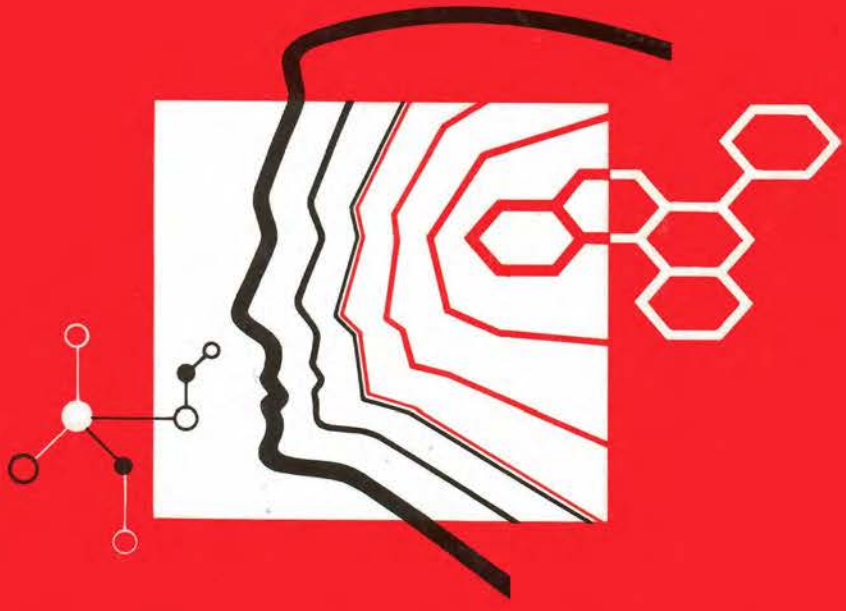
IPCS

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

UNEP

Environmental Health Criteria 200

Copper



IOMC

INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS
A cooperative agreement among UNEP, ILO, FAO, WHO, UNIDO, UNITAR and OECD

WORLD HEALTH ORGANIZATION

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Environmental Health Criteria 200

COPPER

First draft prepared by Dr C. Dameron and colleagues at the National Research Centre for Environmental Toxicology, Australia, and by Mr P.D. Howe, Institute of Terrestrial Ecology, Monks Wood, United Kingdom

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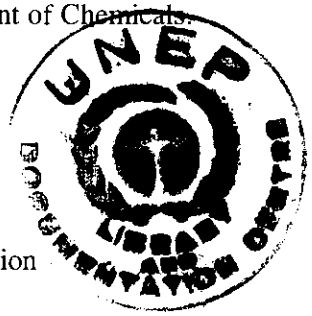

World Health Organization
Geneva, 1998

The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to chemicals, through international peer-review processes, as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sonnd management of chemicals.

The **Inter-Organization Programme for the Sound Management of Chemicals (IOMC)** was established in 1995 by UNEP, ILO, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 UN Conference on Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

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WORLD HEALTH ORGANIZATION

CORRIGENDA

ENVIRONMENTAL HEALTH CRITERIA
No. 202

SELECTED NON-HETEROCYCLIC POLYCYCLIC AROMATIC HYDROCARBONS

Page 11, line 26:
Delete: 26 *Insert*: 17

Page 13: a revised page is attached

Page 21, paragraph 1: The last 3 sentences should read:

Flash-points were available only for three compounds with high molecular mass (for naphthalene, 78.9 °C by the open-cup method and 87.8 °C by the closed cup method; anthracene, 121 °C by the closed-cup method; and phenanthrene, 171 °C by the open cup method). Explosion limits were available only for naphthalene (0.9–5.9 vol %) and ananthrene (0.6 vol %) (Lewis, 1992). Vapour density (air = 1) was 4.42 for naphthalene (IARC, 1973), 5.32 for acenaphthene, 6.15 for anthracene (Lewis, 1992), 6.15 for phenanthrene, and 8.7 for benzo[*a*]pyrene (National Institute for Occupational Safety and Health and Occupational Safety and Health Administration, 1981).

Page 326, line 5:
Delete: *pyrene, triphenylene, perylene, anthanthrene*
Insert: triphenylene, perylene, benzo[ghi]*fluoranthene*

Page 326, line 6:
Delete: *eight* *Insert*: *nine*

Page 399: Following the second entry in Table 90 (Graffi et al., 1953)
Insert: the heading ***Anthanthrene***

Page 400:
Delete: the heading ***Acenaphthene*** (contd)
Insert: the heading ***Anthanthrene*** (contd)

Page 473, entry 1:
Delete: Benzo[*a*]fluorene *Insert*: Benzo[*b*]fluorene

Page 575
Delete: the heading ***Fluoranthene*** *Insert*: the heading ***Fluorene***

Table 2. Summary of results of tests for genotoxicity and carcinogenicity for the 33 polycyclic aromatic hydrocarbons studied

