IARC Handbooks of Cancer Prevention

International Agency for Research on Cancer
World Health Organization

Volume 3

Vitamin A

1998

WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

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This publication represents the views and expert opinions
of an IARC Working Group on the
Evaluation of Cancer-preventive Agents,
which met in Lyon,

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International Agency For Research On Cancer

The International Agency for Research on Cancer (IARC) was established in 1965 by the World Health Assembly, as an independently financed organization within the framework of the World Health Organization. The headquarters of the Agency are in Lyon, France.

The Agency conducts a programme of research concentrating particularly on the epidemiology of cancer and the study of potential carcinogens in the human environment. Its field studies are supplemented by biological and chemical research carried out in the Agency's laboratories in Lyon and, through collaborative research agreements, in national research institutions in many countries. The Agency also conducts a programme for the education and training of personnel for cancer research.

The publications of the Agency contribute to the dissemination of authoritative information on different aspects of cancer research. A complete list is printed at the back of this book. Information about IARC publications, and how to order them, is also available via the Internet at: **http://www.iarc.fr/**

Note to the Reader

Anyone who is aware of published data that may influence any consideration in these *Handbooks* is encouraged to make the information available to the Unit of Chemoprevention, International Agency for Research on Cancer, 150 Cours Albert Thomas, 69372 Lyon Cedex 08, France

Although all efforts are made to prepare the *Handbooks* as accurately as possible, mistakes may occur. Readers are requested to communicate any errors to the Unit of Chemoprevention, so that corrections can be reported in future volumes.

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Abbreviations

ALAT	Alanine aminotransferase
apoB48	Apolipoprotein B-48
apo-RBP	Unbound retinol-binding protein
apoE	Apolipoprotein E
ARAT	Acyl-coenzyme A:retinol acyltransferase
AUC	Area under the concentration-time curve
BBN	*N*-Butyl-*N*-(4-hydroxybutyl)-nitrosamine
BCC	Basal cell carcinoma
bFGF	Basic fibroblast growth factor
BP	Benzo[*a*]pyrene
BPV	Bovine papillomavirus
CARET	Beta-Carotene and Retinol Efficacy Trial
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CHEL	Chinese hamster epithelial liver
CHO	Chinese hamster ovary
CI	95% Confidence interval
CIN	Cervical intraepithelial neoplasia
CRABP	Cellular retinoic acid-binding protein
CralBP	Cellular retinal-binding protein
CRBP	Cellular retinol-binding proteins
CSF	Colony-stimulating factor
CYP	Cytochrome P450
DEN	*N*-Nitrosodiethylamine
DMBA	7,12-Dimethylbenz[*a*]anthracene*
DPT	Diphtheria/pertussis/tetanus
EGF	Epidermal growth factor
FANFT	*N*-[4-(5-Nitro-2-furyl)-2-thiazolyl]-formamide
αFGF	α-Fibroblast growth factor
GGT	γ-Glutamyltranspeptidase
HDL	High-density lipoprotein
HGPRT	Hypoxanthine phosphoribosyl transferase
HIV	Human immunodeficiency virus
Holo-RBP	Retinol–RBP complex
4-HP	*N*-(4-Hydroxyphenyl)retinamide
HPLC	High-performance liquid chromatography
14-HRR	14-Hydroxy-4,14-retro-retinol
HSPG	Heparin sulfate proteoglycans
IR	Infrared
IRBP	Interphotoreceptor retinol-binding protein
IU	International unit
LD_{50}	Dose that is lethal to 50% of individuals
LDL	Low-density lipoprotein
LDL-R	Low-density lipoprotein receptor
LPL	Lipoprotein lipase
LRAT	Lecithin:retinol acyltransferase
LRP	LDL receptor-related protein
LSR	Lipolysis-stimulated receptor
MCA	3-Methylcholanthrene
MNNG	*N*-Methyl-*N'*-nitro-*N*-nitroso-guanidine
MNU	*N*-Methyl-*N*-nitrosourea
MRDR	Modified relative dose response
NK	Natural killer
NMR	Nuclear magnetic resonance
ODC	Ornithine decarboxylase
PASI	Psoriasis Area and Severity Index
PDGF	Platelet-derived growth factor
RAP	Receptor-associated protein
RAR	Retinoic acid receptor
RARE	Retinoic acid response element
RBP	Retinol-binding protein
RDA	Recommended dietary allowance
RDR	Relative dose response
RE	Retinol equivalents
RPE	Retinal pigment epithelium
RR	Relative risk
RXR	Retinoid X receptor
SCC	Squamous cell carcinoma
SCE	Sister chromatid exchanges
SCID	Severe combined immunodeficient
TGF	Transforming growth factor
TNF	Tumour necrosis factor
TPA	12-*O*-Tetradecanoylphorbol 13-acetate
TTR	Transthyretin
UDS	Unscheduled DNA synthesis
UNICEF	United Nations Children's Fund
UV	Ultraviolet
VLDL	Very low-density lipoprotein

*Alternative nomenclature: 9,12-dimethyl-1,2-benzanthracene

Preamble to the *IARC Handbooks of Cancer Prevention*

The prevention of cancer is one of the key objectives of the International Agency for Research on Cancer (IARC). This may be achieved by avoiding exposures to known cancer-causing agents, by increasing host defences through immunization or chemoprevention or by modifying lifestyle. The aim of the *IARC Monographs* programme is to evaluate carcinogenic risks of human exposure to chemical, physical and biological agents, providing a scientific basis for national or international decisions on avoidance of exposures. The aim of the series of *IARC Handbooks of Cancer Prevention* is to evaluate scientific information on agents and interventions that may reduce the incidence of or mortality from cancer. This preamble is divided into two parts. The first addresses the general scope, objectives and structure of the *Handbooks*. The second describes the procedures for evaluating cancer-preventive agents.

Part One

Scope

Cancer-preventive strategies embrace chemical, immunological, dietary and behavioural interventions that may retard, block or reverse carcinogenic processes or reduce underlying risk factors. The term 'chemoprevention' is used to refer to interventions with pharmaceuticals, vitamins, minerals and other chemicals to reduce cancer incidence. The *IARC Handbooks* address the efficacy, safety and mechanisms of cancer-preventive strategies and the adequacy of the available data, including those on timing, dose, duration and indications for use.

Preventive strategies can be applied across a continuum of: (1) the general population; (2) subgroups with particular predisposing host or environmental risk factors, including genetic susceptibility to cancer; (3) persons with precancerous lesions; and (4) cancer patients at risk for second primary tumours. Use of the same strategies or agents in the treatment of cancer patients to control the growth, metastasis and recurrence of tumours is considered to be patient management, not prevention, although data from clinical trials may be relevant when making a *Handbooks* evaluation.

