The Risk Assessment and Safety of Bioactive Substances in Food Supplements
The International Alliance of Dietary/Food Supplement Associations (IADSA) brings together over 50 associations of dietary/food supplement manufacturers and distributors from across the world. IADSA’s central goal is to ensure a greater exchange of information about the science and regulation of dietary supplements and ingredients among scientists, regulators, industry and consumers.

“The Risk Assessment and Safety of Bioactive Substances in Food Supplements” has been researched and drafted by IADSA’s Scientific Group, Dr. John Hathcock, Prof. David Richardson, Dr. Derek Shrimpton and Dr. Hirobumi Ohama, assisted by Dr. Andrew Shao and Sam Jennings BSc (Hons).
The Risk Assessment and Safety of Bioactive Substances in Food Supplements

Dr. John Hathcock PhD, Professor David Richardson, Dr. Andrew Shao PhD, Sam Jennings BSc (Hons)
June 2006
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>Safety methodology for non-essential nutrients</td>
<td>7</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>15</td>
</tr>
<tr>
<td>Chondroitin</td>
<td>21</td>
</tr>
<tr>
<td>Coenzyme Q10 (Ubiquinone)</td>
<td>26</td>
</tr>
<tr>
<td>Lutein</td>
<td>36</td>
</tr>
<tr>
<td>Lycopene</td>
<td>44</td>
</tr>
<tr>
<td>Omega-3</td>
<td>52</td>
</tr>
<tr>
<td>Creatine</td>
<td>62</td>
</tr>
<tr>
<td>Carnitine</td>
<td>72</td>
</tr>
</tbody>
</table>
Introduction

The emerging importance of the bioactive components of food, also known as non-essential nutrients, and their increased use in food supplements is recognised by research institutes, industry and regulatory officials. While many bioactive substances, such as glucosamine, chondroitin, lutein, etc., are generally considered to be non-toxic, there has not been a common approach to evaluating their safety and setting, where necessary, maximum levels for use. In order to fill the needs of regulators and scientific bodies for quantitative safety assessment of such bioactive substances, this report details risk assessments performed on a selection of bioactive substances in food. It describes the method used in detail and sets out the results of its application to a selection of bioactive ingredients.

In their risk assessment of vitamins and minerals, neither the United States of America (USA) nor the European Union (EU) established Upper Level (UL) values for the vitamins and minerals without established adverse effects. As a result, for vitamin B\textsubscript{12} in particular, the absence of a UL has been misinterpreted to indicate that risk assessment is not a useful approach to identifying maximum values\textsuperscript{1}. The Observed Safe Level (OSL) method developed by the Council for Responsible Nutrition (CRN USA) and IADSA in 2004 was intended to fill this methodological gap\textsuperscript{2}. This method was recommended only for those substances that had substantial evidence relating to safety but with no evidence establishing any potential toxicity.

Recently, the Food and Agriculture and World Health Organizations released a report of a workshop titled A Model for Establishing Upper Levels of Intake for Nutrients and Related Substances\textsuperscript{3} (FAO/WHO Technical Workshop on Nutrient Risk Assessment, Geneva, 13 January 2006). This FAO/WHO report recommended a Highest Observed Intake (HOI) approach for substances without recognised adverse effect. The HOI approach to safety evaluation is extremely similar in concept to the OSL method, but the HOI approach was not applied to any specific substance by the FAO/WHO workshop participants.

This report has been developed by members of the IADSA scientific group with the guidance of experts in the bioactive substances covered. It is intended to complement IADSA's earlier publication on the safety of vitamins and minerals\textsuperscript{4} and is the first in a series of risk assessments that it is hoped will provide important guidance to governments and scientific bodies worldwide.

Notes

Observed Safe Levels are not recommended daily intakes but are an indicator of levels of consumption for healthy individuals that should not be exceeded.

Issues such as the purity of ingredients are outside the scope of this report.

The use for medical purposes of any of the nutrients reviewed is outside the scope of this report, which is focused on supplements that are available on free sale.
References


IADSA Safety Methodology for Bioactive Substances

Most of the approaches to upper safe levels of essential and non-essential nutrients are based on widely applicable risk assessment models similar to those used by the Food and Nutrition Board (FNB) in its Dietary Reference Intakes documents published in 1997 and after\(^1\)-\(^5\). The FNB method and reviews are a formalisation and extension of the quantitative methods widely used earlier in risk assessment of other substances, and by the food and supplement industry. Because of the systematic, comprehensive and authoritative character of the FNB risk assessment method for nutrients, this approach has gathered widespread support and adoption by others such as the Scientific Committee on Food (SCF)\(^6\) and the Food Standards Agency’s Expert Group on Vitamins and Minerals (EVM)\(^7\), with some slight modifications. All current methodology emphasises the concept of nutrient specific, quantitative risk assessment, but the difference in the selection and interpretation of the available scientific literature on safety has sometimes resulted in large differences in the safety values for various nutrients, as derived by the FNB, SCF and EVM.

The safety evaluation method underlying this document is that in the updated and expanded *Vitamin and Mineral Safety, 2nd Edition* (by John Hathcock, CRN, Washington, D.C., USA)\(^8\), with the basic features from the methods of the FNB, SCF and EVM. This document, like that of CRN USA, emphasises the direct evaluation of the safety of supplemental intakes of nutrients, rather than total intakes from all sources, where such data are available.
Nutrient-Appropriate Scientific Risk Assessment for Vitamins and Minerals

The term “nutrient-appropriate” used to describe risk assessment for vitamins and minerals and non-essential but beneficial dietary substances indicates that some risk assessment methods are not appropriate. Certain risk assessment methods use default uncertainty factors (sometimes called safety factors) that, although they are generally considered acceptable for identifying safe intakes of food additives and environmental contaminants, are unacceptably large for application in risk assessment of vitamins and minerals. That is, application of these factors leads to identification of “safety limits” that are below the recommended or beneficial daily intakes for some nutrients for certain age-gender groups. For example, the Acceptable Daily Intake (ADI)9 and the Reference Dose (RfD) used by the U.S. Environmental Protection Agency (US EPA)10 involve arbitrary uncertainty factors that calculate "safety limits" for zinc that are below the Recommended Dietary Allowance (RDA) for some groups. Arbitrary factors that reflect an excessive concern for scientific uncertainty, which is always present to one degree or another, can imply that the only “safe” intake is so low as to have no value.
The IADSA Approach to Supplement Safety

Safety evaluation for dietary/food supplement ingredients is properly determined on a case-by-case basis through nutrient-appropriate risk assessment, not as arbitrary multiples of the RDA for essential nutrients, and also not as arbitrarily restrictive limits for the non-essential nutrients.

The IADSA approach to nutrient-appropriate risk assessment requires the safety evaluation to depend on or be identified as:

1. identification of a hazard related to excessive intake, assessment of the dose-response relationship for the identified hazard, consideration of uncertainty, and finally derivation of a supplementation level that is not only safe but includes a reasonable margin of safety; or
2. if no data exist that establish adverse effects in humans, the highest intake level with sufficient scientific evidence of safety at that intake; or
3. if no scientific evidence is available related to safety of high intakes by humans, then animal data may be used with appropriate risk assessment extrapolation, or
4. if no scientific clinical or animal data exist, a compelling pattern of history of safe intake as components of foods commonly consumed by humans.

In the identification of a hazard related to excessive consumption of a nutrient, care must be taken to distinguish between effects that represent a genuine hazard and those that are merely a nuisance. For example, the minor gastrointestinal distress that can occur when supplements are taken on an empty stomach should not be considered equivalent to any risk of a serious effect, such as liver toxicity.

In the dose-responses assessment of each bioactive substance, we noted that the test materials were not necessarily the same from one clinical trial to another and took the identity and quantitative differences into account. These considerations were particularly important in the risk assessments of carnitine, creatine, glucosamine, lutein, lycopene, and the omega-3 fatty acid sources.

Levels for supplements that can be expected to be safe can be identified by either of two related but different approaches. The direct approach, the preferred option, is subject to less uncertainty because it contains fewer steps.
Option 1: Direct Safety Evaluation of Supplemental Intakes:
If appropriate data on supplemental intakes of a specific vitamin or mineral or non-essential nutrient are available, the tolerable upper intake level from supplements (ULS) may be determined directly from those data related to supplemental intakes. If the supplemental intake dose-response relationship is identified from the strongest data and assessed conservatively, no additional uncertainty factor is needed (that is, the implicit UF is 1.0). Option 1, the direct method, identifies the No Adverse Effect Level (NOAEL) and ULS from data related to the use of supplemental amounts of the vitamin or mineral or non-essential nutrients, above and beyond the amounts contributed by the diet, and therefore does not require any additional consideration of amount contributed by consumption of conventional foods. That is, the

- $\text{ULS} = \text{human supplemental intake NOAEL}$ (conservatively selected to justify a UF of 1.0)

OR

Option 2: Indirect or Difference Method for Supplement Safety:
If appropriate data on supplemental intakes of a vitamin or mineral are not available, a difference procedure, similar to that identified by the EU, may be used.

The difference method involves:

- Determination of the tolerable upper intake level (UL) for total intake from all sources
- Identification of the usual intakes from conventional foods (ICF) from appropriate food intake surveys and food composition tables, taking consumption of fortified foods into account, and
- Calculation of the tolerable upper intake from supplements (ULS) as a difference.

That is, $\text{ULS} = \text{UL} - \text{ICF}$.

In contrast to Option 1, this approach identifies NOAEL and UL for total intake of the vitamin or mineral from all sources, including conventional foods and dietary supplements, thereby requiring subtraction of the expected contribution of conventional foods, if this amount is not trivial compared with the NOAEL, in calculation of the ULS for supplemental intake.
**Option 3**: Observed Safe Level:
For some nutrients, without established hazard at high intakes, the toxic potential is so low that there is no credible evidence of adverse effects at any level of intake that has been widely consumed or used in a clinical trial. For such nutrients, the maximum level with sufficient evidence of safety can be identified as an Observed Safe Level (OSL), and this OSL can be used as a ULS. That is,

- $\text{ULS} = \text{OSL}$ (the highest level with convincing evidence of safety, if there are no established adverse effects at any level)

The OSL concept has been incorporated into the report titled *A Model for Establishing Upper Levels of Intake for Nutrients and Related Substances* by the Food and Agriculture Organization/World Health Organization and given the name Highest Observed Intake (HOI) (11). The HOI is described as:

*The highest observed intake (HOI) is derived only when no adverse health effects have been identified. It is the highest level of intake observed or administered as reported within (a) study(ies) of acceptable quality.*

Thus, the OSL and HOI concepts and descriptions are functionally equivalent. With the authority and recognition embodied in the FAO/WHO report, the HOI term is likely to be widely accepted in the future. In this report, however, we continue use of OSL as the primary term to provide continuity in terminology with the earlier CRN and IADSA reports on vitamin and mineral safety.

The FAO/WHO report included the UL method with some slight modifications, careful accounting for intakes from conventional foods, and use of the HOI concept when a UL could not be identified.