Compound	Genotoxicity	Carcinogenicity
Acenaphthene	(?)	?
Acenaphthylene	(?)	No studies
Anthanthrene	(+)	+
Anthracene	-	-
Benz[*a*]anthracene	+	+
Benzo[*b*]fluoranthene	+	+
Benzo[*j*]fluoranthene	+	+
Benzo[*ghi*]fluoranthene	(+)	(-)
Benzo[*k*]fluoranthene	+	+
Benzo[*a*]fluorene	(?)	(?)
Benzo[*b*]fluorene	(?)	(?)
Benzo[*ghi*]perylene	+	-
Benzo[*c*]phenanthrene	(+)	(+)
Benzo[*a*]pyrene	+	+
Benzo[*e*]pyrene	+	?
Chrysene	+	+
Coronene	(+)	(?)
Cyclopenta[*cd*]pyrene	+	+
Dibenz[*a,h*]anthracene	+	+
Dibenzo[*a,e*]pyrene	+	+
Dibenzo[*a,h*]pyrene	(+)	+
Dibenzo[*a,i*]pyrene	+	+
Dibenzo[*a,l*]pyrene	(+)	+
Fluoranthene	+	(+)
Fluorene	-	-
Indeno[1,2,3-*cd*]pyrene	+	+
5-Methylchrysene	+	+
1-Methylphenanthrene	+	(-)
Naphthalene	-	(?)
Perylene	+	(-)
Phenanthrene	(?)	(?)
Pyrene	(?)	(?)
Triphenylene	+	(-)

+, positive; -, negative; ?, questionable
Parentheses, result derived from small database

reported to induce skin cancer. The lung is now the main site of PAH-induced cancer, whereas skin tumours have become more rare because of better personal hygiene.

CONTENTS

NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

* * *

A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Case postale 356, 1219 Châtelaine, Geneva, Switzerland (telephone no. + 41 22 - 9799111, fax no. + 41 22 - 7973460, E-mail irptc@unep.ch).

* * *

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Environmental Health Criteria

PREAMBLE

Objectives

In 1973 the WHO Environmental Health Criteria Programme was initiated with the following objectives:

(i) to assess information on the relationship between exposure to environmental pollutants and human health, and to provide guidelines for setting exposure limits;

(ii) to identify new or potential pollutants;

(iii) to identify gaps in knowledge concerning the health effects of pollutants;

(iv) to promote the harmonization of toxicological and epidemiological methods in order to have internationally comparable results.

The first Environmental Health Criteria (EHC) monograph, on mercury, was published in 1976 and since that time an ever-increasing number of assessments of chemicals and of physical effects have been produced. In addition, many EHC monographs have been devoted to evaluating toxicological methodology, e.g. for genetic, neurotoxic, teratogenic and nephrotoxic effects. Other publications have been concerned with epidemiological guidelines, evaluation of short-term tests for carcinogens, biomarkers, effects on the elderly and so forth.

Since its inauguration the EHC Programme has widened its scope, and the importance of environmental effects, in addition to health effects, has been increasingly emphasized in the total evaluation of chemicals.

The original impetus for the Programme came from World Health Assembly resolutions and the recommendations of the 1972 UN Conference on the Human Environment. Subsequently the work became an integral part of the International Programme on Chemical Safety (IPCS), a cooperative programme of UNEP, ILO and WHO. In

this manner, with the strong support of the new partners, the importance of occupational health and environmental effects was fully recognized. The EHC monographs have become widely established, used and recognized throughout the world.

The recommendations of the 1992 UN Conference on Environment and Development and the subsequent establishment of the Intergovernmental Forum on Chemical Safety with the priorities for action in the six programme areas of Chapter 19, Agenda 21, all lend further weight to the need for EHC assessments of the risks of chemicals.

Scope

The criteria monographs are intended to provide critical reviews on the effect on human health and the environment of chemicals and of combinations of chemicals and physical and biological agents. As such, they include and review studies that are of direct relevance for the evaluation. However, they do not describe *every* study carried out. Worldwide data are used and are quoted from original studies, not from abstracts or reviews. Both published and unpublished reports are considered and it is incumbent on the authors to assess all the articles cited in the references. Preference is always given to published data. Unpublished data are used only when relevant published data are absent or when they are pivotal to the risk assessment. A detailed policy statement is available that describes the procedures used for unpublished proprietary data so that this information can be used in the evaluation without compromising its confidential nature (WHO (1990) Revised Guidelines for the Preparation of Environmental Health Criteria Monographs. PCS/90.69, Geneva, World Health Organization).

In the evaluation of human health risks, sound human data, whenever available, are preferred to animal data. Animal and *in vitro* studies provide support and are used mainly to supply evidence missing from human studies. It is mandatory that research on human subjects is conducted in full accord with ethical principles, including the provisions of the Helsinki Declaration.

The EHC monographs are intended to assist national and international authorities in making risk assessments and subsequent risk

management decisions. They represent a thorough evaluation of risks and are not, in any sense, recommendations for regulation or standard setting. These latter are the exclusive purview of national and regional governments.

Content

The layout of EHC monographs for chemicals is outlined below.