Objective

The objective of the *Handbooks* programme is the preparation of critical reviews and evaluations of evidence for cancer-prevention and other relevant properties of a wide range of potential cancer-preventive agents and strategies by international working groups of experts. The resulting *Handbooks* may also indicate where additional research is needed.

The *Handbooks* may assist national and international authorities in devising programmes of health promotion and cancer prevention and in making benefit–risk assessments. The evaluations of IARC working groups are scientific judgements about the available evidence for cancer-preventive efficacy and safety. No recommendation is given with regard to national and international regulation or legislation, which are the responsibility of individual governments and/or other international authorities. No recommendations for specific research trials are made.

IARC Working Groups

Reviews and evaluations are formulated by international working groups of experts convened by the IARC. The tasks of each group are: (1) to ascertain that all appropriate data have been collected; (2) to select the data relevant for the evaluation on the basis of scientific merit; (3) to prepare accurate summaries of the data to enable the reader to follow the reasoning of the Working Group; (4) to evaluate the significance of the available data from human studies and experimental models on cancer-preventive activity, carcinogenicity and other beneficial and adverse effects; and (5) to evaluate data relevant to the understanding of mechanisms of action.

Working Group participants who contributed to the considerations and evaluations within a particular *Handbook* are listed, with their addresses, at the beginning of each publication. Each participant serves as an individual scientist and not as a representative of any organization, government or industry. In addition, scientists nominated by national and international agencies, industrial associations and consumer and/or environmental organizations may be invited as observers. IARC staff involved in the preparation of the *Handbooks* are listed.

Working procedures

Approximately 13 months before a working group meets, the topics of the *Handbook* are announced, and participants are selected by IARC staff in consultation with other experts. Subsequently, relevant clinical, experimental and human data are collected by the IARC from all available sources of published information. Representatives of producer or consumer associations may assist in the preparation of sections on production and use, as appropriate.

About eight months before the meeting, the material collected is sent to meeting participants to prepare sections for the first drafts of the *Handbooks*. These are then compiled by IARC staff and sent, before the meeting, to all participants of the Working Group for review. There is an opportunity to return the compiled specialized sections of the draft to the experts, inviting preliminary comments, before the complete first-draft document is distributed to all members of the Working Group.

Data for *Handbooks*

The *Handbooks* do not necessarily cite all of the literature on the agent or strategy being evaluated. Only those data considered by the Working Group to be relevant to making the evaluation are included. In principle, meeting abstracts and other reports that do not provide sufficient detail upon which to base an assessment of their quality are not considered.

With regard to data from toxicological, epidemiological and experimental studies and from clinical trials, only reports that have been published or accepted for publication in the openly available scientific literature are reviewed by the Working Group. In certain instances, government agency reports that have undergone peer review and are widely available are considered. Exceptions may be made on an ad-hoc basis to include unpublished reports that are in their final form and publicly available, if their inclusion is considered pertinent to making a final evaluation. In the sections on chemical and physical properties, on production, on use, on analysis and on human exposure, unpublished sources of information may be used.

The available studies are summarized by the Working Group, with particular regard to the qualitative aspects discussed below. In general, numerical findings are indicated as they appear in the original report; units are converted when necessary for easier comparison. The Working Goup may conduct additional analyses of the published data and use them in their assesment of the evidence; the results of such supplementary analyses are given in square brackets. When an important aspect of a study, directly impinging on its interpretation, should be brought to the attention of the reader, a comment is given in square brackets.

Criteria for selection of topics for evaluation

Agents, classes of agents and interventions to be evaluated in the *Handbooks* are selected on the basis of one or more of the following criteria.

- The available evidence suggests potential for significantly reducing the incidence of cancers.
- There is a substantial body of human, experimental, clinical and/or mechanistic data suitable for evaluation.
- The agent is in widespread use and of putative protective value, but of uncertain efficacy and safety.
- The agent shows exceptional promise in experimental studies but has not been used in humans.
- The agent is available for further studies of human use.

Outline of data presentation scheme for evaluating cancer-preventive agents

1. **Chemical and physical characteristics**

2. **Occurrence, production, use, analysis and human exposure**
   - 2.1 Occurrence
   - 2.2 Production
   - 2.3 Use
   - 2.4 Analysis
   - 2.5 Human exposure

3. **Metabolism, kinetics and genetic variation**
   - 3.1 Human studies
   - 3.2 Experimental models

4. **Cancer-preventive effects**
   - 4.1 Human studies
     - 4.1.1 Epidemiology studies
     - 4.1.2 Intervention trials
     - 4.1.3 Intermediate end-points
   - 4.2 Experimental models
     - 4.2.1 Tumour induction
     - 4.2.2 Intermediate biomarkers
     - 4.2.3 *In-vitro* models
   - 4.3 Mechanisms of cancer-prevention

5. **Other beneficial effects**

6. **Carcinogenicity**
   - 6.1 Human studies
   - 6.2 Experimental models

7. **Other toxic effects**
   - 7.1 Adverse effects
     - 7.1.1 Human studies
     - 7.1.2 Experimental studies
   - 7.2 Reproductive and developmental effects
     - 7.2.1 Human studies
     - 7.2.2 Experimental studies
   - 7.3 Genetic and related effects
     - 7.3.1 Human studies
     - 7.3.2 Experimental studies

8. **Summary of data**
   - 8.1 Chemistry, occurrence and human exposure
   - 8.2 Metabolism and kinetic properties
   - 8.3 Cancer-preventive effects
     - 8.3.1 Human studies
     - 8.3.2 Experimental studies
     - 8.3.3 Mechanisms of cancer-prevention
   - 8.4 Other beneficial effects
   - 8.5 Carcinogenic effects
     - 8.5.1 Human studies
     - 8.5.2 Experimental animals
   - 8.6 Toxic effects
     - 8.6.1 Human studies
     - 8.6.2 Experimental studies

9. **Recommendations for research**

10. **Evaluation**
    - 10.1 Cancer-preventive activity
      - 10.1.1 Humans
      - 10.1.2 Experimental animals
    - 10.2 Overall evaluation

11. **References**

Part Two

Evaluation of cancer-preventive agents

A wide range of findings must be taken into account before a particular agent can be recognized as preventing cancer. On the basis of experience from the *IARC Monographs* programme, a systematized approach to data presentation is adopted for *Handbooks* evaluations.