For the bioactive non-vitamin and non-mineral substances found in food, the published clinical trials did not adjust the amount of the administered substance in relation to dietary intakes or endogenous biosynthesis. In this circumstance, the total exposures of the subjects in the clinical trials collectively would have included the administered dosages, intakes from conventional foods consistent with the normal ranges of composition and food intake, and a normal range of exposure from endogenous biosynthesis for those substances produced in the human system. Thus, the OSL or HOI identified does not require adjustment for these sources to calculate an acceptable maximum from supplements. Accordingly, the Upper Level for Supplements (ULS) is equivalent to the OSL or HOI:

$$\text{ULS} = \text{OSL}$$
Safety Evaluation Based on Animal Data

If adequate quality and appropriate scientific data from human subjects are not available but animal toxicology data are available, a safe intake level for human consumption may be estimated through extrapolation from the animal data. Because of the uncertainties about the magnitude of uncertainty factors and body size extrapolations to use, IADSA prefers to depend on human data, if available. In some instances, such as lutein and lycopene, human data are adequate to demonstrate safety up to certain intake levels, but extrapolation from well-conducted preclinical toxicology studies with animals suggests much higher safe levels for human consumption. In such instances, IADSA cites the human data OSL and ULS but also cites the UL as extrapolated from animal data, if that extrapolation has been performed by an authoritative group of scientists, such as the Joint Expert Committee on Food Additives (JECFA) or a GRAS Panel (a panel of experts convened to examine whether the substance is “Generally Recognised as Safe” under national or international guidelines).
History of Safe Food Use

If appropriate and sufficient clinical or preclinical toxicology data are not available, a history of safe use may be helpful if the following conditions are met:

- The chemical identity of the supplemental form is the same as that found in foods.
- The intake level, frequency of intake and duration of use are similar to those that occurred through food consumption.
- The population on which the history of use is based had sufficient health care to provide a good chance of observing any adverse effects.
- No established pattern of adverse effects has credibly related to intake of the substance.
References


Glucosamine
Glucosamine is an aminomonosaccharide. It is the principal component of O-linked and N-linked glycosaminoglycans, which form the matrix of all connective tissues, including cartilage. The evidence that orally administered glucosamine compounds may be effective in ameliorating pain due to osteoarthritis has been accumulating for 30 to 40 years\(^1\). Dietary supplements may contain glucosamine hydrochloride, glucosamine sulphate or N-acetyl-glucosamine. Numerous clinical trials have investigated the efficacy of oral glucosamine compounds, often in combination with chondroitin, in individuals with osteoarthritis. Long-term (3-year) clinical trials\(^2,3\) and meta-analysis clinical trials\(^4,5\) of various durations\(^6-11\) support the efficacy and safety of oral glucosamine in osteoarthritis. In general, the evidence suggests that glucosamine, at the range of dosages commonly consumed, is not toxic and produces no recognisable pattern of adverse effects. Most of the data relate to a single intake level, namely 1,500mg, although this is sometimes divided into three or more individual doses. While most studies have been on the sulphate form, a few have used the hydrochloride form. One twelve-week clinical trial involved a daily dose of 2,000mg of glucosamine hydrochloride\(^12\).
Evidence Related to Safety

Human Studies

The publications on the clinical trials of glucosamine for effectiveness in osteoarthritis also contained much useful information relating to safety. None of the clinical trials has found significant patterns of adverse effects related to glucosamine consumption\(^1\)-\(^5\),\(^11\). In the clinical trials of three years’ duration, substantial numbers of several different adverse health events occurred in both the placebo and the treatment groups, but none of the small differences in adverse event frequency approached statistical significance\(^2\),\(^3\). The conclusions from these studies are further supported by the absence of significant adverse effects in other clinical trials\(^6\)-\(^12\). Human clinical trial data have shown no cause for concern about the safety of oral glucosamine at current and plausible intakes\(^13\),\(^14\).

Apparently, due to implications of the name glucosamine rather than any evidence of a causal relationship between glucosamine and diabetes, indicators of diabetes or tests for diabetes, the possible effects on insulin function and glucose metabolism have been investigated, but not always with an appropriate experimental protocol. Infused glucosamine can increase the hexosamine pathway flux, suggesting an adverse effect of this supplement on glucose homeostasis\(^15\). The hexosamine pathway activation leads to deterioration of pancreatic beta-cell function, thereby posing the possibility that glucosamine could enhance the risk of diabetes\(^16\),\(^17\).

Concerns about a possible adverse effect of glucosamine on glucose homeostasis or diabetes have prompted direct evaluation of these endpoints in clinical trials. One clinical trial administered a daily dose of 1500mg glucosamine hydrochloride for 90 days and found no effects on haemoglobin A\(_{1c}\) concentrations in diabetic subjects\(^18\), and another found no effects of 1500mg of glucosamine sulphate per day on blood glucose or serum insulin in normal volunteers after 12 weeks\(^19\). Thorough review of the evidence on this relation of glucosamine to glucose metabolism and function reveals no adverse effects\(^20\),\(^21\). Thus, concerns about a possible diabetogenic effect of glucosamine that arose from biochemical studies have been investigated in clinical trials, and the human data directly demonstrate that this effect does not occur in normal or diabetic subjects who consume 1500mg of glucosamine per day during 12 weeks of exposure. Because of the small size of the clinical trials involved, the possibility of an effect in sensitive individuals cannot be excluded.

The highest glucosamine dosage utilised in a double-blind, placebo-controlled, randomised clinical trial was 2,000mg of glucosamine hydrochloride per day for 12 weeks in subjects with osteoarthritis of the knee\(^12\). Subjects (24 assigned to glucosamine and 22 to placebo) were monitored for the side effects of nausea/vomiting, gastrointestinal upset/cramps, headache, bloating, dry mouth and tenderness in the knee. The total side effects reported were not significantly different, with 11 among the 24 subjects in the glucosamine group and 10 among the 22 placebo controls, with no significant differences in any category.
The National Institutes of Health (NIH)-sponsored glucosamine/chondroitin arthritis intervention trial (GAIT) involved nearly 1500 osteoarthritis patients who ingested 1500mg per day glucosamine hydrochloride, 1200mg per day chondroitin sulphate, the combination of the two, 200mg per day of the prescription pain medication Celebrex™ or placebo for twenty four weeks. Adverse effects were closely monitored throughout the study period. A total of 634 patients were exposed to glucosamine hydrochloride. Results showed no significant difference in the incidence of adverse effects between any of the treatment arms.

The glucosamine fraction of total weight is higher with the hydrochloride than with the sulphate; the bioavailability of both forms exceeds 90 percent, with glucosamine hydrochloride approaching 100 percent. With these chemical differences and bioavailability similarities, the safety conclusions reached for hydrochloride can be appropriately extrapolated to the sulphate.

Animal and In Vitro Studies
The large number of animal and in vitro studies addressing the safety as well as the metabolism and metabolic effects of glucosamine have been reviewed in detail. The LD50 of glucosamine hydrochloride is greater than 5000mg/kg, and the NOAEL is 2700mg/kg in rats and 2149mg/kg in dogs. Assuming a 60kg adult body weight, the 1500mg daily dose in humans amounts to 25mg/kg, and the 2000mg dose equals 33mg/kg. Thus, extrapolation of the extensive data obtained included in the animal and in vitro toxicology studies suggests that none of these effects is likely in humans.
Risk Assessment

NOAEL or LOAEL
None of the clinical trials found adverse effects related to glucosamine administration, and therefore there is, by definition, no basis for identifying a LOAEL. In the absence of a LOAEL, a NOAEL is not usually set. Without either of these two values the establishment of a UL is not appropriate.

OSL
The glucosamine dosage that was utilised in most clinical trials is 1500mg/day. The single clinical trial that used 2000mg of glucosamine hydrochloride found no adverse effects. There are ample data to identify 1500mg of glucosamine sulphate as the OSL. The absence of adverse effects in clinical trial at 2000mg of glucosamine hydrochloride, together with the huge margins of safety indicated by animal studies and the direct evidence against a diabetogenic effect in humans is sufficient grounds for setting the OSL at 2,000mg of glucosamine hydrochloride. Further, the differences in glucosamine content and bioavailability allow this value 2000mg OSL to be applied to glucosamine sulphate as well.

In one placebo-controlled double-blind randomised clinical trial of glucosamine hydrochloride (1500mg) in combination with chondroitin sulphate (1200mg), two subjects in the active group had allergic responses, compared with none in the placebo group. The combined treatment prevents attribution of the effect to a specific ingredient, but allergic responses related to glucosamine of shellfish origin have been reported previously. Many supplement products containing glucosamine from this source carry an allergy warning statement. Glucosamine derived from plant sources would not need such warnings.

Identification of 2000mg/day as the OSL for oral consumption of glucosamine (either the hydrochloride or the sulphate) carries no significant uncertainty, due to the confidence gained from extreme safety in animal and in vitro tests.

The subjects in the clinical trials would have been consuming little to no glucosamine in their diet, and therefore the quantities of glucosamine added in clinical trials discussed were supplemental amounts well above the very small amount in the foods consumed. Therefore, this risk assessment represents a direct approach to ULS. No correction is needed for the glucosamine in the food supply. Therefore, the OSL for glucosamine is set at 2000mg and is identified as the ULS. Allergy warnings are appropriate only for products including glucosamine of shellfish origin.

NOAEL and LOAEL: No toxicological basis
OSL: 2000mg glucosamine compound (hydrochloride or sulphate)
ULS: 2000mg per day
References
Chondroitin
Chondroitin

Background

Chondroitin sulfate is an essential part of a large protein molecule (proteoglycan) that gives cartilage elasticity. The concept that orally administered chondroitin sulphate, along with glucosamine, might slow the process of osteoarthritis has been around for decades. Numerous clinical trials have investigated the efficacy of oral chondroitin and/or glucosamine in individuals with osteoarthritis. Meta-analysis supports the efficacy\textsuperscript{1,2}. In general, the evidence suggests that chondroitin sulphate, at the range of dosages commonly consumed, is not toxic and produces no recognisable pattern of adverse effects. Most of the data relate to single intake levels, and no systematic study of the dose-response relationship has been conducted.
Evidence Related to Safety

Human Studies

Several clinical trials have involved the oral administration of chondroitin sulphate\textsuperscript{1-7}. The age, health conditions, dosage, duration and monitoring and evaluations methods have differed greatly. For confidence in the results, those studies with stronger designs carry more weight regarding a conclusion of safety at that dosage. In a risk assessment, the studies with strong designs and involving higher dosages deserve greater weight in identifying the highest dosage that can be confidently concluded to carry no identifiable risk of adverse effects.

The highest oral chondroitin sulphate dosage administered in the published clinical trials is 1,200mg per day\textsuperscript{2,4,7}. Other trials have utilised dosages of 1,000mg\textsuperscript{3} and 800mg\textsuperscript{1,5,6}. The duration of study varied from 3 months to 3 years, and the number of subjects from 12 to 635. Similarly, the clinical monitoring that could have detected adverse effects ranged from sparse to extensive (e.g., self-reports of possible adverse effect to clinical evaluation combined with extensive haematological and clinical chemistry indices). None of these clinical trials found any significant adverse effects.

The clinical trial by Verbruggen and coworkers\textsuperscript{4} is especially convincing regarding the safety of oral chondroitin sulphate. It involved 165 subjects treated for 3 years with an oral dose of 1,200mg per day. The monitoring included examination by three physicians. The only adverse effect reported was a single case of gastritis in one chondroitin-treated subject. The subject withdrawals were fewer among those treated with chondroitin sulphate compared with placebo-treated controls. These results are consistent with the most recent trial conducted on chondroitin sulphate. The NIH-sponsored glucosamine/chondroitin arthritis intervention trial (GAIT) involved nearly 1500 osteoarthritis patients who ingested 1500mg/day glucosamine hydrochloride, 1200mg/day chondroitin sulphate, the combination of the two, 200mg/day of the prescription pain medication Celebrex\textsuperscript{TM} or placebo for twenty four weeks\textsuperscript{7}. Adverse effects were closely monitored throughout the study period. A total of 635 patients were exposed to chondroitin sulphate. Results showed no significant difference in the incidence of adverse effects between any of the treatment arms. None of the clinical trials found any clinical chemistry (blood and urine) or haematological effects of oral chondroitin sulphate.