- Summary – a review of the salient facts and the risk evaluation of the chemical
- Identity – physical and chemical properties, analytical methods
- Sources of exposure
- Environmental transport, distribution and transformation
- Environmental levels and human exposure
- Kinetics and metabolism in laboratory animals and humans
- Effects on laboratory mammals and *in vitro* test systems
- Effects on humans
- Effects on other organisms in the laboratory and field
- Evaluation of human health risks and effects on the environment
- Conclusions and recommendations for protection of human health and the environment
- Further research
- Previous evaluations by international bodies, e.g. IARC, JECFA, JMPR

Selection of chemicals

Since the inception of the EHC Programme, the IPCS has organized meetings of scientists to establish lists of priority chemicals for subsequent evaluation. Such meetings have been held in Ispra, Italy, 1980; Oxford, United Kingdom, 1984; Berlin, Germany, 1987; and North Carolina, USA, 1995. The selection of chemicals has been based on the following criteria: the existence of scientific evidence that the substance presents a hazard to human health and/or the environment; the possible use, persistence, accumulation or degradation of the substance shows that there may be significant human or environmental exposure; the size and nature of populations at risk (both human and other species) and risks for euvironment; international concern, i.e. the

substance is of major interest to several countries; adequate data on the hazards are available.

If an EHC monograph is proposed for a chemical not on the priority list, the IPCS Secretariat consults with the Cooperating Organizations and all the Participating Institutions before embarking on the preparation of the monograph.

Procedures

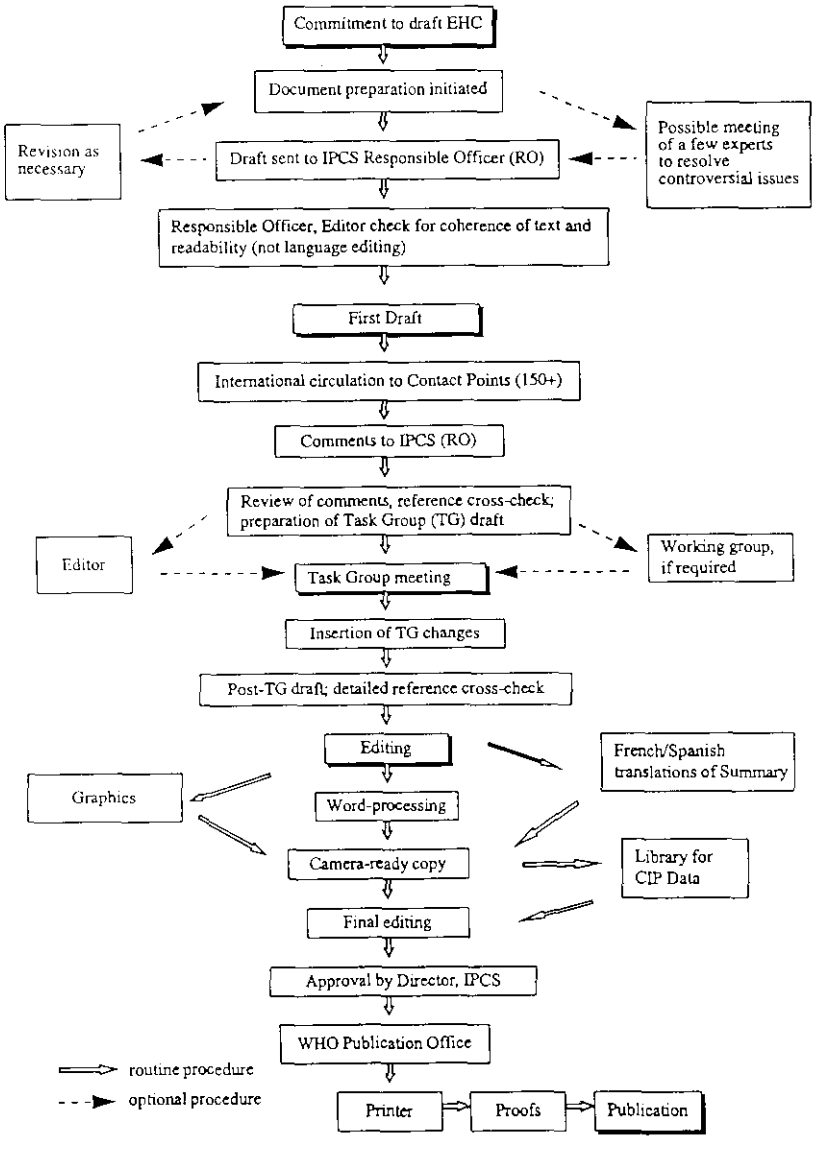
The order of procedures that result in the publication of an EHC monograph is shown in the flow chart on p. xv. A designated staff member of IPCS, responsible for the scientific quality of the document, serves as Responsible Officer (RO). The IPCS Editor is responsible for layout and language. The first draft, prepared by consultants or, more usually, staff from an IPCS Participating Institution, is based initially on data provided from the International Register of Potentially Toxic Chemicals, and reference data bases such as Medline and Toxline.

The draft document, when received by the RO, may require an initial review by a small panel of experts to determine its scientific quality and objectivity. Once the RO finds the document acceptable as a first draft, it is distributed, in its unedited form, to well over 150 EHC contact points throughout the world who are asked to comment on its completeness and accuracy and, where necessary, provide additional material. The contact points, usually designated by governments, may be Participating Institutions, IPCS Focal Points, or individual scientists known for their particular expertise. Generally some four months are allowed before the comments are considered by the RO and author(s). A second draft incorporating comments received and approved by the Director, IPCS, is then distributed to Task Group members, who carry out the peer review, at least six weeks before their meeting.