1. Chemical and physical characteristics of the agent

The Chemical Abstracts Services Registry Number, the latest Chemical Abstracts Primary Name, the IUPAC Systematic Name and other definitive information (such as genus and species of plants) are given as appropriate. Information on chemical and physical properties and, in particular, data

relevant to identification, occurrence and biological activity are included. A description of technical products of chemicals includes trade names, relevant specifications and available information on composition and impurities. Some of the trade names given may be those of mixtures in which the agent being evaluated is only one of the ingredients.

2. Occurrence, production, use, analysis and human exposure

2.1 Occurrence

Information on the occurrence of an agent or mixture in the environment is obtained from data derived from the monitoring and surveillance of levels in occupational environments, air, water, soil, foods and animal and human tissues. When available, data on the generation, persistence and bioaccumulation of the agent are included. For mixtures, information is given about all agents present.

2.2 Production

The dates of first synthesis and of first commercial production of a chemical or mixture are provided; for agents that do not occur naturally, this information may allow a reasonable estimate to be made of the date before which no human use of, or exposure to, the agent could have occurred. The dates of first reported occurrence of an exposure are also provided. In addition, methods of synthesis used in past and present commercial production and methods of production that may give rise to different impurities are described.

2.3 Use

Data on production, international trade and uses and applications are obtained for representative regions. Some identified uses may not be current or major applications, and the coverage is not necessarily comprehensive. In the case of drugs, mention of their therapeutic applications does not necessarily represent current practice, nor does it imply judgement as to their therapeutic efficacy.

2.4 Analysis

An overview of current methods of analysis or detection is presented. Methods for monitoring human exposure are also given, when available.

2.5 Human exposure

Human uses of, or exposure to, the agent are described. If an agent is used as a prescribed or over-the-counter pharmaceutical product, then the type of person receiving the product in terms of health status, age, sex and medical condition being treated are described. For nonpharmaceutical agents, particularly those taken because of cultural traditions, the characteristics of use or exposure and the relevant populations are described. In all cases, quantitative data, such as dose–response relationships, are considered to be of special importance.

3. Metabolism, kinetics and genetic variation

In evaluating the potential utility of a suspected cancer-preventive agent or strategy, a number of different properties, in addition to direct effects upon cancer incidence, are described and weighed. Furthermore, as many of the data leading to an evaluation are expected to come from studies in experimental animals, information that facilitates interspecies extrapolation is particularly important; this includes metabolic, kinetic and genetic data. Whenever possible, quantitative data, including information on dose, duration and potency, are considered.

Information is given on absorption, distribution (including placental transfer), metabolism and excretion in humans and experimental animals. Kinetic properties within the target species may affect the interpretation and extrapolation of dose–response relationships, such as blood concentrations, protein binding, tissue concentrations, plasma half-lives and elimination rates. Comparative information on the relationship between use or exposure and the dose that reaches the target site may be of particular importance for

extrapolation between species. Studies that indicate the metabolic pathways and fate of the agent in humans and experimental animals are summarized, and data on humans and experimental animals are compared when possible. Observations are made on inter-individual variations and relevant metabolic polymorphisms. Data indicating long-term accumulation in human tissues are included. Physiologically based pharmacokinetic models and their parameter values are relevant and are included whenever they are available. Information on the fate of the compound within tissues and cells (transport, role of cellular receptors, compartmentalization, binding to macromolecules) is given.

Genotyping will be used increasingly, not only to identify subpopulations at increased or decreased risk for cancers but also to characterize variation in the biotransformation of, and responses to, cancer-preventive agents.

This subsection can include effects of the compound on gene expression, enzyme induction or inhibition, or pro-oxidant status, when such data are not described elsewhere. It covers data obtained in humans and experimental animals, with particular attention to effects of long-term use and exposure.

4. Cancer-preventive effects

4.1 Human studies

Types of study considered. Human data are derived from experimental and non-experimental study designs and are focused on cancer, precancer or intermediate biological end-points. The experimental designs include randomized controlled trials and short-term experimental studies; non-experimental designs include cohort, case–control and cross-sectional studies.

Cohort and case–control studies relate individual use of, or exposure to, the agents under study to the occurrence of cancer in individuals and provide an estimate of relative risk (ratio of incidence or mortality in those exposed to incidence or mortality in those not exposed) as the main measure of association. Cohort and case–control studies follow an observational approach, in which the use of, or exposure to, the agent is not controlled by the investigator.

Intervention studies are experimental in design — that is, the use of, or exposure to, the agent is assigned by the investigator. The intervention study or clinical trial is the design that can provide the strongest and most direct evidence of a protective or preventive effect; however, for practical and ethical reasons, such studies are limited to observation of the effects among specifically defined study subjects of interventions of 10 years or fewer, which is relatively short when compared with the overall lifespan.

Intervention studies may be undertaken in individuals or communities and may or may not involve randomization to use or exposure. The differences between these designs is important in relation to analytical methods and interpretation of findings.

In addition, information can be obtained from reports of correlation (ecological) studies and case series; however, limitations inherent in these approaches usually mean that such studies carry limited weight in the evaluation of a preventive effect.

Quality of studies considered. The *Handbooks* are not intended to summarize all published studies. It is important that the Working Group consider the following aspects: (1) the relevance of the study; (2) the appropriateness of the design and analysis to the question being asked; (3) the adequacy and completeness of the presentation of the data; and (4) the degree to which chance, bias and confounding may have affected the results.

Studies that are judged to be inadequate or irrelevant to the evaluation are generally omitted. They may be mentioned briefly, particularly when the information is considered to be a useful supplement to that in other reports or when it provides the only data available. Their inclusion does not imply acceptance of the adequacy of the study design, nor of the analysis and interpretation of the results, and their limitations are outlined.

Assessment of the cancer-preventive effect at different doses and durations. The Working Group gives special attention to quantitative assessment of the preventive effect of the agent under study, by assessing data from studies at different doses. The Working Group also addresses issues of timing and duration of use or exposure. Such quantitative assessment is important to clarify the circumstances under which a preventive effect can be achieved, as well as the dose at which a toxic effect has been shown.

Criteria for a cancer-preventive effect. After summarizing and assessing the individual studies, the Working Group makes a judgement concerning the evidence that the agent in question prevents cancer in humans. In making their judgement, the Working Group considers several criteria for each relevant cancer site.

Evidence of protection derived from intervention studies of good quality is particularly informative. Evidence of a substantial and significant reduction in risk, including a dose–response relationship, is more likely to indicate a real effect. Nevertheless, a small effect, or an effect without a dose–response relationship, does not imply lack of real benefit and may be important for public health if the cancer is common.