The clinical trial evidence has been the subject of four published meta-analyses\textsuperscript{8-11} and one review/commentary\textsuperscript{12}. These publications focused primarily on the benefits of oral chondroitin sulphate in limiting the progression of osteoarthritis, but they also have relevance to the safety of this ingredient. The meta-analyses also support the safety of oral chondroitin at 1,200mg/day, the highest intake systematically studied.
Risk Assessment

**NOAEL or LOAEL**
The absence of adverse effects at any of the dosages in the clinical trials does not support identification of a LOAEL or NOAEL. The evidence indicates no adverse effect of 1,200mg of oral chondroitin per day, but does not suggest at what dosage adverse effects might occur. Therefore there is, by definition, no basis for identifying a LOAEL. In the absence of a LOAEL, a NOAEL is not usually set. Without either of these two values the establishment of a UL is not appropriate.

**OSL**
The highest chondroitin sulphate dosage that has been utilised in clinical trials is 1,200mg/day. There are sufficient data at this level to identify it as the OSL. The nearly complete absence of any adverse effects of chondroitin sulphate within the range of the clinical trials reviewed (800 to 1,200mg/day) suggest that the highest level, 1,200mg/day, is not a true NOAEL and that any LOAEL is likely to be much higher. The single case of gastritis among hundreds of subjects treated suggests that this one case is not causally related to chondroitin sulphate, or that the individual had a very unusual sensitivity. Thus, the single case of gastritis should not influence the outcome of the risk assessment.

The subjects in the clinical trials most probably consumed little to no chondroitin in their diets, and therefore the quantities of chondroitin discussed were supplemental amounts well above that which could be obtained from dietary sources. Therefore, this risk assessment represents a direct approach to a ULS. No correction is needed for the chondroitin sulphate in the food supply.

Identification of 1,200mg/day as the OSL for oral consumption of chondroitin sulphate up to three years by adults carries little uncertainty - there are no known adverse effects to be avoided. Therefore, the OSL of 1,200mg is identified as the ULS.

**Animal Studies**
Because the human clinical trial data are judged sufficient to support identification of an OSL value, the data from animal experiments were not reviewed.

**NOAEL and LOAEL:** No toxicological basis

**OSL:** 1200mg chondroitin sulphate

**ULS:** 1200mg per day
References


Coenzyme Q10
(Ubiquinone)
Background

Coenzyme Q is a biosynthesised quinine structure that occurs widely in living organisms such as yeasts, plants and animals, and hence is also known as ubiquinone (ubiquitously occurring quinone)\(^1\). In higher organisms, including humans, this compound has 10 isoprenoid units in the side chain and is named coenzyme Q10 (CoQ10)\(^2\).

CoQ10 has two major physiological activities: (a) mitochondrial electron-transport activity necessary for the efficient production of high-energy phosphates necessary for muscle contraction and other functions\(^3\), and (b) an antioxidant activity\(^4\). The antioxidant activity results only with the reduced form (ubiquinol), which is produced physiologically from CoQ10. The mitochondrial electron-transport function to provide the energy for muscle function or the antioxidant activity may account for the reported benefits in persons with acute myocardial infarction\(^5\). The antioxidant activity or improved immune function may relate to evidence of anti-cancer activity by CoQ10\(^6\).

CoQ10 is not recognised as a vitamin\(^7\) but has some vitamin-like qualities, including benefits under some circumstances when ingested even though the small quantities that are synthesised in the tissues\(^8\). The potential benefits of oral intake have led to extensive interest in the health benefits of CoQ10. Its widespread use in dietary supplements gives reason to evaluate the safety of CoQ10 through quantitative risk assessment.

Most upper safe levels of nutrients and related substances are based on widely applicable risk assessment models based on that published by the US Food and Nutrition Board (FNB) in its Dietary Reference Intakes documents in 1997 and after\(^7,9-12\). The FNB method and reviews are a formalisation and extension of the quantitative methods widely used earlier in risk assessment of other substances, and by the food and supplement industry. Because of the systematic, comprehensive and authoritative character of the FNB risk assessment method for nutrients, this approach has gathered widespread support and adoption by others such as the European Commission Scientific Committee on Food (SCF)\(^13\), the United Kingdom Expert Group on Vitamins and Minerals (EVM)\(^14\) and more recently by the Food and Agriculture Organization/World Health Organization project report *A Model for Establishing Upper Levels of Intake for Nutrients and Related Substances*\(^15\) with some slight modifications. All these reports reflect the concepts and procedures established much earlier for the risk assessment of non-carcinogenic chemicals\(^16\).
Evidence Related to Safety

Clinical Trial Data
Several double-blind, placebo-controlled clinical trials have found no systematic pattern of adverse effects of CoQ10 in dosages of 2,400mg/day in Parkinson's Disease patients\textsuperscript{18}, 1,200mg/day in early Parkinson's Disease patients\textsuperscript{19}, 900mg/day in healthy adults\textsuperscript{20}, 600mg/day in Parkinson's Disease patients\textsuperscript{21,22} and in a number of trials at dosages from 390mg/day down to 100mg/day in subjects who were healthy or had a variety of disease conditions\textsuperscript{4-6,23-67}. The size, duration, observations made and the overall quality and power of these studies varied considerably. Considered together, they provide strong evidence that there is no consistent pattern or incidence of nausea, related gastrointestinal effect or other adverse effect of CoQ10 over a period of up to a few months. Higher doses up to 3,000mg/day have been administered without adverse effects in three small groups of patients without control groups\textsuperscript{18,68,69}. The largest of these three trials\textsuperscript{69} provides substantial but not conclusive evidence of safety at an oral intake of 3,000mg/day.

Some clinical trials with CoQ10 administration found CoQ10 treatment possibly to produce nausea, heartburn, upset stomach or related effects at dosages of 1,200mg/day\textsuperscript{70} and 600mg day in Huntington’s disease patients\textsuperscript{71} and 600mg/day in myocardial infarction patients\textsuperscript{72}. Similar effects have been observed at dosages of 180mg/day (specifically 1.5mg/kg) in patients with stable angina pectoris\textsuperscript{73}, at 150mg/day in heart failure patients\textsuperscript{74,75}, at 120mg/day in acute myocardial infarction patients\textsuperscript{5,76} and as low as 60mg/day in oligospermia subjects\textsuperscript{77}.

The incidence of stomach upset with CoQ10 was similar that with placebo in one study at 120mg dosage\textsuperscript{35} but greater with the same dosage of CoQ10 in another\textsuperscript{76}. In a 16-month clinical trial involving 80 subjects, much higher doses of CoQ10 (300 to 1,200mg/day) did not produce significant nausea or other adverse effects\textsuperscript{19}.

Neither the incidence nor the severity of nausea increases with the dosage. No adverse impacts other than these mild and transient gastrointestinal effects have been reported. The recurrent pattern of nausea and related effects suggest causality by some component of the administered capsules, but there is no dose-response relationship to the CoQ10 content. Nausea has been reported in clinical trials with CoQ10 at dosages of 60, 120, 150, 180, 600 and 1,200mg/day, but its incidence and severity in the placebo group was just as great as in the treated group in most of the trials. No nausea or related gastrointestinal effect was reported in a much larger number of clinical trials in a dosage range up to 3,000mg/day, although one uncontrolled trial at 3,000mg/day was only 10 days and involved a small number of subjects\textsuperscript{18} and the other uncontrolled trial at 3,000mg/day involved long-term administration but had no control group\textsuperscript{68}. 

Coenzyme Q10 (Ubiquinone)
The absence of a dose-response relationship between CoQ10 and nausea strongly suggests that the capsule or oil vehicle, and not the CoQ10 itself, may have been responsible for the nausea effect. Also, concomitant pain in some of the disease conditions in some trial subjects, especially angina and myocardial infarction, might have predisposed toward the occurrence of nausea.

A possible adverse effect of oral CoQ10 would be a decreased endogenous biosynthesis and decreased blood and tissue levels resulting in a “rebound” deficiency if the oral supply should be discontinued. There is no scientific evidence to support this hypothetical concern, and there is significant evidence, in both humans and animals, that this mechanism does not present a significant problem. A well conducted human trial with oral intakes up to 900mg/day included a multiple-month follow-up period that provided direct evidence that rebound deficiency did not occur\textsuperscript{20}. More detailed metabolic evidence is available showing that endogenous synthesis of CoQ9 in rats is not decreased by an oral intake of 103mg/kg\textsuperscript{78} or 20mg/kg\textsuperscript{79}. Overall, the scientific evidence strongly indicates that conditioned, rebound deficiency is not a problem with CoQ10.

In conclusion, the clinical trial database shows no adverse effects causally or plausibly related to CoQ10. Any effects that have occurred with CoQ10 also occurred with similar frequency in the placebo-group subjects in the same clinical trials.
Human NOAEL or OSL (HOI)

No adverse effect causally related to CoQ10 consumption by humans has been identified, and thus a NOAEL (or LOAEL) cannot be identified, and a UL cannot be derived. The dosages used in clinical trials are evaluated for adequacy to establish with confidence a lack of adverse effect at that level of CoQ10 intake. Therefore, the clinical trial data were evaluated to identify an OSL (termed HOI in the FAO/WHO report).

3,000 mg/day
This level of CoQ10 intake was evaluated in an open-label study in 31 ALS patients, who were evaluated with an extensive series of clinical and laboratory indices that might have shown any adverse effects, but the open-label design of this trial limits the confidence in the finding of no adverse effects. Other data at this dosage level come from case reports on two groups of six subjects each. The lack of control groups and the small number of subjects precludes use of these data to identify a low uncertainty OSL for healthy adults.

2,400 mg/day
This level of CoQ10 intake was administered to 16 Parkinson’s Disease patients for 8 weeks without any adverse effect. The short duration and small cohort increase the uncertainty in application of these data to healthy adults, although the data are consistent with this level being an OSL. Selection of this level as an OSL would require application of a UF greater than unity to calculate a UL and ULS. The size of a UF for this calculation is not apparent from these data. These limitations do not allow the identification of this level of intake as a high-confidence OSL with no correction for uncertainty, i.e., allow a value of unity (1.0) to be assigned to the uncertainty.

1,200 mg/day
This level has been studied in a relatively large trial study (n = 80) of early Parkinson’s Disease patients in a strongly designed clinical trial of 16 months duration that found no adverse effect. Another small clinical trial (n = 10) of six months’ duration at this intake resulted in “heartburn”, possibly related to this level of CoQ10 administration. Because there is no consistent pattern of this or related adverse effects with a dose-response relationship, this finding is judged not to establish an adverse effect of CoQ10 at this level of intake. The 16-month trial provides strong evidence of the lack of an adverse effect of this level of CoQ10. There is no known mechanism that suggests that Huntington's Disease or early Parkinson's Disease patients would be less susceptible to any adverse effect of CoQ10, and therefore these data are judged appropriate to establish an OSL.

900 mg/day
The absence of adverse effects in a well conducted clinical trial in a healthy cohort (n = 88) over a four-week period is completely consistent with and supports the OSL of 1,200mg/day identified above. This trial found no effects of CoQ10 on a spectrum of safety indices.
600 mg/day and lower intakes

The only adverse effect reported (nausea) with CoQ10 intake has no apparent dose-response relationship, which suggests that it is not causally related. Collectively, this large number of clinical trials provides strong evidence that is consistent with the OSL identified. Nothing in these reports contradicts the OSL identified.
Coenzyme Q10 (Ubiquinone)

Risk Assessment

NOAEL and LOAEL
No toxicological basis was found.