The Task Group members serve as individual scientists, not as representatives of any organization, government or industry. Their function is to evaluate the accuracy, significance and relevance of the information in the document and to assess the health and environmental risks from exposure to the chemical. A summary and recommendations for further research and improved safety aspects are also required. The composition of the Task Group is dictated by the range of expertise required for the subject of the meeting and by the need for a balanced geographical distribution.

Flow Chart

EHC PREPARATION FLOW CHART



The three cooperating organizations of the IPCS recognize the important role played by nongovernmental organizations. Representatives from relevant national and international associations may be invited to join the Task Group as observers. Although observers may provide a valuable contribution to the process, they can only speak at the invitation of the Chairperson. Observers do not participate in the final evaluation of the chemical; this is the sole responsibility of the Task Group members. When the Task Group considers it to be appropriate, it may meet *in camera*.

All individuals who as authors, consultants or advisers participate in the preparation of the EHC monograph must, in addition to serving in their personal capacity as scientists, inform the RO if at any time a conflict of interest, whether actual or potential, could be perceived in their work. They are required to sign a conflict of interest statement. Such a procedure ensures the transparency and probity of the process.

When the Task Group has completed its review and the RO is satisfied as to the scientific correctness and completeness of the document, it then goes for language editing, reference checking and preparation of camera-ready copy. After approval by the Director, IPCS, the monograph is submitted to the WHO Office of Publications for printing. At this time a copy of the final draft is sent to the Chairperson and Rapporteur of the Task Group to check for any errors.

It is accepted that the following criteria should initiate the updating of an EHC monograph: new data are available that would substantially change the evaluation; there is public concern for health or environmental effects of the agent because of greater exposure; an appreciable time period has elapsed since the last evaluation.

All Participating Institutions are informed, through the EHC progress report, of the authors and institutions proposed for the drafting of the documents. A comprehensive file of all comments received on drafts of each EHC monograph is maintained and is available on request. The Chairpersons of Task Groups are briefed before each meeting on their role and responsibility in ensuring that these rules are followed.

WHO TASK GROUP ON ENVIRONMENTAL HEALTH CRITERIA FOR COPPER

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WHO TASK GROUP ON ENVIRONMENTAL HEALTH CRITERIA FOR COPPER

A WHO Task Group on Environmental Health Criteria for Copper met in Brisbane, Australia, from 24 to 28 June 1996. The meeting was sponsored by a consortium of Australian Commonwealth and State Governments through a national steering committee chaired by Dr K. Bentley, Director, Health and Environmental Policy, Department of Health and Family Services, Canberra. The meeting was co-hosted and organized by the Department of Health and Family Services, Commonwealth of Australia, the Queensland Departments of Health, Environment and Heritage, and the National Research Centre for Environmental Toxicology. Participants were welcomed by Dr G.R. Neville, Principal Medical Adviser, Queensland Health on behalf of the host organizations. In opening the meeting, Dr G.C. Becking, on behalf of Dr M. Mercier, Director of the IPCS and the three cooperating organizations (UNEP/ILO/WHO), thanked the Australian Commonwealth and State Governments for their longstanding generous support in providing funding for this Task Group as well as several previous IPCS Task Groups and consultations over the last four years. He thanked the Staff of Queensland Health and the National Research Centre for Environmental Toxicology for their excellent work in organizing the Task Group for Copper. The Task Group reviewed and revised the draft criteria monograph, and made an evaluation of the risks to human health and the environment from exposure to copper.

The first draft of this monograph was prepared by Dr C. Dameron and colleagues at the National Research Centre for Environmental Toxicology, Australia, and by Mr P.D. Howe, Institute of Terrestrial Ecology, Monks Wood, United Kingdom. The Task Group draft, incorporating the comments received from the IPCS Contact Points for Environmental Health Criteria monographs, was prepared by Mr P.D. Howe and the Secretariat.

Dr G.C. Becking (IPCS Central Unit, Interregional Research Unit) and Ms K. Lyle (Sheffield, England) were responsible for the overall scientific content and technical editing, respectively, of this monograph.

The efforts of all who helped in the preparation and finalization of this publication are gratefully acknowledged.

ABBREVIATIONS

AAS	atomic absorption spectroscopy
ALAD	aminolaevulinic acid dehydratase
ALAT	alanine aminotransferase
AROI	acceptable range of oral intake
ASAT	aspartate aminotransferase
ASV	anodic stripping voltammetry
AVS	acid volatile sulfides
CEC	cation exchange capacity
CNS	central nervous system
CSV	cathodic stripping voltammetry
CT_{MAX}	critical thermal maxima
DT-OCEE	deficiency toxicity optimum concentration for essential elements
EDTA	ethylene diamine tetraacetic acid
EPA	Environmental Protection Agency (USA)
ER	endoplasmic reticulum
FI-AAS	flow-injection atomic absorption spectroscopy
GF-AAS	graphite furnace atomic absorption spectroscopy
GLC	gas liquid chromatography
GLC-MS	gas liquid chromatography–mass spectrometry
HDL	high density lipoprotein
HPLC	high performance liquid chromatography
IC	ion chromatography
ICC	Indian childhood cirrhosis
ICP-AES	inductively coupled plasma–atomic emission spectroscopy
ICP-ES	inductively coupled plasma–emission spectroscopy
ICP-MS	inductively coupled plasma–mass spectrometry
ICT	idiopathic copper toxicosis
LBW	low birth weight