Evidence is frequently available from different types of study and is evaluated as a whole. Findings that are replicated in several studies of the same design or using different approaches are more likely to provide evidence of a true protective effect than isolated observations from single studies.

The Working Group evaluates possible explanations for inconsistencies across studies, including differences in use of, or exposure to, the agent, differences in the underlying risk of cancer and metabolism and genetic differences in the population.

The results of studies judged to be of high quality are given more weight. Note is taken of both the applicability of preventive action to several cancers and of possible differences in activity, including contradictory findings, across cancer sites.

Data from human studies (as well as from experimental models) that suggest plausible mechanisms for a cancer-preventive effect are important in assessing the overall evidence.

The Working Group may also determine whether, on aggregate, the evidence from human studies is consistent with a lack of preventive effect.

4.2 Experimental models

4.2.1 Experimental animals

Animal models are an important component of research into cancer prevention. They provide a means of identifying effective compounds, of carrying out fundamental investigations into their mechanisms of action, of determining how they can be used optimally, of evaluating toxicity and, ultimately, of providing an information base for developing intervention trials in humans. Models that permit evaluation of the effects of cancer-preventive agents on the occurrence of cancer in most major organ sites are available. Major groups of animal models include: those in which cancer is produced by the administration of chemical or physical carcinogens; those involving genetically engineered animals; and those in which tumours develop spontaneously. Most cancer-preventive agents investigated in such studies can be placed into one of three categories: compounds that prevent molecules from reaching or reacting with critical target sites (blocking agents); compounds that decrease the sensitivity of target tissues to carcinogenic stimuli; and compounds that prevent evolution of the neoplastic process (suppressing agents). There is increasing interest in the use of combinations of agents as a means of improving efficacy and minimizing toxicity. Animal models are useful in evaluating such combinations. The development of optimal strategies for human intervention trials can be facilitated by the use of animal models that mimic the neoplastic process in humans.

Specific factors to be considered in such experiments are: (1) the temporal requirements of administration of the cancer-preventive agents; (2) dose–response effects; (3) the site-specificity of

cancer-preventive activity; and (4) the number and structural diversity of carcinogens whose activity can be reduced by the agent being evaluated.

An important variable in the evaluation of the cancer-preventive response is the time and the duration of administration of the agent in relation to any carcinogenic treatment, or in transgenic or other experimental models in which no carcinogen is administered. Furthermore, concurrent administration of a cancer-preventive agent may result in a decreased incidence of tumours in a given organ and an increase in another organ of the same animal. Thus, in these experiments it is important that multiple organs be examined.

For all these studies, the nature and extent of impurities or contaminants present in the cancer-preventive agent or agents being evaluated are given when available. For experimental studies of mixtures, consideration is given to the possibility of changes in the physicochemical properties of the test substance during collection, storage, extraction, concentration and delivery. Chemical and toxicological interactions of the components of mixtures may result in nonlinear dose–response relationships.

As certain components of commonly used diets of experimental animals are themselves known to have cancer-preventive activity, particular consideration should be given to the interaction between the diet and the apparent effect of the agent being studied. Likewise, restriction of diet may be important. The appropriateness of the diet given relative to the composition of human diets may be commented on by the Working Group.

Qualitative aspects. An assessment of the experimental prevention of cancer involves several considerations of qualitative importance, including: (1) the experimental conditions under which the test was performed (route and schedule of exposure, species, strain, sex and age of animals studied, duration of the exposure, and duration of the study); (2) the consistency of the results, for example across species and target organ(s); (3) the stage or stages of the neoplastic process, from preneoplastic lesions and benign tumours to malignant neoplasms, studied and (4) the possible role of modifying factors.

Considerations of importance to the Working Group in the interpretation and evaluation of a particular study include: (1) how clearly the agent was defined and, in the case of mixtures, how adequately the sample composition was reported; (2) the composition of the diet and the stability of the agent in the diet; (3) whether the source, strain and quality of the animals was reported; (4) whether the dose and schedule of treatment with the known carcinogen were appropriate in assays of combined treatment; (5) whether the doses of the cancer-preventive agent were adequately monitored; (6) whether the agent(s) was absorbed, as shown by blood concentrations; (7) whether the survival of treated animals was similar to that of controls; (8) whether the body and organ weights of treated animals were similar to those of controls; (9) whether there were adequate numbers of animals, of appropriate age, per group; (10) whether animals of each sex were used, if appropriate; (11) whether animals were allocated randomly to groups; (12) whether appropriate respective controls were used; (13) whether the duration of the experiment was adequate; (14) whether there was adequate statistical analysis; and (15) whether the data were adequately reported. If available, recent data on the incidence of specific tumours in historical controls, as well as in concurrent controls, are taken into account in the evaluation of tumour response.

Quantitative aspects. The probability that tumours will occur may depend on the species, sex, strain and age of the animals, the dose of carcinogen (if any), the dose of the agent and the route and duration of exposure. A decreased incidence and/or decreased multiplicity of neoplasms in adequately designed studies provides evidence of a cancer-preventive effect. A dose-related decrease in incidence and/or multiplicity further strengthens this association.

Statistical analysis. Major factors considered in the statistical analysis by the Working Group include

the adequacy of the data for each treatment group: (1) the initial and final effective numbers of animals studied and the survival rate; (2) body weights; and (3) tumour incidence and multiplicity. The statistical methods used should be clearly stated and should be the generally accepted techniques refined for this purpose. In particular, the statistical methods should be appropriate for the characteristics of the expected data distribution and should account for interactions in multifactorial studies. Consideration is given as to whether the appropriate adjustment was made for differences in survival.

4.2.2 Intermediate biomarkers

Other types of study include experiments in which the end-point is not cancer but a defined preneoplastic lesion or tumour-related, intermediate biomarker.

The observation of effects on the occurrence of lesions presumed to be preneoplastic or the emergence of benign or malignant tumours may aid in assessing the mode of action of the presumed cancer-preventive agent. Particular attention is given to assessing the reversibility of these lesions and their predictive value in relation to cancer development.

4.2.3 In-vitro models

Cell systems *in vitro* contribute to the early identification of potential cancer-preventive agents and to elucidation of mechanisms of cancer prevention. A number of assays in prokaryotic and eukaryotic systems are used for this purpose. Evaluation of the results of such assays includes consideration of: (1) the nature of the cell type used; (2) whether primary cell cultures or cell lines (tumorigenic or nontumorigenic) were studied; (3) the appropriateness of controls; (4) whether toxic effects were considered in the outcome; (5) whether the data were appropriately summated and analysed; (6) whether appropriate quality controls were used; (7) whether appropriate concentration ranges were used; (8) whether adequate numbers of independent measurements were made per group; and (9) the relevance of the end-points, including inhibition of mutagenesis, morphological transformation, anchorage-independent growth, cell–cell communication, calcium tolerance and differentiation.