OSL
The OSL identified came from a clinical trial with a substantial cohort of 80 and fairly long duration of 16 months\(^{18}\) and a shorter, smaller clinical trial\(^{70}\). Plasma levels of CoQ10 in subjects consuming 3,000mg/day reach a plateau by 4 months\(^{69}\), indicating that a bioaccumulation effect is not likely to precipitate chronic toxicity. Animal data\(^{78}\) and human data\(^{79}\) indicate that rebound deficiency after cessation of high CoQ10 intake is not a problem. The complete absence of a significant pattern of adverse effects that are causally related to CoQ10 at higher and lower intakes provides confidence in the extrapolation of the data from Parkinson’s Disease patients to healthy adults.

The OSL was identified from data on subjects consuming a variety of diets and having \textit{in vivo} synthesis of CoQ10. Thus, these additional sources of CoQ10 are already considered and do not need to be subtracted from the OSL to identify a ULS. Thus, a ULS based on the toxicological evidence in human clinical trials is 1,200mg CoQ10 per day.

NOAEL and LOAEL: No toxicological basis
OSL: 1200 mg per day
ULS: 1200 mg per day
References

Coenzyme Q10 (Ubiquinone)


71 Study Group Huntington, “Randomized, Placebo-Controlled Trial of Coenzyme Q10 and Remacemide in Huntington’s Disease,” Neurology 57, 397-404 (2001).
Lutein
Background

Lutein, and its stereo isomer zeaxanthin, are lipid-soluble members of the xanthophyll family of carotenoids. Lutein, the second most prevalent carotenoid in human serum, is concentrated in ocular tissues such as the lens and the macula lutea, and is present in the human diet primarily in dark, leafy green vegetables. Known mostly for its role in eye health, consumption and serum levels of lutein have been shown to be inversely related to the risk for ocular diseases, including age-related macular degeneration (AMD) and cataracts. Findings from a growing collection of placebo-controlled intervention trials indicate that ingestion of lutein-containing foods or supplements results in increased macular pigment optical density, and may help to improve visual function in patients suffering from AMD and other ocular diseases.
Evidence Related to Safety

Human Studies

There have been more than thirty peer-reviewed, published human clinical trials involving lutein. Of these, the ten most relevant studies regarding safety are presented in this review. Criteria for study inclusion were study duration (at least one week) and lutein dose utilised (greater than 2mg/day). Studies investigating acute bioavailability, pharmacokinetics or post-prandial responses from single bolus doses were excluded from this analysis, and are used solely as supportive information. Also excluded were studies that did not quantify the dosage of lutein being administered (such as feeding studies). Human trial data are limited to the use of all-trans lutein, both in the “free” form and in the fatty acid esterified form. Only a few of the studies undertaken to assess the beneficial effects of lutein have monitored any possible adverse side effects, and then primarily through self-reporting. There are no published human studies that have focused specifically on the safety of lutein supplementation.

The highest lutein dosage utilised in a human clinical trial was 40mg/d for nine weeks (63 days) followed by an additional 17 weeks (119 days) at 20mg/day in retinitis pigmentosa patients. The longest duration trial was 12 months (365 days) at a lutein dose of 10mg/day in AMD patients. Where measured, serum lutein levels increased in a dose-dependent manner. No adverse effects were observed in any of the reviewed studies.

Mean level of lutein consumption from foods in the U.S. is estimated at less than 2mg/day, suggesting that the doses used in the reviewed trials (5 to 20-fold higher than what is typically consumed in the diet) are adequate to assess safety of supplementation. The absence of any pattern of adverse effects related to lutein consumption in any of the published human trials provides support for a high level of confidence in the safety of this compound.

Concerns that excess lutein might contribute to vitamin A toxicity are unfounded, as it is not a substrate for the 15,15-monooxygenase enzyme that cleaves β-carotene into vitamin A, and therefore it possesses no pro-vitamin A activity. Thus, since lutein does not serve as a source of vitamin A for the body, it is unlikely to exert adverse effects analogous to vitamin A toxicity. Concerns about a link to lung cancer also do not apply to lutein, as the mode of that effect of β-carotene appears to be related to pro-vitamin A activity. There are reports in the literature of extremely high carotenoid doses resulting in the precipitation of crystals in the retina of exposed monkeys. However, these have not involved lutein and are limited to a carotenoid (canthaxanthin) with chemical and physical characteristics distinct from that of lutein. The only documented side effect of lutein supplementation is carotenodermia, a condition characterised by a yellowish discoloration of the skin resulting from elevated serum carotenoid levels. The condition is most often associated with high β-carotene intake from foods or supplements (>30 mg/day), and has been reported in only two clinical trials involving lutein. The U.S. Institute of Medicine recognises carotenodermia as a harmless biological effect of high carotenoid intake.
Animal and In Vitro Studies

Animal and in vitro studies addressing the safety as well as the metabolism and metabolic effects of lutein have been reviewed in detail\(^1\). The LD\(_{50}\) of all trans lutein has not yet been formally identified, but rat studies using doses ranging from 35mg/day\(^3\) up to 639mg/kg/day\(^3\) with no associated toxicity are described in the literature. The results of teratogenicity and mutagenicity studies conducted also showed no irreversible adverse effects at comparable dosages. Assuming a 60kg adult body weight, a 10mg daily dose in humans amounts to 170µg/kg, and a 40mg dose equals 670µg/kg. These doses appear to be infinitesimally small in comparison to those used in the toxicity studies, which themselves did not cause adverse effects.
Risk Assessment

**Human NOAEL**
None of the clinical trials found an adverse effect related to lutein administration and therefore there is, by definition, no basis for identifying a LOAEL. In the absence of a LOAEL, a NOAEL is not usually set. Without either of these two values the establishment of a UL is not usually set.

**Human OSL**
Published relevant human clinical trials involved lutein doses of 8, 10, 12, 15, 20, 20.5 and 40mg/day\(^{13,16,27,28,33-38}\). All human studies reviewed, with the exception of that by Dagnelie et al.\(^{16}\), are double-blind, randomised, controlled trials. A series of non-randomised, open-label clinical trials has also been published. The dosages involved in these studies range from 2.4mg/day to 30mg/day, and the results are consistent with respect to safety, showing no observed or reported adverse effects\(^{14,39-41}\).

Although the absence of adverse effects with 40mg/day in the study by Dagnelie et al.\(^{16}\) suggests this dose is appropriate for setting the OSL, the lack of a control group argues against use of these data to identify an OSL for healthy adults. The next highest dose tested in a published, double-blind, placebo-controlled trial is at 20mg/day for 180 days in 29 adults\(^{35}\). Subjects receiving this level of lutein experienced a 4.6-fold increase in serum lutein (to 1062.5nmol/L), with no adverse effects. The complete absence of adverse effects in all the published human trials using lutein doses above, at and below the 20mg level provides sufficient support for this value to be confidently designated as the OSL.

Populations exist in which the habitual consumption of lutein and other carotenoids is extremely high: in the Fiji Islands the daily consumption of dark green leafy vegetables exceeds 200g, providing nearly 25mg of lutein\(^{42}\). The quantities of lutein involved in these trials are supplemental amounts well above the average amount consumed in foods in, for example, the U.S. (less than 2mg/day\(^{19}\)). Therefore, this risk assessment represents a direct approach to ULS.
Uncertainty Evaluation

The highest lutein dose from published animal toxicity studies is reported by Kruger et al. A dose of 639mg/kg/day in rats for four weeks caused no adverse effects. This equates to approximately 38,000mg in a healthy 60kg adult. Application of a 1000-fold uncertainty factor (UF) would result in a ULS of 38mg.

NOAEL and LOAEL: >40mg all trans lutein

OSL: 20mg all trans lutein

ULS: 38mg all trans lutein based on extrapolation from animal data, or 20mg/day based on randomised, controlled human trials.
References


Lycopene
Background

Of the 600 or so carotenoids that exist in nature, only a fraction are consumed in the diet, and of these only a handful are present in human serum. Lycopene has no oxygen atoms and is therefore a member of the hydrocarbon family of carotenoids\(^1\). It is also the most prevalent carotenoid in human serum\(^2\). Lycopene is present in the human diet primarily in dark red fruits and vegetables such as tomatoes\(^3\), and consumption and serum levels of lycopene have been linked to a reduced risk of cardiovascular disease\(^4,5\) and prostate cancer\(^6,7\). Findings from a growing collection of placebo-controlled intervention trials suggests that consumption of lycopene (either as a dietary supplement or in the form of processed tomatoes) can reduce DNA damage\(^8,9\) and may have beneficial effects on prostate cancer\(^10-13\).
Evidence Related to Safety

**Human Studies**

There have been more than thirty peer-reviewed, published human intervention trials involving various forms of lycopene. Of these, the sixteen most relevant studies regarding safety are presented in this review. Criteria for study inclusion were study duration (at least one week), lycopene dose utilised (greater than 8mg/day), and studies had to be randomised, placebo-controlled intervention trials. Studies that were uncontrolled and unblinded, those investigating acute bioavailability, pharmacokinetics or post-prandial responses from single bolus doses were excluded from this analysis, and are used solely as supportive information. Also excluded were studies that did not quantify the dosage of lycopene being administered (such as certain feeding studies). Lycopene exists in nature in several isomeric forms, with the majority of both natural and synthetic sources of supplemental lycopene being in the trans form. For the purposes of this review, human trial data are limited to the use of total or trans lycopene, as specified. Only a few of the studies undertaken to assess the beneficial effects of lycopene have monitored any possible adverse side effects, and then primarily through self-reporting. There are no human studies that have focused specifically on the safety issues of lycopene supplementation. Two comprehensive reviews have been published examining the safety of natural and synthetic sources of supplemental lycopene. Both reviews concluded, based on the available human and animal toxicity data, that there is no indication of any significant adverse effects of lycopene.

The highest lycopene dosage utilised in a randomised, controlled human clinical trial was 150mg/day for seven days. The longest duration trial was 20 weeks (140 days) at a lycopene dose of 13.3mg/day in healthy adults. Due to differences in study duration and variations in bioavailability from different lycopene sources, serum lycopene levels did not appear to follow a consistent dose-dependent response. Although supplemental forms of lycopene derived from synthetic and natural sources appear to have comparable bioavailability, this is not the case with other dietary lycopene sources, such as raw or processed tomatoes or tomato juice. Other potential confounders include the inconsistencies in the amount of time allowed for depletion and/or wash-out phases, which varied considerably between studies, and the baseline lycopene status of the subjects being tested. However, irrespective of these inter-study inconsistencies, there were no adverse effects observed or reported at any intake in any of the reviewed studies.

Mean level of lycopene consumption from food in, for example, the U.S., has been estimated at just over 8mg/day, suggesting that the doses used in the reviewed trials (up to many times higher than what is typically consumed in the diet) are adequate to assess safety of supplementation. The absence of any pattern of adverse effects related to lycopene consumption in any of the published human trials provides support for a high level of confidence in the safety of this compound.
Since lycopene possesses no pro-vitamin A activity, there is no concern for vitamin A toxicity. There are reports in the literature of extremely high carotenoid doses resulting in the precipitation of crystals in the retina of exposed monkeys\textsuperscript{21,22}. However, these have not involved lycopene and are limited to canthaxanthin, a xanthophyll with chemical and physical characteristics distinct from that of lycopene. The only documented side effect of lycopene supplementation is carotenodermia, a condition characterised by a yellowish discoloration of the skin resulting from elevated serum levels. The condition is most often associated with high β-carotene intake from foods or supplements (\textgreater{}30mg/day)\textsuperscript{23}, and has been reported in only a few instances involving lycopene\textsuperscript{18,24,25}. The U.S. Institute of Medicine recognises carotenodermia as a harmless biological effect of high carotenoid intake\textsuperscript{2}.