LDL	low density lipoprotein
LEC	Long–Evans Cinnamon (rat)
LOEC	lowest-observed-effect concentration
MATC	maximum acceptable toxicant concentration
MRE	metal responsive element
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NTA	nitrilotriacetic acid
OCEE	optimal concentration of essential elements
PIXE	proton-induced X-ray fluorescence
PTDI	provisional tolerable daily intake
RER	rough endoplasmic reticulum
SAAM	standard algal assay medium
SER	smooth endoplasmic reticulum
SOD	superoxide dismutase
TIMS	thermal ionization mass spectrometry
UV	ultraviolet
XRF	X-ray fluorescence

1. SUMMARY AND CONCLUSIONS

1.1 Identity, physical and chemical properties

Copper is a reddish-brown, ductile and malleable metal. It belongs to group IB of the Periodic Table. In compounds found in the environment it usually has a valence of 2 but can exist in the metallic, +1 and +3 valence states. Copper is found naturally in a wide variety of mineral salts and organic compounds, and in the metallic form. The metal is sparingly soluble in water, salt or mildly acidic solutions, but can be dissolved in nitric and sulfuric acids as well as basic solutions of ammonium hydroxide or carbonate.

Copper possesses high electrical and thermal conductivity and resists corrosion.

1.2 Analytical methods

The wide range of copper species, inorganic and organic, has led to the development of an array of sampling techniques, preparation and analytical methods to quantify the element in environmental and biological samples. Contamination of the samples with copper from air, dusts, vessels or reagents during sampling and preparation is a major source of analytical errors, and "clean" techniques are essential.

Colorimetric and gravimetric methods for the measurement of copper are simple to use and are inexpensive; however, their usefulness is limited to situations where extreme sensitivity is not essential. For measurement of low concentrations of copper in various matrices, atomic absorption spectrophotometric (AAS) methods are the most widely used. A dramatic increase in sensitivity is obtained by the utilization of graphite furnace atomic absorption spectrophotometry (GF-AAS) rather than flame AAS. Depending upon sample pre-treatment, separation and concentration procedures, detection limits of about 1 µg/litre in water by GF-AAS and 20 µg/litre by AAS have been reported and levels of 0.05–0.2 µg/g of tissue have been detected by GF-AAS. Greater sensitivities can be achieved through the use of emission techniques such as high temperature inductively coupled argon plasma techniques followed by atomic emission spectroscopy

(ICP-AES) or a mass spectrometer (ICP-MS). Other more sensitive and specialized methodologies are available such as X-ray fluorescence, ion-selective electrodes and potentiometric methods, and anodic stripping and cathodic stripping voltametry.

1.3 Sources of human and environmental exposure

Natural sources of copper exposure include windblown dust, volcanoes, decaying vegetation, forest fires and sea spray. Anthropogenic emissions include smelters, iron foundries, power stations and combustion sources such as municipal incinerators. The major release of copper to land is from tailings and overburdens from copper mines and sewage sludge. Agricultural use of copper products accounts for 2% of copper released to soil.

Copper ores are mined, smelted and refined to produce many industrial and commercial products. Copper is widely used in cooking utensils and water distribution systems, as well as fertilizers, bactericides, fungicides, algicides and antifouling paints. It is also used in animal feed additives and growth promoters, as well as for disease control in livestock and poultry. Copper is used in industry as an activator in froth flotation of sulfide ores, production of wood preservatives, electroplating, azo-dye manufacture, as a mordant for textile dyes, in petroleum refining and the manufacture of copper compounds.

1.4 Environmental transport, distribution and transformation

Copper is released to the atmosphere in association with particulate matter. It is removed by gravitational settling, dry deposition, washout by rain and rainout. Removal rate and distance travelled from the source depend on source characteristics, particle size and wind velocity.

Copper is released to water as a result of natural weathering of soil and discharges from industries and sewage treatment plants. Copper compounds may also be intentionally applied to water to kill algae. Several processes influence the fate of copper in the aqueous environment. These include complex formation, sorption to hydrous

metal oxides, clays and organic materials, and bioaccumulation. Information on the physicochemical forms of copper (speciation) is more informative than total copper concentrations. Much of the copper discharged to water is in particulate form and tends to settle out, precipitate out or be adsorbed by organic matter, hydrous iron, manganese oxides and clay in the sediment or water column. In the aquatic environment the concentration of copper and its bioavailability depend on factors such as water hardness and alkalinity, ionic strength, pH and redox potential, complexing ligands, suspended particulate matter and carbon, and the interaction between sediments and water.

The largest release of copper is to land; the major sources of release are mining operations, agriculture, solid waste and sludge from treatment works. Most copper deposited in soil is strongly adsorbed and remains in the upper few centimetres of soil. Copper adsorbs to organic matter, carbonate minerals, clay minerals, hydrous iron and manganese oxides. The greatest amount of leaching occurs from sandy acidic soils. In the terrestrial environment a number of important factors influence the fate of copper in soil. These include the nature of the soil itself, pH, presence of oxides, redox potential, charged surfaces, organic matter and cation exchange.