4.3 Mechanisms of cancer prevention

Data on mechanisms can be derived from both human studies and experimental models. For a rational implementation of cancer-preventive measures, it is essential not only to assess protective end-points but also to understand the mechanisms by which the agents exert their anticarcinogenic action. Information on the mechanisms of cancer-preventive activity can be inferred from relationships between chemical structure and biological activity, from analysis of interactions between agents and specific molecular targets, from studies of specific end-points *in vitro*, from studies of the inhibition of tumorigenesis *in vivo*, from the effects of modulating intermediate biomarkers, and from human studies. Therefore, the Working Group takes account of data on mechanisms in making the final evaluation of cancer prevention.

Several classifications of mechanisms have been proposed, as have several systems for evaluating them. Cancer-preventive agents may act at several distinct levels. Their action may be: (1) extracellular, for example, inhibiting the uptake or endogenous formation of carcinogens, or forming complexes with, diluting and/or deactivating carcinogens; (2) intracellular, for example, trapping carcinogens in non-target cells, modifying transmembrane transport, modulating metabolism, blocking reactive molecules, inhibiting cell replication or modulating gene expression or DNA metabolism; or (3) at the level of the cell, tissue or organism, for example, affecting cell differentiation, intercellular communication, proteases, signal transduction, growth factors, cell adhesion molecules, angiogenesis, interactions with the extracellular matrix, hormonal status and the immune system.

Many cancer-preventive agents are known or suspected to act by several mechanisms, which

may operate in a coordinated manner and allow them a broader spectrum of anticarcinogenic activity. Therefore, multiple mechanisms of action are taken into account in the evaluation of cancer-prevention.

Beneficial interactions, generally resulting from exposure to inhibitors that work through complementary mechanisms, are exploited in combined cancer-prevention. Because organisms are naturally exposed not only to mixtures of carcinogenic agents but also to mixtures of protective agents, it is also important to understand the mechanisms of interactions between inhibitors.

5. Other beneficial effects

This section contains mainly background information on preventive activity; use is described in Section 2.3. An expanded description is given, when appropriate, of the efficacy of the agent in the maintenance of a normal healthy state and the treatment of particular diseases. Information on the mechanisms involved in these activities is described. Reviews, rather than individual studies, may be cited as references.

The physiological functions of agents such as vitamins and micronutrients can be described briefly, with reference to reviews. Data on the therapeutic effects of drugs approved for clinical use are summarized.

6. Carcinogenicity

Some agents may have both carcinogenic and anticarcinogenic activities. If the agent has been evaluated within the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, that evaluation is accepted, unless significant new data have appeared that may lead the Working Group to reconsider the evidence. When a re-evaluation is necessary or when no carcinogenic evaluation has been made, the procedures described in the Preamble to the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* are adopted as guidelines.

7. Other toxic effects

Toxic effects are of particular importance in the case of agents that may be used widely over long periods in healthy populations. Data are given on acute and chronic toxic effects, such as organ toxicity, increased cell proliferation, immunotoxicity and adverse endocrine effects. If the agent occurs naturally or has been in clinical use previously, the doses and durations used in cancer-prevention trials are compared with intakes from the diet, in the case of vitamins, and previous clinical exposure, in the case of drugs already approved for human use. When extensive data are available, only summaries are presented; if adequate reviews are available, reference may be made to these. If there are no relevant reviews, the evaluation is made on the basis of the same criteria as are applied to epidemiological studies of cancer. Differences in response as a consequence of species, sex, age and genetic variability are presented when the information is available.

Data demonstrating the presence or absence of adverse effects in humans are included; equally, lack of data on specific adverse effects is stated clearly.

Findings in human and experimental studies are presented sequentially under the headings 'Adverse effects', 'Reproductive and developmental effects' and 'Genetic and related effects'.

The section 'Adverse effects' includes information on immunotoxicity, neurotoxicity, cardiotoxicity, haematological effects and toxicity to other target organs. Specific case reports in humans and any previous clinical data are noted. Other biochemical effects thought to be relevant to adverse effects are mentioned.

The section on 'Reproductive and developmental effects' includes effects on fertility, teratogenicity, foetotoxicity and embryotoxicity. Information from nonmammalian systems and *in vitro* are presented only if they have clear mechanistic significance.

The section 'Genetic and related effects' includes results from studies in mammalian and nonmammalian systems *in vivo* and *in vitro*. Information on

whether DNA damage occurs via direct interaction with the agent or via indirect mechanisms (e.g. generation of free radicals) is included, as is information on other genetic effects such as mutation, recombination, chromosomal damage, aneuploidy, cell immortalization and transformation, and effects on cell–cell communication. The presence and toxicological significance of cellular receptors for the cancer-preventive agent are described.

The adequacy of epidemiological studies of toxic effects, including reproductive outcomes and genetic and related effects in humans, is evaluated by the same criteria as are applied to epidemiological studies of cancer. For each of these studies, the adequacy of the reporting of sample characterization is considered and, where necessary, commented upon. The available data are interpreted critically according to the end-points used. The doses and concentrations used are given, and, for experiments *in vitro*, mention is made of whether the presence of an exogenous metabolic system affected the observations. For studies *in vivo*, the route of administration and the formulation in which the agent was administered are included. The dosing regimens, including the duration of treatment, are also given. Genetic data are given as listings of test systems, data and references; bar graphs (activity profiles) and corresponding summary tables with detailed information on the preparation of genetic activity profiles are given in appendices. Genetic and other activity in humans and experimental mammals is regarded as being of greater relevance than that in other organisms. The *in-vitro* experiments providing these data must be carefully evaluated, since there are many trivial reasons why a response to one agent may be modified by the addition of another.

Structure–activity relationships that may be relevant to the evaluation of the toxicity of an agent are described.

Studies on the interaction of the suspected cancer-preventive agent with toxic and subtoxic doses of other substances are described, the objective being to determine whether there is inhibition or enhancement, additivity, synergism or potentiation of toxic effects over an extended dose range.

Biochemical investigations that may have a bearing on the mechanisms of toxicity and cancer-prevention are described. These are carefully evaluated for their relevance and the appropriateness of the results.

8. Summary of data

In this section, the relevant human and experimental data are summarized. Inadequate studies are generally not summarized; such studies, if cited, are identified in the preceding text.

