i>From food (all sources)</i>				
Adults (16-64 years) ¹¹	66.5 (74.6)	62.0 (73.1)		
Adults 65 years and over ¹²				
free living	66.9 (71.5)	60.7 (68.1)		
institutionalised	49.6 (52.1)	47.6 (54.9)		
EU labelling RDA ¹³	60mg			
Supplemental doses	60-3000			
Regulations				
Infant formula and Follow-on formula ¹⁴			Minimum – 8mg	Not less than 25mg
Cereal-based baby foods ¹⁵				
Weight reduction ¹⁶ whole daily diet replacement meal replacement			45 15	
<i>Maximum total safe daily intake</i> COMA 1991 ¹	Possible risks of diarrhoea at intakes of g/day			
EHPM 1997 ¹⁷	Upper safe level – 1,000mg Long term consumption No Upper limit established			

¹⁰ Committee on Medical Aspects of Food and Nutrition Policy (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects 41. London: HMSO.

¹¹ Dietary and nutritional survey of British adults. 1986/7

¹² National Diet and Nutrition Survey of people aged 65 years and over. 1994/5

¹³ The Food Labelling Regulations 1996

¹⁴ The Infant Formula and Follow-on Formula Regulations 1995

¹⁵ The Processed Cereal-based Foods and Baby Foods for Infants and Young Children Regulations 1997.

¹⁶ The Foods Intended for Use in Energy Restricted Diets for Weight Reduction Regulations 1997.

¹⁷ Vitamins and Minerals A Scientific Evaluation of the Range of Safe Intakes. European Federation of Health Product Manufacturers 1997.