Bioaccumulation of copper from the environment occurs if the copper is biologically available. Accumulation factors vary greatly between different organisms, but tend to be higher at lower exposure concentrations. Accumulation may lead to exceptionally high body burdens in certain animals (such as bivalves) and terrestrial plants (such as those growing on contaminated soils). However, many organisms are capable of regulating their body copper concentration.

1.5 Environmental levels and human exposure

The concentration of copper in air depends on the proximity of the site to major sources such as smelters, power plants and incinerators. Copper is widely distributed in water because it is a naturally occurring element. However, care must be taken when interpreting copper concentrations in the aquatic environment. In aquatic systems the environmental levels of copper are usually measured as either total or dissolved concentrations, with the latter being more representative of the bioavailability of the metal.

Average background concentrations of copper in air in rural areas range from 5 to 50 ng/m^3. Copper levels in seawater of 0.15 µg/litre and in fresh water of 1–20 µg/litre are found in uncontaminated areas. Sediment is an important sink and reservoir for copper. Background levels of copper in natural freshwater sediments range from 16 to 5000 mg/kg (dry weight). Copper levels in marine sediments range from 2 to 740 mg/kg (dry weight). In anoxic sediments copper is bound strongly by sulfide and therefore not bioavailable. Median copper concentrations in uncontaminated soil were reported to be 30 mg/kg (range 2–250 mg/kg). Copper is accumulated by plants, invertebrates and fish. Higher concentrations of copper have been reported in organisms from copper-contaminated sites than in those from non-contaminated sites.

For healthy, non-occupationally-exposed humans the major route of exposure to copper is oral. The mean daily dietary intake of copper in adults ranges between 0.9 and 2.2 mg. A majority of studies have found intakes to be at the lower end of that range. The variation reflects different dietary habits as well as different agricultural and food processing practices used worldwide. In some cases, drinking-water may make a substantial additional contribution to the total daily intake of copper, particularly in households where corrosive waters have stood in copper pipes. In homes without copper piping or with noncorrosive water, copper intake from drinking-water seldom exceeds 0.1 mg/day, although intakes greater than a few mg per day can result from corrosive water distributed through copper pipes. In general, total daily oral intakes of copper (food plus drinking-water) are between 1 and 2 mg/day, although they may occasionally exceed 5 mg/day. All other intakes of copper (inhalation and dermal) are insignificant in comparison to the oral route. Inhalation adds 0.3–2.0 µg/day from dusts and smoke. Women using copper IUDs are exposed to only 80 µg or less of copper per day from this source.

1.6 Kinetics and metabolism in laboratory animals and humans

The homoeostasis of copper involves the dual essentiality and toxicity of the element. Its essentiality arises from its specific incorporation into a large number of proteins for catalytic and structural purposes. The cellular pathways of uptake, incorporation

into protein and export of copper are conserved in mammals and modulated by the metal itself.

Copper is mainly absorbed through the gastrointestinal tract. From 20 to 60% of the dietary copper is absorbed, with the rest being excreted through the faeces. Once the metal passes through the basolateral membrane it is transported to the liver bound to serum albumin. The liver is the critical organ for copper homoeostasis. The copper is partitioned for excretion through the bile or incorporation into intra- and extracellular proteins. The primary route of excretion is through the bile. The transport of copper to the peripheral tissues is accomplished through the plasma attached to serum albumin, ceruloplasmin or low-molecular-weight complexes.

The methods used to study copper homoeostasis in mammals include dietary analyses and balance studies. Isotope and standardized biochemical analyses of these processes are essential to understand copper deficiency and excess.

The biochemical toxicity of copper, when it exceeds homoeostatic control, is derived from its effects on the structure and function of biomolecules such as DNA, membranes and proteins directly or through oxygen-radical mechanisms.

1.7 Effects on laboratory animals and *in vitro* test systems

The toxicity of a single oral dose of copper varies widely between species (LD_{50} range 15–1664 mg Cu/kg body weight). The more soluble salts (copper(II) sulfate, copper(II) chloride) are generally more toxic than the less soluble salts (copper(II) hydroxide, copper(II) oxide). Death is preceded by gastric haemorrhage, tachycardia, hypotension, haemolytic crisis, convulsions and paralysis. LD_{50} values for dermal exposure were reported at > 1124 and > 2058 mg Cu/kg body weight in rats and rabbits respectively. The inhalation LC_{50} (exposure duration unspecified) was > 1303 mg Cu/kg body weight in rabbits, and respiratory function was impaired in guinea-pigs exposed to 1.3 mg Cu/m^3 for 1 h.

Rats given up to 305 mg Cu/kg per day orally in the diet as copper(II) sulfate for 15 days showed alterations in blood biochemistry

and haematology (particularly anaemia) and adverse effects on the liver, kidney and lungs. Effects were qualitatively similar with other copper compounds and in other species. The no-observed-effect level (NOEL) in this study was 23 mg Cu/kg body weight per day. However, sheep were particularly sensitive and repeated doses of 1.5–7.5 mg Cu/kg body weight per day as copper(II) sulfate or copper(II) acetate resulted in progressive liver damage, haemolytic crisis and ultimately death.