8.1 Chemistry, occurrence and human exposure

Human exposure to an agent is summarized on the basis of elements that may include production, use, occurrence in the environment and determinations in human tissues and body fluids. Quantitative data are summarized when available.

8.2 Metabolism and kinetic properties

Data on metabolism and kinetic properties in humans and in experimental animals are given when these are considered relevant to the possible mechanisms of cancer-preventive, carcinogenic and toxic activity.

8.3 Cancer-preventive effects

8.3.1 Human studies

The results of relevant studies are summarized, including case reports and correlation studies when considered important.

8.3.2 Experimental studies

Data relevant to an evaluation of cancer-preventive activity in experimental models are summarized. For each animal species and route of administration, it is stated whether a change in the incidence of neoplasms or preneoplastic lesions was observed, and the tumour sites are indicated. Negative findings are also summarized. Dose– response relationships and other quantitative data may be given when available.

8.3.3 Mechanism of cancer-prevention

Data relevant to the mechanisms of cancer-preventive activity are summarized.

8.4 Other beneficial effects

When beneficial effects other than cancer prevention have been identified, the relevant data are summarized.

8.5 Carcinogenic effects

Normally, the agent will have been reviewed and evaluated within the *IARC Monographs* programme, and that summary is used with the inclusion of more recent data, if appropriate.

8.5.1 Human studies

The results of epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also summarized.

8.5.2 Experimental animals

Data relevant to an evaluation of carcinogenic effects in animal models are summarized. For each animal species and route of administration, it is stated whether a change in the incidence of neoplasms or preneoplastic lesions was observed, and the tumour sites are indicated. Negative findings are also summarized. Dose–response relationships and other quantitative data may be mentioned when available.

8.6 Toxic effects

Adverse effects in humans are summarized, together with data on general toxicological effects and cytotoxicity, receptor binding and hormonal and immunological effects. The results of investigations on the reproductive, genetic and related effects are summarized. Toxic effects are summarized for whole animals, cultured mammalian cells and non-mammalian systems. When available, data for humans and for animals are compared.

Structure–activity relationships are mentioned when relevant to toxicity.

9. Recommendations for research

During the evaluation process, it is likely that opportunities for further research will be identified. These are clearly stated, with the understanding that the areas are recommended for future investigation. It is made clear that these research opportunities are identified in general terms on the basis of the data currently available.

10. Evaluation

Evaluations of the strength of the evidence for cancer-preventive activity and carcinogenic effects from studies in humans and experimental models are made, using standard terms. These terms may also be applied to other beneficial and adverse effects, when indicated. When appropriate, reference is made to specific organs and populations.

It is recognized that the criteria for these evaluation categories, described below, cannot encompass all factors that may be relevant to an evaluation of cancer-preventive activity. In considering all the relevant scientific data, the Working Group may assign the agent or other intervention to a higher or lower category than a strict interpretation of these criteria would indicate.

10.1 Cancer-preventive activity

The evaluation categories refer to the strength of the evidence that an agent prevents cancer. The evaluations may change as new information becomes available.

Evaluations are inevitably limited to the cancer sites, conditions and levels of exposure and length of observation covered by the available studies. An evaluation of degree of evidence, whether for a single agent or a mixture, is limited to the materials tested, as defined physically, chemically or biologically. When the agents evaluated are considered by the Working Group to be sufficiently closely related, they may be grouped for the purpose of a single evaluation of degree of evidence.

Information on mechanisms of action is taken into account when evaluating the strength of

evidence in humans and in experimental animals, as well as in assessing the consistency of results between studies in humans and experimental models.

10.1.1 Cancer-preventive activity in humans

The evidence relevant to cancer prevention in humans is classified into one of the following categories.

- *Sufficient evidence of cancer-preventive activity*
  The Working Group considers that a causal relationship has been established between use of the agent and the prevention of human cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.
- *Limited evidence of cancer-preventive activity*
  The data suggest a reduced risk for cancer with use of the agent but are limited for making a definitive evaluation either because chance, bias or confounding could not be ruled out with reasonable confidence or because the data are restricted to intermediary biomarkers of uncertain validity in the putative pathway to cancer.
- *Inadequate evidence of cancer-preventive activity*
  The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding a cancer-preventive effect of the agent, or no data on the prevention of cancer in humans are available.
- *Evidence suggesting lack of cancer-preventive activity*
  Several adequate studies of use or exposure are mutually consistent in not showing a preventive effect.

The strength of the evidence for any carcinogenic effect is assessed in parallel.

Both cancer-preventive activity and carcinogenic effects are identified and, when appropriate, tabulated by organ site. The evaluation also cites the population subgroups concerned, specifying age, sex, genetic or environmental predisposing risk factors and the relevance of precancerous lesions.

10.1.2 Cancer-preventive activity in experimental animals

Evidence for cancer prevention in experimental animals is classified into one of the following categories.

- *Sufficient evidence of cancer-preventive activity*
  The Working Group considers that a causal relationship has been established between the agent and a decreased incidence and/or multiplicity of neoplasms.
- *Limited evidence of cancer-preventive activity*
  The data suggest a cancer-preventive effect but are limited for making a definitive evaluation because, for example, the evidence of cancer prevention is restricted to a single experiment, the agent decreases the incidence and/or multiplicity only of benign neoplasms or lesions of uncertain neoplastic potential or there is conflicting evidence.
- *Inadequate evidence of cancer-preventive activity*
  The studies cannot be interpreted as showing either the presence or absence of a preventive effect because of major qualitative or quantitative limitations (unresolved questions regarding the adequacy of the design, conduct or interpretation of the study), or no data on cancer prevention in experimental animals are available.
- *Evidence suggesting lack of cancer-preventive activity*
  Adequate evidence from conclusive studies in several models shows that, within the limits of the tests used, the agent does not prevent cancer.

10.2 Overall evaluation

Finally, the body of evidence is considered as a whole, and summary statements are made that encompass the effects of the agents in humans with regard to cancer-preventive activity, carcinogenic effects and other beneficial and adverse effects, as appropriate.

General Remarks

Introduction and definitions

In much of the scientific literature on nutrition, 'vitamin A' is used as a generic term referring to both preformed vitamin A (largely all-trans-retinol and its esters) and some of the carotenoids. This Handbook, the third in the IARC series of *Handbooks of Cancer Prevention*, focuses on the cancer-preventive effects of the preformed vitamin A compounds, principally retinol and retinyl esters. Volume 2 of the series reviewed the carotenoids (IARC, 1998) and the forthcoming Volume 4 will review in detail retinoic acid, metabolites of retinol and related synthetic retinoid compounds. The full scientific terminology, common abbreviated names and relevant general terms used to describe both individual compounds and broad classes of vitamin A compounds are summarized in Table 1.