\textbf{Animal and In Vitro Studies}

Animal and \textit{in vitro} studies addressing the safety as well as the metabolism and metabolic effects of lycopene have been reviewed in detail\textsuperscript{15,16}. For natural lycopene (derived from tomato oleoresin), the LD\textsubscript{50} in rats was not established but was determined to be more than 5000mg/kg body weight, and the NOAEL established to be more than 4500mg/kg body weight (based on a 13 week study)\textsuperscript{15}. For synthetic lycopene, no adverse effects were detected up to the highest dose of 1000mg/kg body weight for four weeks or 500mg/kg body weight for 13 weeks\textsuperscript{16}. The results of teratogenicity and mutagenicity studies conducted on both lycopene forms also showed no irreversible adverse effects at comparable dosages. Such a large margin of safety from animal studies provides a high level of confidence that supplemental lycopene, regardless of the form, can be consumed safely at relatively high doses by humans.
Risk Assessment

**Human NOAEL**
None of the clinical trials found an adverse effect related to lycopene administration and therefore there is, by definition, no basis for identifying an LOAEL. In the absence of an LOAEL, a NOAEL is not usually set. Without either of these two values the establishment of a UL is not usually set \(^{26}\).

**Human OSL**
Published relevant human clinical trials involved lycopene doses of up to 150mg/day \(^{17}\). All human studies reviewed were double-blind, randomised, controlled trials. A series of non-randomised, open-label clinical trials has also been published. The dosages involved in these studies range from 20 to 37mg/day, the results of which are consistent with respect to safety, showing no observed or reported adverse effects \(^{13,20,27,28}\).

Although the absence of adverse effects with 150mg/day in the study by Rao and Agarwal \(^{17}\) suggests this dose is appropriate for setting the OSL, the relatively short duration (1 week) and small sample size (n = 20) argue against use of this study for identification of an OSL for healthy adults.

The next highest dose tested in a published, double-blind, placebo-controlled trial is at 75mg/day for 4 weeks (28 days) in 15 healthy adults \(^{29,30}\). Subjects receiving lycopene experienced a significant 41% increase in serum lycopene and significant 2-fold increase in buccal mucosa cell lycopene level, with no adverse effects reported. The longer duration and the combined result of no adverse effects from this study and that of Rao and Agarwal \(^{17}\), which also implemented a 75mg/day dose, provide sufficient support for the designation of this study as the basis for the human OSL of 75mg/day.

The remainder of the reviewed trials include lycopene doses ranging from 12 to 47mg/day for durations ranging from 1 to 20 weeks \(^{8-11,14,18,31-39}\). The complete absence of adverse effects in all the published human trials using lycopene doses above, equal to and below the 75mg level provides sufficient support for this value to be confidently designated as the OSL.

The quantities of lycopene involved in these trials are supplemental amounts well above the estimated average amount consumed in foods consumed in, for example, the U.S. (8mg/day \(^{15}\)). Therefore, this risk assessment represents a direct approach to ULS.
Uncertainty Evaluation

The highest lycopene dose from published animal toxicity studies is reported by Matulka et al. using lycopene derived from tomato oleoresin\textsuperscript{15}. An acute dose of 5000mg/kg in rats failed to produce any clinical signs of toxicity or death and a subchronic dose of up to 4500mg/kg body weight per day for 13 weeks also caused no adverse effects. This NOAEL in rats equates to approximately 270g/day in a healthy 60kg adult. Application of a 1000-fold uncertainty factor (UF) would result in a ULS of 270mg.

**NOAEL and LOAEL:** >150mg lycopene

**OSL:** 75mg lycopene

**ULS:** 270mg per day lycopene based on extrapolation from animal data
References


Omega-3
Background

According to the definition by Gunstone\(^1\), omega-3 fatty acids (n-3 polyunsaturated fatty acids, n-3 PUFA) are a family of polyenoic acids with three or more \textit{cis}-unsaturated centres separated from each other by one methylene group, and with the first unsaturated centre being three carbon/atoms from the end methyl group.

There are two categories within the omega-3 fatty acids\(^2\): the first contains C18:3 (\(\alpha\)-linolenic acid, ALA); and the second category, derived from ALA by chain-elongation and desaturation, contains the long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA). The two most commonly studied of these compounds are C20:5 (eicosopentaenoic acid, EPA) and C22:6 (docosahexaenoic acid, DHA), both of which are found in fish oils and, in the case of DHA, some marine algae. Further members of the group include C22:5 (docosapentaenoic acid, DPA), also abundant in fish oils, and 18:4(n-3) (stearidonic acid) and 16:4(n-3) (hexadecatetraenoic acid), both found in some edible marine algae\(^3\).

For the purposes of this report, the term ‘omega-3’ will be used to describe the two most commonly studied LC n-3 PUFA, namely EPA and DHA. These two fatty acids are the most abundant LC n-3 PUFA found in fish oil, usually comprising over 50% of the n-3 fatty acids in unmodified fish oils. They are believed to be the fatty acids responsible for the beneficial effects of fish oil on cardiovascular health, and hence are of the most interest commercially in supplements. In recent years companies have investigated alternative production procedures, modification and restructuring of the fatty acids to increase the ratio of omega-3 in their oils, and different processes have been developed to produce ‘high omega-3’ oils.

Omega-3 are primarily sourced from fish oils but, due to the extremely high demand of these fatty acids, research is now being undertaken into the possible use of alternative sources such as edible marine algae and the production of omega-3 oils using genetically modified higher plants\(^4\).

Omega-3 are almost completely absorbed from the diet and are either oxidised, incorporated into tissue lipids or converted to eicosanoids\(^2\). Reported beneficial effects of omega-3 in humans include improving lipid profiles, lowering blood pressure, reducing platelet aggregation, anti-inflammatory and immunological effects and reducing aggression as well as beneficial effects on some other psychiatric disorders.
Evidence Related to Safety

Human Studies
Numerous studies have been undertaken to assess the apparent beneficial effects of oral intake of omega-3 on human health issues such as serum lipid profiles\(^5\)\(^-\)\(^12\), blood pressure control\(^13\)\(^-\)\(^16\), platelet function\(^17\)\(^-\)\(^19\) and psychiatric disorders\(^20\)\(^-\)\(^24\). These human studies have varied greatly in size, duration, age and health of the subjects and level of omega-3 administered. Only a few of the studies undertaken to assess the beneficial effects of omega-3 have monitored any possible adverse side effects, and then primarily through self-reporting. There are few studies that have focused specifically on the safety issues of omega-3. The primary concerns with regard to the safety of omega-3 are its effect on glycaemic control in diabetes, reduced platelet aggregation/increased bleeding time and adverse immunological effects.

Glycaemic control in diabetes
The effect of omega-3 on glycaemic control in diabetes has been studied, often with conflicting results. An adverse effect on glycaemic control was reported in a study investigating the effect of 4g/day omega-3 on patients with non-insulin-dependent diabetes mellitus (NIDDM)\(^25\), and in a study administering 7.5g/day omega-3 to patients with NIDDM\(^26\). However, no adverse effect on glycaemic control in NIDDM patients was reported in four studies with omega-3 at doses of 1.8g/day for 2 months\(^5\), 2g/day for 48 weeks\(^8\), 3g/day for 6 months\(^27\) and 3.84g/day for 6 weeks\(^28\). A meta-analysis\(^29\) of 26 studies ranging in dose of omega-3 from 0.89g/day to 7.7g/day concluded that there was a dose-response relationship, particularly in NIDDM, between EPA and DHA on various measured parameters, but that there were no deleterious effects of omega-3 administration on glycaemic control in either NIDDM or insulin-dependent (IDDM) subjects. The conclusions further suggested that 3g/day omega-3 was a safe level of intake for NIDDM patients.

Platelet aggregation/bleeding time
The main focus of research into the effect of omega-3 on platelet aggregation and bleeding time occurred in the 1980s and early 1990s. Again, there are conflicting results between the different studies, with some reporting a statistically significant reduction of platelet aggregation\(^10\)\(^,\)\(^17\)\(^,\)\(^18\) or increase in bleeding time\(^32\), but others finding no significant changes occurring\(^11\)\(^,\)\(^30\)\(^,\)\(^31\). Levels of omega-3 varied widely in the studies, with effects on platelet aggregation being reported with as little as 1.8g/day omega-3 over 3 weeks, but no effect being reported with 6g/day omega-3 over 6 weeks. A review of studies investigating the effects of omega-3 on haemostatic variables\(^33\) found that those that did report significant changes in platelet aggregation often presented conflicting combinations of effects with different agonists. It also noted that, in many of the studies investigating both platelet aggregation and bleeding time, changes in in vitro aggregation did not correspond with increased bleeding time or with changes in other haemostatic variables. The same author reviewed studies investigating bleeding times and again found conflicting results, although the majority of those showing no or non-significant changes tended to be those using a lower level of omega-3 for a shorter period of time. However, it was noted that the prolongation of bleeding time was usually moderate and there were no reports of serious bleeding.
Although reduced platelet aggregation and/or prolonged bleeding time are often reported in studies on omega-3, it is rarely stated whether the degree of change, whilst statistically significant, is sufficient to have a deleterious effect on the subjects’ health. Effects have been described at wide-ranging levels of omega-3 for greatly differing periods of time, but it is worth noting that there appears to be a lack of significant bleeding in subjects taking large doses of omega-3 and who undergo major surgical procedures or childbirth\textsuperscript{33}. A study investigating whether fish oil might prevent restenosis following angioplasty recorded that the patients taking 6.9g/day omega-3 for 6 months who underwent an invasive angioplasty procedure displayed no difference in clinically significant bleeding compared to the control group\textsuperscript{34}.

**Immunological effects**

A number of studies have investigated the effects of omega-3 on different inflammatory cell functions in humans\textsuperscript{35-42}, and concerns have been raised that a reduction in inflammatory cell functions could compromise host defence and cause detrimental immunological effects in humans\textsuperscript{40,41}. There are conflicting results between the studies as to the effect, if any, of omega-3 on various characteristics indicative of inflammatory cell function. A review on the effect of omega-3 on various immune response factors\textsuperscript{43} suggested that the conflicting results may be related to different experimental protocols used and/or to different subject characteristics: there is a wide variation in immune response in healthy human subjects, which is influenced by age, gender and general lifestyle. There are no clear definitions in the literature of a dose-response effect of omega-3 on inflammatory cell functions, but effects have been seen in studies providing as little as 1g/day omega-3. However, in the studies where an effect of omega-3 on inflammatory cell functions is reported, it is not stated whether the degree of effect, although statistically significant, is sufficient to have a deleterious effect on the subjects’ health.
Other adverse effects
In a 28-day study where 18 women received 4g/day omega-3, two complained of occasional nausea. In a later study of 70 subjects where the study group received omega-3 at levels ranging between 1g/day to 4g/day for 12 weeks, the only events that were considered treatment-related by the physicians were gastrointestinal. These affected 4 of 18 in the control group and 20 of 52 in the study groups. However, these events were attributed to the intake of 4g/day of an oily substance rather than to the omega-3 itself. Only one required the cessation of treatment, the other events being mild and self-limited. A 6-month study with 935 subjects taking either placebo or 3g/day omega-3 stated that only 39 subjects reported side effects, which were mainly mild gastrointestinal events: 18 subjects in the study group and 21 in the placebo group. Again this is probably attributable to the large intake of an oily substance. Other reported events in studies on omega-3 include a ‘fish aftertaste’ and ‘belching’. There have been no serious adverse events attributable to treatment with omega-3 at levels ranging up to 7.5g/day.