Long-term exposure in rats and mice showed no overt signs of toxicity other than a dose-related reduction in growth after ingestion of 138 mg Cu/kg body weight per day (rats) and 1000 mg Cu/kg body weight per day (mice). The no-observed-adverse-effect level (NOAEL) was 17 mg Cu/kg body weight per day in rats, and 44 and 126 mg Cu/kg body weight per day in male and female mice, respectively. The effects included inflammation of the liver and degeneration of kidney tubule epithelium.

Studies of reproductive and developmental toxicity were limited. Some testicular degeneration and reduced neonatal body and organ weights were seen in rats at dose levels in excess of 30 mg Cu/kg body weight per day over extended time periods, and fetotoxic effects and malformations were seen at high dose levels (> 80 mg Cu/kg body weight per day).

Copper(II) sulfate was not mutagenic in bacterial assays. However, a dose-related increase in unscheduled DNA synthesis was seen in rat hepatocytes. In the mouse micronucleus assay, one study showed a significant increase in chromosome breaks at the highest intravenous dose (1.7 mg Cu/kg body weight) but no effect was seen in another study at intravenous doses up to 5.1 mg Cu/kg body weight.

Studies of neurotoxicity have not shown effects on behaviour but neurochemical changes have been reported after oral administration of 20–40 mg Cu/kg body weight per day. A limited number of immunotoxicity studies showed humoral and cell-mediated immune function impairment in mice after oral intakes from drinking-water of about 10 mg Cu/kg body weight per day.

1.8 Effects on humans

Copper is an essential element and adverse health effects are related to deficiency as well as excess. Copper deficiency is associated with anaemia, neutropenia and bone abnormalities but clinically evident deficiency is relatively infrequent in humans. Balance data may be used to anticipate clinical effects, whereas serum copper and ceruloplasmin levels are useful measures of moderate to severe deficiency but less sensitive measures of marginal deficiency.

Except for occasional acute incidents of copper poisoning, few effects are noted in normal populations. Effects of single exposure following suicidal or accidental oral exposure have been reported as metallic taste, epigastric pain, headache, nausea, dizziness, vomiting and diarrhoea, tachycardia, respiratory difficulty, haemolytic anaemia, haematuria, massive gastrointestinal bleeding, liver and kidney failure, and death. Gastrointestinal effects have also resulted from single and repeated ingestion of drinking-water containing high copper concentrations, and liver failure has been reported following chronic ingestion of copper. Dermal exposure has not been associated with systemic toxicity but copper may induce allergic responses in sensitive individuals. Metal fume fever from inhalation of high concentrations in the air in the occupational setting has been reported and, although other respiratory effects have been attributed to exposure to mixtures containing copper (e.g. Bordeaux mix, mining and smelting), the role of copper has not been demonstrated. Workers apparently exposed to high air levels resulting in an estimated intake of 200 mg Cu/day developed signs suggesting copper toxicity (e.g. elevated serum copper levels, hepatomegaly). Available data on reproductive toxicity and carcinogenicity are inadequate for risk assessment.

A number of groups are described where apparent disorders in copper homoeostasis result in greater sensitivity to copper deficit or excess than the general population. Some disorders have a well-defined genetic basis. These include Menkes disease, a generally fatal manifestation of copper deficiency; Wilson disease (hepatolenticular degeneration), a condition leading to progressive accumulation of copper; and hereditary aceruloplasminaemia, with clinical symptoms of iron overload. Indian childhood cirrhosis (ICC) and idiopathic copper toxicosis (ICT) are conditions related to excess copper which

may be associated with genetically based copper sensitivity, although this has not been demonstrated unequivocally. These are fatal liver conditions in early childhood where copper accumulates in the liver. Incidences of the diseases were related to high copper intake, at least in some cases.

Other groups potentially sensitive to copper excess are haemodialysis patients and subjects with chronic liver disease. Groups at risk of copper deficiency include infants (particularly low birth weight/preterm babies, children recovering from malnutrition, and babies fed exclusively with cow's milk), people with malabsorption syndromes (e.g. coeliac disease, sprue, cystic fibrosis), and patients on total parenteral nutrition. Copper deficiency has been implicated in the pathogenesis of cardiovascular disease.

1.9 Effects on other organisms in the laboratory and field

The adverse effects of copper must be balanced against its essentiality. Copper is an essential element for all biota, and care must be taken to ensure the copper nutritional needs of organisms are met. At least 12 major proteins require copper as an integral part of their structure. It is essential for the utilization of iron in the formation of haemoglobin, and most crustaceans and molluscs possess the copper-containing haemocyanin as their main oxygen-carrying blood protein. In plants copper is a component of several enzymes involved in carbohydrate, nitrogen and cell wall metabolism.

A critical factor in assessing the hazard of copper is its bioavailability. Adsorption of copper to particles and complexation by organic matter can greatly limit the degree to which copper will be accumulated and elicit effects. Other cations and pH can also significantly affect bioavailability.

Copper has been shown to exert adverse reproductive, biochemical, physiological and behavioural effects on a variety of aquatic organisms. Copper concentrations as low as 1–2 µg/litre have been shown to have adverse effects on aquatic organisms; however, large variations due to species sensitivity and bioavailability must be considered in the interpretation and application of this information.