Compounds in the vitamin A, or retinol, family, as defined by the International Union of Pure and Applied Chemistry/International Union of Biology (IUPAC-IUB) Joint Commission on Biochemical Nomenclature, belong to a class derived from four isoprenoid units joined in a head-to-tail manner to produce a monocyclic parent having five carbon–carbon double bonds and a functional group at the acyclic terminus. The parent compound in preformed vitamin A, from which retinal and retinyl esters are derived, is all-*trans*-retinol (hereinafter referred to as retinol). The structures, physical properties and sources of retinol, retinyl esters, retinal and retinoic acid are listed in Section 1 of this Handbook.

Many of the significant discoveries in chemistry and biology that have led to our understanding of the critical roles in nutrition played by retinol, retinal, retinoic acid and their analogues, have been thoroughly reviewed (Moore, 1957; Tee, 1992; Blomhoff, 1994; Sporn *et al.*, 1994). In 1913, McCollum and Davies as well as, independently, Osborne and Mendel identified a lipid-soluble dietary factor needed for the growth of rats. It was later termed 'fat-soluble A'. In 1931, Karrer and co-workers determined

Table 1. Nomenclature of vitamin A compounds

Full name	Common abbreviated names	Included in class known as: 'Preformed vitamin A'	'Vitamin A precursors'	'Total vitamin A'
All-*trans*-retinol	Retinol	Yes		Yes
All-*trans*-retinol palmitate	Retinyl palmitate	Yes		Yes
All-*trans*-retinol acetate	Retinyl acetate[a]	Yes		Yes
Provitamin A carotenoids [b]			Yes	Yes
All-*trans*-retinal	Retinal, retinaldehyde[c]			
All-*trans*-retinoic acid	Retinoic acid			
9-*cis*-Retinoic acid	9-*cis*-Retinoic acid			
Other natural metabolites				
Retinoid[d]				
Synthetic retinoids[e]				

[a] Retinyl acetate is a usable form of preformed vitamin A, but it is found in only small amounts in the diet.

[b] Carotenoids that are metabolized to retinol in vivo. In the diet, these are largely α- and β-carotene and β-cryptoxanthin.

[c] The term retinaldehyde is used in this volume only if confusion could occur with the adjective 'retinal' relating to the retina of the eye.

[d] A member of the vitamin A family the structure of which is related to those of retinol, retinal or retinoic acid or their derivatives.

[e] A synthetic retinoid having structural modifications not found in natural compounds. Generally, this term is used in reference to analogues of retinoic acid and its double-bond isomers. These compounds will be covered in detail in Volume 4 of the IARC Handbook series.

the structure of retinol, and soon thereafter the structure of β-carotene. In 1937, retinol was first crystallized from fish liver oil, from which the first crystalline esters were also isolated five years later. In 1946, Van Dorp and Arens synthesized retinoic acid and in 1947 the Isler group reported a commercially feasible synthesis of retinol. In 1950, Karrer and Eugster synthesized β-carotene. Synthetic retinol serves as the precursor for the retinyl esters used widely in vitamin A supplements and in food fortificants. These early developments have been summarized by Moore (1957).

Both plants and animals are sources of vitamin A for humans. Plants contain carotenoids, some of which are provitamin A compounds, but they contain no preformed vitamin A. Over 600 naturally occurring carotenoids have been identified, but only about 10 are major sources of vitamin A (Olson, 1994). Animal products contain vitamin A predominantly in the form of retinyl esters, but also as retinol and, in small amounts, as provitamin A carotenoids originating from plants consumed by the animals. Both synthetic and natural vitamin A and carotenoids are also found in pharmaceuticals and cosmetics. Section 2 of this Handbook covers the major sources of vitamin A.

Because of the low water-solubility of retinol, retinal and retinoic acid, the retinoid-binding proteins play an important role in providing a source of stable, solubilized retinoids for target tissues. Specific binding proteins selectively compartmentalize and regulate retinol levels. The nuclear retinoic acid receptor proteins function as transcription factors, permitting the retinoids to act as hormones that modulate the activity of retinoid-responsive genes. The binding proteins are therefore thought both to regulate the metabolic transformations of vitamin A and to prevent toxic effects that could be caused by high levels of unbound retinoids. The distribution of retinoids in tissues and their metabolic transformations, functions and characteristics are further described in Section 3 of this Handbook.

Vitamin A in human nutrition and cancer

In 1922, Mori observed corneal and conjunctival keratinization and interference with tear production in vitamin A-deficient rats. In 1925, Wolbach and Howe observed metaplastic changes in gastrointestinal, genitourinary, ocular and respiratory epithelia (replacement of columnar and transitional cells by squamous, keratinized cells) in animals deprived of vitamin A. These changes were morphologically similar to, but distinct from, certain pre-neoplastic lesions (reviewed by Shapiro, 1986). The observation of inhibitory action of vitamin A on the induction of tumours of the forestomach and cervix (Chu & Malmgren, 1965) and of bronchotracheal tumours (Saffiotti *et al.*, 1967) stimulated much interest in the relationship between vitamin A-dependent cell differentiation and cancer. In the next 15 years, the interactive roles of retinoids in the chemoprevention of carcinogenesis were carefully explored (Sporn & Roberts, 1983). Subsequently, the roles of retinoic acid and its 9-*cis* isomer in controlling cell differentiation, growth and reproduction were established (reviewed in Gudas *et al.*, 1994; Mangelsdorf *et al.*, 1994; Blomhoff, 1994). Several reviews on cancer prevention studies using vitamin A in cell culture, in animals and in man have been published in recent years (Hong & Itri, 1994; Moon *et al.*, 1994; Alberts & Garcia, 1995; Kelloff *et al.*, 1996; Minna & Mangelsdorf, 1997; Sankaranarayanan *et al.*, 1997).

'Chemoprevention' is a term that has been widely used to describe intentional chemical interference with the process of carcinogenesis by inducing a variety of biological mechanisms. Chemoprevention can be achieved by preventing the onset of carcinogenesis by protecting against initiation or by arresting or reversing stages of carcinogenesis at various steps in the processes of promotion and progression. The use of agents to prevent cancer in healthy individuals falls within the framework of 'primary prevention'. Chemoprevention used to arrest or reverse carcinogenesis in individuals who have been identified as having a pre-malignant lesion falls within the framework of 'secondary prevention' (the prevention of disease at a preclinical stage). 'Tertiary prevention', defined as the prevention of complications (recurrence, invasion, metastases) among people already diagnosed with symptomatic disease, is best regarded in the context of clinical management

of the cancer patient. Tertiary prevention is not considered in this Handbook.