It is worth noting that the omega-3 supplements currently on the market contain on average from between 100-150mg omega-3 per soft gel capsule (for supplements aimed at consumption by children) up to around 660mg omega-3 per capsule for an adult’s ‘high dose’ supplement (based on modified oils), with the majority of capsules containing between 250-450mg omega-3. Whilst the maximum size of soft gel capsule currently on the market contains 1750mg of fill, these are difficult to swallow by a large proportion of consumers and thus the majority of soft gel capsules aimed at the adult market contain 1000mg of fill. The children’s supplements tend to be considerably smaller, with less than 500mg of fill. Therefore, in order to achieve a supplemental intake of 4g omega-3, the number of capsules required to be ingested would range from around 6 (for the highest dose supplement) up to around 40 (for the children’s supplement), which would entail consumption of up to 15g or more of oily substance.

Dose-response studies
There have been very few specific dose-response safety studies on omega-3. One early study investigated the dose-response effects of supplementation of omega-3 on 45 healthy male subjects at levels of 0g/day (control group), 1.5g/day, 3g/day and 6g/day for 12 weeks. Blood samples were taken at baseline, after 12 weeks of supplementation and 12 weeks after cessation of supplementation, and were used to examine haematological and biochemical variables, lipid profile, plasma phospholipids and inflammatory cell function. At baseline and after 12 weeks of supplementation bleeding time measurements and erythrocyte deformability studies were also carried out. No dose-dependent effects were observed in the majority of the tests and where there was a response (serum triglycerides and HDL3-cholesterol concentrations reduced and HDL2 cholesterol increased dose dependently), results for 3g and 6g omega-3 were similar. The authors concluded that, taking into account possible adverse effects on subjects with e.g. impaired glucose tolerance, a level of 3g/day omega-3 was a safe supplementation dose in humans.
A later study investigated the maximum tolerated dose and dose-limiting toxicities of omega-3 on 22 patients with cancer cachexia\(^\text{45}\). The level of dose was calculated on a g/kg of body weight basis, and the starting dose of omega-3 for a 70kg patient was just over 4g. Physical assessments and haematological and biochemical measurements were performed during the study. Levels of omega-3 were increased every two weeks if no toxicity was observed. Dose-limiting toxicity was found to be gastrointestinal; there was no haematological toxicity. The study concluded that the maximum tolerated dose of omega-3 for a 70kg patient was 13.1g/day, \textit{i.e.} approximately 0.187g/kg body weight. The omega-3 was given to the patients in capsule form and, in the case of a 70kg patient, 21g/day of oily substance was ingested in order to receive 13.1g omega-3. The gastrointestinal events were therefore most likely in response to the ingestion of such large volumes of oily substance as opposed to the actual omega-3.

**Modified and restructured omega-3 fatty acids**

From the available literature, there is clearly a need to characterise better the exact nature of the omega-3 fatty acids. Whereas most of the early studies on omega-3 were carried out on expressed and refined fish oils without apparent modification, many of the more recent studies have been carried out using ‘purified’ ethyl esters of EPA and DHA that have been developed to produce ‘high omega-3’ oils. There is insufficient evidence that these chemically modified forms of omega-3 can be considered to have the same safety profiles as the original unmodified fish oils. However, the results of studies that have been identified as using omega-3 in ethyl ester, ‘purified’ or ‘concentrated’ form\(^\text{6-9,12-17,19-27,31-34,37,39,42}\) suggest that levels of or below 3g/day are unlikely to cause serious adverse effects.
**Risk Assessment**

**Human NOAEL**

The data from the studies indicate that there is a dose-response effect between omega-3 and various haematological and immunological parameters. The data do not, however, indicate whether the effects observed would be deleterious to human health. Gastrointestinal events have been reported, particularly at higher levels of omega-3, but these appear to be due to the ingestion of large quantities of oily substance and not causally related to consumption of omega-3. Taking into account the different forms of omega-3 used in the studies, and the lack of information as to the actual clinical degree of haematological and immunological effect, it is not possible to define accurately a Lowest Observed Adverse Effect Level (LOAEL) or No Observed Adverse Effect Level (NOAEL).

The dose-response effect of omega-3 appears to be particularly observed at levels of 4g/day and above. The results of the majority of the studies suggest that levels of omega-3 up to 3g/day would be a safe intake for most humans, with conclusions drawn by the authors as to a safe level of omega-3, they appear to agree on 3g/day omega-3 as an acceptable level of intake, with one study specifying 3g/day as an acceptable supplemental intake. There are sufficient data to identify 3g/day as the Observed Safe Level (OSL). In their June 1997 final rule affirming menhaden oil as Generally Recognised As Safe (GRAS) for use as a direct human food ingredient, the U.S. Food and Drug Administration (FDA) concluded that 3g/day of EPA and DHA was a safe level with regard to bleeding time and glycaemic control. This level was not altered in the proposed amendment to the rule in 2002 nor in the tentative final rule in 2004.

Typical recommendations for omega-3 dietary intake are 0.3 to 0.5g/day, but generally the level of omega-3 consumed in the diet is very low, with mean dietary intake less than 0.3g/day. The subjects in the studies varied as to their diets during the period of omega-3 supplementation, some being requested to avoid consumption of fish products, others to maintain their normal dietary habits. Thus in some studies the levels of omega-3 stated were in addition to that obtained from the normal diet. With dietary intake so low no correction is needed for omega-3 in the food supply; therefore the OSL of 3g/day is identified as the tolerable upper intake level from supplements (ULS).

**Animal Studies**

The data from animal studies were not reviewed as the data from the human studies are judged sufficient to supported identification of an OSL. Therefore, this risk assessment represents a direct approach to a ULS.

**NOAEL and LOAEL: No toxicological basis**

**OSL: 3g omega-3**

**ULS: 3g per day**
References


46 Nordic Council of Ministers, "Nordic recommendations on n-3 fatty acid preparations from fish oils", Nordic Council of Ministers, Copenhagen, Denmark (1992)
53 Scientific Advisory Committee on Nutrition Fish Inter-Committee SACN/COT Subgroup, "The effects of long chain polyunsaturated fatty acids on early human growth and cognitive function", SACN Members’ Meeting 3 March. FICS(SACN)/04/01 (2004)
Creatine
Background

First discovered in 1832, creatine is a naturally occurring amino acid-like compound made in the liver, kidneys and pancreas from the essential amino acids arginine, glycine and methionine. In humans, over 95% of the total creatine content is located in skeletal muscle. A 70kg male possesses approximately 120g of total creatine, with a daily turnover estimated to be around 2g. Part of this turnover can be replaced through exogenous sources of creatine in foods, including meat, fish and poultry. In its phosphorylated intracellular form as creatine phosphate, it provides the high-energy phosphate for adenosine triphosphate.

As a dietary supplement, creatine is available in powdered, tablet and liquid forms as either creatine monohydrate or creatine ethyl ester, with only the former having been included in published, randomised, controlled trials. In the last ten years, over 700 randomised, controlled trials have been conducted on or with creatine, with the majority examining creatine’s performance-enhancing benefits. The majority of these clinical trials have found beneficial effects from creatine supplementation, particularly during short, repeated bursts of high-intensity activity. Recent research efforts have also focused on the potential benefits of creatine use in patients coping with certain neuromuscular disorders.

Initial recommendations for creatine use stemmed from early research using 5-7 days of “loading” with 20-30g per day (divided into 4-6 equal, 5g doses), resulting in increased muscle creatine content. Based on new research, refinements have been made to this strategy, and now many athletes consume only one 5g dose approximately 60 minutes prior to or immediately after training (exercise is known to enhance creatine uptake by about 10%). Although responses are quite variable from person to person, subjects ingesting creatine average a 2-5 pound greater gain in muscle mass, and 5-15% greater increases in muscle strength and power compared to control (or placebo) subjects. Creatine supplementation does not appear to enhance endurance-related exercise performance.

Research indicates that once muscle stores of creatine are full, they can remain elevated for an additional 4-5 weeks without further supplementation. Normal healthy adults who continue to use creatine after their muscle stores have reached peak levels may find the additional creatine is converted to creatinine and excreted in the urine. Urinary creatinine levels are commonly used as a marker of kidney function. Individuals who ingest creatine will frequently have elevated creatinine levels – this is normal and represents an increased rate of muscle creatine conversion to creatinine rather than an abnormality of kidney function.
Evidence Related to Safety

Reports of links between creatine use and muscle strains, muscle cramps, heat intolerance and other side effects are not supported by the scientific literature. Studies conducted in athletes and military personnel indicate a substantial level of safety of both short- and long-term creatine use in healthy adults\textsuperscript{7-12}. Concerns about high-dose creatine usage causing kidney damage are based solely on a total of two published case reports in which one of the affected individuals was suffering from existing underlying renal disease\textsuperscript{13,14}. Both comprehensive literature reviews and expert panels have maintained that there is no conclusive evidence to support the notion that creatine may adversely affect kidney function in healthy individuals\textsuperscript{6,15-18}.

Human Studies

There have been more than seventy peer-reviewed, published human clinical trials involving creatine. Of these, the most relevant randomised, controlled studies regarding safety are presented in this review. Sample size, dosage and duration, controlling of diet, along with other co-interventions (such as various forms of exercise training), and outcome measures vary considerably between studies. Overall, the literature demonstrates a substantial level of safety with creatine when used in healthy individuals. The primary side effect reported in clinical trials is gastrointestinal upset due to malabsorption of creatine.

The only form of creatine to be studied in randomised, controlled clinical trials is creatine monohydrate, each gram of which provides 0.879g of creatine. Therefore, for the purposes of this review, the term “creatine” and accompanying dosages refers to creatine monohydrate. Other criteria for study inclusion were study duration (more than one week), and studies had to be randomised, placebo-controlled intervention trials. Studies that were uncontrolled and unblinded, observational, those investigating acute bioavailability, pharmacokinetics or postprandial responses from single bolus doses were excluded from this analysis, and are used solely as supportive information.

Nearly all clinical trials conducted with creatine using healthy adults have implemented the use of a “loading phase” (typically 20g/day creatine, for 3 days to 1 week in duration), followed by a “maintenance phase” (typically 5 to 6g/day, varying duration). The rationale is that the large dose for a short period at the outset of supplementation may help facilitate creatine uptake by skeletal muscle\textsuperscript{2}. For the purposes of this review, rather than being used as the basis for short-term recommendations, loading doses are used as support for recommendations for a lower maintenance dose deemed safe for long-term use.
Several human studies have focused specifically on the safety issues of creatine supplementation. In 2003, Kreider et al. and Greenwood et al. published two separate reports on an open-label study involving creatine supplementation in Division IA college football players. Both reports concluded that creatine doses ranging from 5–15g/day for 21 months did not increase the incidence of cramping or injury, and had no adverse effects on hepatic or renal function\textsuperscript{10-12}. Other uncontrolled trials have reported similar findings in athletes with respect to renal function and thermoregulation\textsuperscript{7,19-22}. Although not randomised, double-blind, controlled trials, these reports help to provide confidence in the remaining body of research demonstrating the safety of creatine.

An intake of approximately one gram of creatine per day from the diet, with the equivalent amount produced endogenously\textsuperscript{1}, suggests that the doses used in the reviewed trials are 3 to 12-fold higher and are adequate to assess safety of supplementation. The absence of any pattern of adverse effects related to creatine supplementation in any of the published human trials provides support for a high level of confidence in the safety of this compound.

**Animal and In Vitro Studies**

Although many studies on creatine using animal models have been published, few have specifically focused on safety and toxicity. Of these, only one reported adverse effects using a rat model of renal cystic disease\textsuperscript{23}. Using a creatine loading dose, followed by a maintenance dose (human equivalent of 20g/day, 1 week followed by 5g/day, 5 weeks) analogous to that used in human trials, Edmunds et al. reported that this regimen contributed to reduced renal function (increased kidney weight, increased serum urea level, lower creatinine clearances) in Sprague-Dawley rats with existing cystic kidney disease. These results are in contrast with a study published by Taes et al.\textsuperscript{24}, who reported that creatine supplementation does not affect kidney function in rats with pre-existing renal failure. Researchers provided sham-operated and partially nephrectomised (effectively inducing renal failure) rats either a control diet, or creatine diet (providing 0.9g/kg body weight creatine/day; equivalent to approximately 50g/day in 60kg adult human) for four weeks\textsuperscript{24}. There was no effect of creatine supplementation on inulin or creatinine clearance rates, urinary protein excretion or urea clearance.

A histological assessment of the effect of up to one year of creatine supplementation revealed that 0.05g/kg body weight/day in mice resulted in inflammation of the liver (no other organs affected), while a supraphysiological dose in rats (2% creatine diet) caused no pathological effects in any of the organs analysed\textsuperscript{25}. These results suggest that the effects of creatine are species specific and that the rat model more closely represents humans.

Other creatine studies conducted using animal models (mice, rats, guinea pigs, dogs; doses ranging from 0.05 to 2g/kg body weight/day for between 2 and 8 weeks) examining serum, muscle and organ concentrations, while not focused on safety or toxicity, have not reported any adverse effects\textsuperscript{26-28}. These studies provide additional support that creatine supplementation at doses analogous to or higher than those used in humans do not cause adverse effects in most animals under normal conditions.
Research on creatine safety and toxicity has focused primarily on its effect on renal function. This stems from the knowledge that excess creatine is eliminated from the body via glomerular filtration in the kidney either as creatine or its metabolite, creatinine. Two human case reports and a single study in rats with renal disease have also fuelled concerns about creatine's effect on the kidney. In clinical practice, several marker compounds are used to assess renal function, including serum creatinine and urea levels, urinary albumin and inulin clearance. Elevated serum levels of creatinine well beyond the normal range (50–115 µmol/L) can be indicative of reduced clearance rates, and along with increased urinary albumin denote compromised renal function. Both controlled and uncontrolled clinical trials involving creatine supplementation have demonstrated elevations in one or more of the following markers: serum creatine and creatinine, and urinary creatine and creatinine. In all cases these elevations have been within the normal range.

Although not reviewed in detail here, published clinical trials lasting one week or less, which comprise approximately half of the total number of clinical trials conducted on creatine, are generally supportive of the longer-term trials. Regardless of dosage (ranging from 5 to 30 g/day) or study population, none of the short-term trials (ranging from 3 to 7 day), reports any adverse effects, and where measured, all relevant endpoints are within the normal range. Aside from the case reports, none of the clinical studies involving healthy adults or those using neuromuscular disease patients has found reduced renal clearance rates (as indicated by above normal elevations in serum creatinine or urea), or increased urinary albumin. Other alleged adverse effects of creatine supplementation, such as cramping and increased core body temperature, have not been observed in any of the controlled or uncontrolled human trials.

**Human NOAEL**

Given that none of the clinical trials found a clear adverse effect related to creatine administration, there is, by definition, no basis for identifying an LOAEL. In the absence of an LOAEL, a NOAEL is not usually set. Without either of these two values the establishment of a UL is usually not set.

**Human OSL**

Published relevant human clinical trials involved creatine doses of up to 26 g/day (“loading” phase) and 6 g/day (“maintenance” phase). All human studies reviewed were double-blind, randomised, controlled trials. A series of non-randomised, open-label clinical trials has also been published. The dosages involved in these studies range from 1 to 30 g/day for up to five years, the results of which are consistent with respect to safety, showing no observed or reported adverse effects.
Of the randomised, controlled clinical trials reviewed in this report, two studies reported gastrointestinal side effects. One study involved elderly men and described the side effects as few and minor. The other reported that a few subjects taking creatine left the study due to gastrointestinal upset. The same study reported no significant difference overall in side effects between creatine and placebo. The remaining studies report a complete absence of side effects, do not address the issue of side effects or report no difference in the incidence of side effects between creatine and placebo. With respect to clinically relevant markers, none of the studies reviewed showed clinically relevant changes in serum creatinine or urea, or urinary albumin, or liver enzymes. A total of two studies using healthy adult subjects reported statistically significant increases in serum creatinine. Robinson et al. reported a 33% increase in serum creatinine (to 90µmol/L) in healthy adults who received 20g/day creatine (5 days) followed by 3g/day creatine (8 weeks) combined with a supervised resistance training programme. Kreider et al. reported a 22.5% increase in serum creatinine (to 125µmol/L) in Division IA college football players who received 15.75g/day creatine along with a supervised resistance training programme for 28 days. Although elevated, these values are still recognised as being within the normal range for serum creatinine and likely are reflective of an increased conversion of creatine to creatinine within the body and not renal dysfunction.

Kreider et al. reported no adverse effects in Div. IA college football players who ingested 15.75g/day creatine for 28 days, but did observe a significant increase in serum creatinine levels (to 125µmol/L). The interpretation of these results is confounded by the fact that these athletes were also undergoing an intense exercise training regimen. While the serum creatinine level is still within a normal range for this population, the relatively small sample size (n = 14) and short duration argue against use of this study for identification of an OSL.

Three studies implement the use of a 10g/day maintenance dose. Arciero et al. and Watsford et al. use very similar study designs involving healthy adult male subjects and a 20g/day (5 and 7 days, respectively) and a 10g/day maintenance dose (up to day 28). While neither study reported any adverse effects, they also failed to measure or report any clinically relevant safety outcomes (such as serum or urinary markers). This fact, combined with the relatively short duration, argue against use of this study for identification of an OSL. The final study using a 10g/day dose involved amyotrophic lateral sclerosis (ALS) patients, who were studied for a total of 310 days. Although some minor gastrointestinal side effects were observed, there was no change in serum urea or urinary albumin levels. The long duration and modest sample size (n = 88) provide confidence in the safety of this dose. However, the diseased nature of the study population argues against use of these results for identification of an OSL.
The gastrointestinal side effects reported by Chrusch were described as few and minor\(^\text{58}\). This study utilised the largest dose of creatine for the “loading phase” (26g/day) in healthy subjects and a dose of 6g/day in the “maintenance” phase, but failed to measure or report any clinically relevant safety outcomes. Although it was of moderate duration (84 days), the small sample size, population age (elderly men), and lack of clinically relevant measurements argue against the use of this study for identification of an OSL. Bennet \textit{et al.}\(^\text{9}\) implemented a very similar dosing regimen (20g/day, followed by 6g/day) and found no adverse effects, including no clinically relevant changes in safety markers. Powers \textit{et al.}\(^\text{63}\) observed no increase in serum or urinary creatinine after a dosing regimen of 25g/day (7 days) followed by 5g/day (21 days). Kilduff \textit{et al.}\(^\text{64}\) (22.8 g/day, 7 days, followed by 5.7g/day, 21 days) reported no adverse effects and only a normal rise in urinary creatinine. However, the relatively small sample sizes and short duration employed in these studies argue against their use for identification of an OSL.

Using a relatively small sample size (n = 8), Derave \textit{et al.}\(^\text{65}\) exposed subjects to a relatively large loading dose (20g/day, 7 days) followed by 5g/day for 19 weeks, for a total exposure of 140 days in healthy adults. This study also showed no significant increase in either serum creatinine or urea. Therefore, given the substantial duration of exposure and the findings of the studies including creatine doses at, above and below this level, this study is chosen to serve as the basis for the human OSL of 5g/day.

The remainder of the relevant studies employed creatine maintenance doses of 5g/day or lower for durations of up to one year in varying populations and serve to support and provide confidence in the selected OSL\(^\text{5,66-78}\). In all of these studies, there were either no adverse effects observed by the investigators or none reported. One study\(^\text{8}\) reported a significant increase in serum creatinine, the level of which (90µmol/L) is still well within the normal range. The quantities of creatine involved in these trials are supplemental amounts well above the estimated amount in foods consumed in the U.S. (1g/day\(^\text{1}\)). Therefore, this risk assessment represents a direct approach to ULS.

**Uncertainty Evaluation**

The study chosen as the basis for the human OSL\(^\text{65}\), on its own merit alone, would necessitate the application of a relatively high UF, due to its small sample size (n = 8). However, there is a collection of other randomised controlled trials conducted in healthy adults using creatine doses at, above and below 5g/day, all demonstrating and/or reporting no adverse effects. These trials serve as support for, and provide confidence in, the results of the study by Derave \textit{et al.}.

**ULS**

Because the 5g/day dose was administered to subjects eating normal diets, no correction of the OSL for dietary intake is needed; therefore OSL = ULS = 5g per day.

**NOAEL and LOAEL:** >10g per day

**OSL:** 5g creatine

**ULS:** 5g per day
References

Creatine


Carnitine
Background

Carnitine was first discovered in muscle tissue, as the name implies, in 1905. It is a quaternary amine that functions in the movement of long-chain fatty acids into mitochondria, modulates the proportion of coenzyme A that is acetylated, and some other reactions in cellular metabolism. The natural form and the only one with biological activity is the geometric isomer k-carnitine (LCAR). In its metabolic functions, acetyl and other acyl groups are repeatedly added to and removed from the carnitine molecule. Blood levels respond to endogenous synthesis, dietary intakes and increasing urinary excretion when the threshold plasma concentration is reached. Because LCAR needs sometimes exceed endogenous synthesis, LCAR is known as a “conditionally essential nutrient”.

LCAR facilitates long-chain fatty acid metabolism by catalysing the transfer of these molecules into the mitochondria where oxidation occurs through the β-oxidation process. Although its biological activities are irreplaceable, carnitine is not an essential dietary component because of its synthesis in tissues. Under some circumstances, such as specific genetic defects and inadequate protein in the diet, biosynthesis from the amino acids lysine and methionine does not meet the physiological needs for optimal functions to support a healthy condition. Carnitine supplementation of infant formula is frequently recommended; this is especially true for premature infants. Because of carnitine’s roles in fatty acid oxidation, carnitine supplements are also commonly used in weight loss programmes. Certain drugs, especially valproic acid and pivalic acid prodrugs, negatively affect human carnitine status.

Foods of animal origin contain significant amounts of LCAR, with the values ranging from as low as approximately 3 or 4mg per serving for cheese, chicken and fish to more than 80mg per serving for beef steak.
Carnitine

Evidence Related to Safety

Human Studies

Three forms of carnitine are commonly available as supplements: \( \kappa \)-carnitine (LCAR), acetyl-\( \kappa \)-carnitine (ALCAR) and propionyl-\( \kappa \)-carnitine (PLCAR), the latter in Europe but not the United States. All clinical intervention trials have utilised one or more of these forms of LCAR. Because of the differences in percentage of the total compound weight represented by LCAR, the reported dosages will be adjusted to indicate the net amounts of \( \kappa \)-carnitine. LCAR does not require adjustment, but ALCAR is 72.3 percent LCAR and PLCAR is 68.8 percent LCAR. There is no evidence suggesting that these three forms have inherently different safety characteristics because the body’s systems efficiently remove the acetyl or propionyl groups, leaving free LCAR. The usual mode of administration is oral, but a few studies have used intravenous (IV) administration, especially in a short loading-dose phase that preceded a longer-term oral administration. Trials that involved only intravenous administration are not considered in this risk assessment for oral LCAR.

6,000 mg/day:
A one-year clinical trial of LCAR involved 473 patients with a first acute myocardial infarction (acute MI). The oral dose of 6g/day for one year followed a loading dose of 9g/day by intravenous administration for five days. Patients were intensively monitored, including cardiac function parameters. Few (8%) of the patients received angiotensin-converting enzyme (ACE) inhibitors, but the majority (78%) were given thrombolytic therapy. Although the study was not powered to detect a difference in mortality, the LCAR treatment was associated with a non-significant decrease (from 10% to 4%) in mortality during the year of treatment. In one cardiac function parameter (left ventricular end-diastolic volume, or LVEDV), LCAR produced a statistically significant protective effect. There were no significant adverse effects of LCAR (except the nuisance of a fishy body odour).