The discoveries that have enhanced our understanding of the various phases in the multi-step process of carcinogenesis have also led to the current understanding that preventive agents may act in different ways at different stages of progression. Hence, potential cancer-preventive agents, such as vitamin A, appear to be useful for either primary or secondary cancer prevention, or for both. Potential chemopreventive agents could be useful among individuals at average cancer risk, or among those at high cancer risk due to other behavioural or genetic factors (e.g., smokers or carriers of cancer-relevant genetic mutations). Section 4 reviews human observational and intervention studies using primary and secondary prevention strategies among subjects at various levels of cancer risk, as well as animal experimental studies.

Vitamin A deficiency is clearly a continuing public health problem in many areas of the world (Sommer & West, 1996). Because vitamin A deficiency can lead to increased risk for many health problems including infection and blindness, programmes to supplement and fortify foods for undernourished populations continue to be an important public health priority. The public health problem of vitamin A deficiency and the potential role of vitamin A in cancer prevention are two important but distinctly different issues. The implications and inferences that can be drawn from epidemiological observational studies and from the limited set of intervention studies using supplements of preformed vitamin A are reviewed in Section 4.

Dietary factors are thought to have a major causative role in cancer induction. Though the contribution of diet to cancer risk probably varies across populations, it has been estimated that in the United States possibly one third of all cancer deaths may be attributable to nutritional factors (Doll & Peto, 1981). A consistent finding for many cancer sites has been that diets with a high proportion of fruits and vegetables, and hence high levels of carotenoids, are associated with lower cancer risk (IARC, 1998). This Handbook focuses more specifically on the evidence for a protective effect of preformed vitamin A.

Issues in research on vitamin A

Knowledge of the effects of vitamin A and its molecular mechanisms of action is continually expanding. Thus, over the past several decades, as methods and understanding have advanced, findings from older studies need to be viewed in their historical context. As in other areas of cancer prevention research, studies of vitamin A have been carried out using a wide variety of methods, including animal experiments *in vitro* and *in vivo*, observational human studies, and, ultimately, intervention trials in humans. Thus, it has been a formidable challenge to compare and contrast findings across different study designs and settings to produce a coherent evaluation of the overall potential of vitamin A as a cancer-preventive agent in human populations. Nonetheless, the Working Group has conducted such an evaluation, first summarizing the convergent and divergent data in support of the general conclusions (Section 8), and finally producing an overall evaluation of the cancer-preventive potential of vitamin A (Section 10). These summaries and conclusions afforded the Working Group the opportunity to identify research areas critical for the advancement of cancer prevention that could lead to implementation of successful prophylactic treatments. Thus, future research should be directed to areas listed in Section 9 that the Working Group considered of high priority.

An important limitation of the use of preformed vitamin A in cancer prevention in humans is the toxicity that is seen at high doses. Toxic effects are seen in various organs, including the skin, circulation (e.g., hypertriglyceridaemia), liver, nervous system and bones (see Section 7). Some aspects of toxicity could be explained by overwhelming of the binding capacity of cellular and extracellular binding proteins by excess vitamin A or retinoids, which would result in the presence of unbound ('free') vitamin A, which might interact differently with receptors or other sites of retinoid action. Of particular concern regarding the widespread ingestion of preformed vitamin A supplements is the apparent sensitivity of the

developing embryo to teratogenesis by daily supplemental retinyl palmitate at 25 000 IU per day or possibly less (Nau *et al.*, 1994). Such effects have prompted the development of literally thousands of synthetic retinoids designed to have more specific beneficial properties, but with lower toxic potential. The cancer-preventive potential of these synthetic retinoids will be covered in detail in Volume 4 of the IARC Handbook series. An active retinoid without teratogenic potential is yet to be identified, which suggests that retinoid receptors may play important roles in aspects of both the desired activity and the teratogenicity and reproductive toxicity.

One of the particular challenges in interpreting the effects of vitamin A on cancer is that studies have been performed at widely differing levels of vitamin A nutritive status. Obviously, experimental studies in humans maintained in a vitamin A-deficient state cannot be ethically justified. Therefore, studies have been performed in animals. For instance, several studies have indicated greater effects with supplementation in animals that were first fed vitamin A-deficient diets than in animals on normal diets. In animal studies, it is also possible to test the effects of vitamin A at high doses, where toxicity is often seen, whereas this type of research in human populations is also not justifiable.

A striking feature of vitamin A physiology is the elaborate mechanism of homeostatic control of circulating concentrations of plasma retinol across a broad range of intakes of preformed vitamin A and provitamin A. Therefore, much of the human observational and experimental work, which has been carried out within this homeostatically controlled range, may not be comparable to animal experimental studies with vitamin A at levels of deprivation or excess. Also related to the phenomenon of homeostasis is the limitation of the use of serum retinol levels as a measure of vitamin A status (see Section 2). Circulating retinol concentrations remain fairly constant until liver reserves fall to very low levels (below 0.07 mmol/g) (Olson, 1994), and factors other than intake, especially infection, infestation, malnutrition and acute stress, can affect circulating retinol levels (Section 5.2), thus limiting the value of plasma levels in other than long-term prospective studies. Vitamin A status can now be estimated by the relative dose–response and the modified relative dose–response tests, which have been widely used to assess vitamin A deficiency states. A more precise method of estimating total body stores, namely the isotope dilution method using deuterated retinol (Olson, 1994), has not been widely used, as it is technically demanding.

Purposes of this Handbook

The review and commentary in this Handbook is intended for use by researchers, clinicians, educators, and public health policy makers interested in cancer prevention and nutrition. This handbook is intended to provide a comprehensive review of the relevant information in the published scientific literature available to the Working Group on the role of vitamin A in cancer prevention. The focus of this critical review and commentary is on retinol and the retinyl esters. Some scientific literature in this field overlaps with reports dealing with vitamin A metabolites, vitamin A precursors and total dietary vitamin A (which is a combination of preformed vitamin A and its precursors), so information from this wide range of research is included in the current review when it was deemed relevant to our understanding of the observed effects of retinol or retinyl esters on cancer development. The observed effects of preformed vitamin A in cell and organ culture, in animal models and in human dietary observational epidemiological studies and intervention studies are reviewed. Based on this review, the Working Group offers recommendations for future research on the use of vitamin A in cancer prevention (Section 9) and an overall evaluation of the strength of the evidence for a role of vitamin A for cancer prevention in humans (Section 10).

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