100 mg/kg/day:
This dose of LCAR is equivalent to 6,000mg in a 60kg adult. LCAR or placebo was administered orally for 6 months to hyperactive boys between 6 and 13 years of age, with 10 in the LCAR group and 12 in the placebo group. Adverse events were infrequent but somewhat more common in the placebo than in the LCAR group, except for an unpleasant body odour, which the authors speculated might be due to the metabolic release of trimethylamine from the LCAR.

100 mg/kg/day:
This double-blind placebo controlled cross-over trial of LCAR administration for 8 weeks involved 35 Rett syndrome patients. A large majority were 20 years of age or younger. The dose is equivalent to a 6,000mg/day intake in a 60kg adult. Three subjects experienced very loose bowels while taking the LCAR at 100mg/kg/day. The condition resolved when the dose was decreased to 75mg/kg/day. Three more reported a fishy body or urine odour while taking LCAR. No other side effects were seen.
4,000 mg/day:
This small (n = 10/treatment) clinical trial involved oral administration of 4,000mg/day LCAR for 3 months each for placebo and LCAR in a cross-over design. Although the numbers were small, the subjects were intensively monitored for a wide spectrum of clinical, haematological, metabolic and blood and urine biochemical effects. All clinically obvious adverse events (such as gastralgia, headache, nausea and pruritus) were infrequent in all groups, with no statistical differences. Thus, there was no evidence of adverse effects from LCAR.

3,000 mg/day:
This dose of LCAR was given to 21 acute myocardial infarction patients for 90 days after a 7-day loading dose of 6,000mg/day by intravenous administration. A placebo was given to 24 patients. Standard clinical chemistries and extensive cardiac function monitoring revealed no adverse effects.

3,000 mg/day:
This dose of LCAR was administered for 120 days to 20 patients with severe, ischemically induced cardiac insufficiency, with 21 patients receiving a placebo. Performance on a bicycle ergometer was measured. No adverse effects were reported.

2,732 mg/day:
This LCAR net dosage resulted from the combined administration of 2,000mg LCAR and 1,000mg of ALCAR. The trial involved 60 men with low sperm vitality. The treatment was for 6 months, with an additional follow up period of two months. No adverse effects were reported.

2,196 mg/day:
This LCAR dose was achieved by administration of 3,000mg of ALCAR. The 12-month study involved 431 younger Alzheimer’s disease patients, with 215 of them receiving the placebo. Adverse events were reported in some detail, but there was no evidence that the treatment caused any of them. Three patients in the ALCAR group died compared with four in the placebo group. Three patients died from cancer, two from cardiac causes, one from pneumonia, and one from unknown causes. Six patients receiving LCAR withdrew from the study, due to adverse events, compared with two receiving the placebo. Among those who continued throughout the study, there was no difference between the placebo and treatment groups.

2,196 mg/day:
This 52-week trial with 19 diabetic subjects involved administration of 2,196mg LCAR as 3,000mg ALCAR to 13 subjects and placebo to the others. Standard clinical and chemical evaluations, and specialised diabetes tests found no adverse effects.
50 mg ALCAR/kg/day\textsuperscript{13}: 
This dose is equivalent to 2,196mg LCAR in a 60kg person. The one-year study involved boys aged 6-13 years with hyperactive behaviour in Fragile X syndrome, with 8 receiving ALCAR and 9 receiving the placebo. No side effects were reported.

2,064 mg/day\textsuperscript{14}: 
This dose was administered as 3,000mg PLCAR for 90 days to 22 patients with peripheral arterial obliterative disease of the lower limbs. Some clinical benefit but no adverse effects were reported.

2,000 mg/day or less: 
Several clinical trials\textsuperscript{15-30} have involved administration of LCAR, ALCAR or PLCAR at dosages of 2,000mg or less LCAR per day. The size, duration and clinical measurements monitoring in these trials differed greatly. The duration of treatment ranged from 3 days up to one year. The number of subjects ranged from just a few up to a few hundred. In many research articles, the literature review and discussion presume that LCAR is safe within a broad range of intakes, and many of these articles do not address adverse effects as having been seen, not seen or monitored. The reports that address adverse effects are discussed below.

A relatively large ($n=485$) trial on intermittent claudication patients found no differences in the adverse events and withdrawals for the PLCAR (equivalent to 1,376mg LCAR) and placebo groups\textsuperscript{18}. In a six-month study of 118 intermittent claudication patients receiving PLCAR equivalent of 2,064mg LCAR/day, nausea and diarrhoea were slightly but non-significantly less common than in 127 in the placebo group\textsuperscript{16}. A one-year trial of ALCAR equivalent to 1,464mg LCAR/day in 333 diabetic neuropathy patients found no difference in clinical chemistries or haematological indices, but six withdrew from the ALCAR treatment group, compared with two in the placebo group\textsuperscript{20}. In another trial, one patient was reported to have diarrhoea while taking 2,000mg LCAR/day\textsuperscript{24}. Nine of 18 moderately obese patients given 2,000mg LCAR/day reported having nausea and diarrhoea\textsuperscript{29}. Extensive clinical chemistry and haematological assays found no adverse effects of 2,000mg of LCAR after 21 days of treatment\textsuperscript{25}. Some of these reports do not describe adverse effect monitoring but assert that there were none\textsuperscript{23}.

Animal Studies
A large number of human intervention trials have been published. Although they vary in strength of design and performance, the human data provide a sufficient basis for a risk assessment. Therefore, the animal data are not reviewed in this risk assessment.
Risk Assessment

**Human NOAEL**

No adverse effects of LCAR, ALCAR or PLCAR have been established, except for unpleasant body or urine odour in some individuals. Thus, an LOAEL cannot be identified and no NOAEL is established. In the absence of established adverse effects a UL cannot be set and the OSL approach will be used.

**Human OSL**

No adverse effects were observed after a one-year administration with 6,000mg/day of LCAR to 239 patients with an acute myocardial infarction, who were intensively monitored for cardiac health and function. The acute and extreme diseased condition of these subjects and the fact that no other published trials have used this or a higher level of LCAR argue against selection of this intake as a high-confidence NOAEL. This absence of adverse effects may be applicable to most adults, but the database is not sufficient at this intake level for a firm conclusion.

No adverse effects were observed after three months' treatment with 4,000mg/day of LCAR to 10 hyperthyroidism patients. Although no adverse effects were seen, the small size and modest duration of this trial prevent identification of 4,000mg/day as the NOAEL. The report on 6,000mg/day increases the confidence in this study, but not sufficiently to have a high level of confidence in the absence of adverse effects.

Two trials have administered 100mg/kg/day to small numbers of children for two months with no adverse effects other than unpleasant or fishy body odour. This dose is equivalent to 6,000mg in a 60kg adult, but the small size and short duration of these trials prevent the confident extrapolation of the results to establish an adult NOAEL. One trial involved the administration of 50mg/kg/day (equivalent to 3,000mg in a 60kg adult) ALCAR for a year to boys with Fragile X syndrome. Correction for molecular weight gives the equivalent of 2,562mg LCAR for a 70kg adult. The extremely small size (8 ALCAR, 9 placebos) of the trial and lack of statement about adverse effects prevents the confident extrapolation to establish an adult NOAEL.

Two trials involved administration of 3,000mg/day LCAR to small groups of patients for 3 months. No observed effects were reported but one report did not address the issue and the other simply asserted that there were none without giving any details. Considering other reports without adverse effects at high levels of LCAR, this 3,000mg intake may warrant selection as the adult NOAEL, but the database is not large or robust enough to support that decision.

Another clinical trial involved daily administration of 2,000mg LCAR and 1000mg ALCAR (total equivalent to 2,732mg LCAR) to 56 young men who were healthy except for reduced sperm motility. Adverse effects were not reported but neither discussed. These results might qualify as the adult NOAEL except that the report does not give sufficient detail.
Five clinical trials involved administration of 3,000mg/day of ALCAR (equivalent to 2,196mg LCAR) or PLCAR (equivalent to 2,064mg LCAR)\textsuperscript{11,12,14,16,17}. Two of these studies involved a one-year administration of ALCAR administration. The larger of these studies involved 431 younger Alzheimer’s patients (216 ALCAR and 215 placebo)\textsuperscript{11}. The adverse events were body odour, flatulence, increased appetite and rash but the drop out rate for the LACAR and placebo group was not different. The other one-year study, with myocardial infarction patients, was small: 13 diabetics and a similar placebo group of 6\textsuperscript{12}.

A six-month study with PLCAR at 1,000mg for two months, 2,000mg for two months, and 3,000mg for two months (LCAR equivalents of 688, 1,376 and 2,064mg, respectively) involved a substantial cohort (118 PLCAR, 127 placebos) of intermittent claudication patients\textsuperscript{16}. Nausea and gastric pain were non-significantly more common with the placebo than with the PLCAR treatment.

A small 90-day trial administered PLCAR (equivalent to 2,064mg LCAR) to 11 patients with peripheral arterial obliterative disease, plus a parallel placebo group, but the report did not address adverse effects\textsuperscript{14}.

Three shorter-term, modest-size clinical trials with 2,000mg LCAR did not report adverse effects\textsuperscript{22,26,31}.

Collectively, the trials at LCAR equivalents of 2,064mg, 2,196mg or 2,000mg LCAR provide strong evidence that LCAR in this intake range is without adverse effects in patients with a variety of unrelated diseases. From these data, an adult human OSL of 2,000mg of LCAR equivalents is selected. This is equivalent to 2,000mg LCAR, 2,732mg ALCAR and 2,906mg PLCAR.

**Uncertainty Evaluation**

The existence of clinical trials that administered 4,000mg and 6,000mg LCAR provides additional confidence in the data at 2,000mg LCAR equivalents. The use of diseased patients in most or all of the clinical trials would tend to create uncertainty in the extrapolation of the results to normal adults. This possible conclusion, however, is countered by the wide variety of unrelated diseases of the patients in the different trials. Because there is no pattern of side effects occurring in patients with specific diseases, there seems to be no reasonable possibility that the different diseases would all protect against any adverse effects of LCAR. Therefore, it is reasonable to extrapolate the results to normal, healthy adults.

Uncertainty is markedly reduced for the OSL by the existence of clinical trials that involved much higher intakes and by the multiplicity of trials at or just above the OSL. Therefore, a UF of unity is selected, thus making no adjustment of the OSL.
None of the clinical trials adjusted the administered amount of LCAR, ALCAR, or PLCAR for the intakes from foods or for endogenous biosynthesis. Therefore, the OSL identified applies to supplemental amounts of the compounds.

**ULS**

None of the clinical trials adjusted the administered amount of LCAR, ALCAR, or PLCAR for the intakes from foods or for endogenous biosynthesis. Therefore, the OSL identified applies to supplemental amounts of the compounds.

**NOAEL and LOAEL:** No toxicological basis

<table>
<thead>
<tr>
<th>OSL</th>
<th>2000mg LCAR equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2000mg LCAR</td>
</tr>
<tr>
<td></td>
<td>2732mg ALCAR</td>
</tr>
<tr>
<td></td>
<td>2906mg PLCAR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ULS</th>
<th>2000mg per day LCAR equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2000mg per day LCAR</td>
</tr>
<tr>
<td></td>
<td>2732mg per day ALCAR</td>
</tr>
<tr>
<td></td>
<td>2906mg per day PLCAR</td>
</tr>
</tbody>
</table>
Carnitine

References


3. J. Higdon, "L-Carnitine", in The Linus Pauling Institute Micronutrient Information Center (Oregon State University), edited by T. M. Hagen (Linus Pauling Institute, Corvallis, OR, 2002)