In natural phytoplankton communities chlorophyll *a* and nitrogen fixation were significantly reduced at copper concentrations of ≥ 20 µg/litre and carbon fixation was significantly reduced at > 10 µg/litre. EC_{50}s (72 h) for algae, based on growth inhibition, range from 47 to 120 µg Cu/litre.

For freshwater invertebrates, 48-h $L(E)C_{50}$s range from 5 µg Cu/litre for a daphnid species to 5300 µg Cu/litre for an ostracod. For marine invertebrates 96-h LC_{50}s range from 29 µg Cu/litre for the bay scallop to 9400 µg Cu/litre for the fiddler crab. The acute toxicity of copper to freshwater and marine fish is highly variable. For freshwater fish 96-h LC_{50}s range from 3 µg Cu/litre (Arctic grayling) to 7340 µg Cu/litre (bluegill). For marine fish 96-h LC_{50}s range from 60 µg Cu/litre for chinook salmon to 1400 µg Cu/litre for grey mullet.

Although plants require copper as a trace element, at high soil levels copper can be extremely toxic. Generally visible symptoms of metal toxicity are small chlorotic leaves and early leaf fall. Growth is stunted and initiation of roots and development of root laterals are poor. Reduced root development may result in a lowered water and nutrient uptake which leads to disturbances in the metabolism and growth retardation. At the cellular level, copper inhibits a large number of enzymes and interferes with several aspects of plant biochemistry (including photosynthesis, pigment synthesis and membrane integrity) and physiology (including interference with fatty acids, protein metabolism and inhibition of respiration and nitrogen fixation processes).

Toxic effects have been observed in laboratory studies of earthworms exposed to copper in soil; cocoon production is the most sensitive parameter measured, with significant adverse effects at 50–60 mg Cu/kg.

Adverse field effects on soil microorganisms have been correlated with enhanced copper concentrations in areas where copper-containing fertilizers have been applied and in areas near to copper–zinc smelters. In citrus-growing areas, to which copper-containing fungicides have been applied, leaf chlorosis has been found to be significantly correlated with soil copper levels.

Tolerance to copper has been demonstrated in the environment for phytoplankton, aquatic and terrestrial invertebrates, fish and terrestrial plants. Tolerance mechanisms which have been proposed in plants include binding of metal to cell wall material, presence of metal-tolerant enzymes, complex formation with organic acids with subsequent removal to the vacuole, and binding to specialized thiol-rich proteins or phytochelatins.

1.10 Conclusions

1.10.1 Human health

The lower limit of the acceptable range of oral intake (AROI) is 20 µg Cu/kg body weight per day. This figure is arrived at from the adult basal requirement with an allowance for variations in copper absorption, retention and storage (WHO, 1996). In infancy, this figure is 50 µg Cu/kg body weight per day.

The upper limit of the AROI in adults is uncertain but it is most likely in the range of several but not many mg per day in adults (several meaning more than 2–3 mg/day). This evaluation is based solely on studies of gastrointestinal effects of copper-contaminated drinking-water. A more specific value for the upper AROI could not be confirmed for any segment of the general population. We have limited information on the level of ingestion of copper from food that would provoke adverse health effects.

The available data on toxicity in animals were considered unhelpful in establishing the upper limit of the AROI, owing to uncertainty about an appropriate model for humans. Moreover, traditional methodology for safety assessment, based on application of uncertainty factors to data in animals, does not adequately address the special attributes of essential elements such as copper.

From available data on human exposures worldwide, but particularly in Europe and the Americas, there is greater risk of health effects from deficiency of copper intake than from excess copper intake.

1.10.2 Environmental effects

Protection of aquatic life in waters with high bioavailability will require limiting total dissolved copper to some concentration less than 10 µg/litre; however, the appropriate concentration limit will depend on the biota and exposure conditions at sites of concern and should be set based on further evaluation of all relevant data.

At many sites, physicochemical factors limiting bioavailability will warrant higher copper limits. Regulatory criteria should take into account the speciation of copper if dischargers can demonstrate that the bioavailability of copper in the receiving water can be measured reliably.

When sampling and analysing environmental media for copper, it is essential that "clean" techniques be employed.

Because copper is an essential element, procedures to prevent toxic levels of copper should not incorporate safety factors that result in recommended concentrations being below natural levels.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES AND ANALYTICAL METHODS

2.1 Identity

Copper, the 29th element and the first in group IB of the Periodic Table, displays four oxidation states: metallic copper Cu^0, cuprous ion Cu^+, cupric Cu^{2+} and trivalent copper ion Cu^{3+}. Copper also forms organometallic compounds. The natural isotopic abundance is 69.17% ^{63}Cu and 30.83% ^{65}Cu, giving the element an average relative atomic mass of 63.546 (Lide & Frederikse, 1993b). The limited range of stable isotopes and their common distribution has inhibited isotopic distribution studies. Useful radioactive copper isotopes are ^{64}Cu (12.701 h half-life) and ^{67}Cu (61.92 h half-life); they decay with the production of β-particles and γ-rays (Lide & Frederikse, 1993b) and are produced in synchrotrons for physical and biological studies.

Copper is found in a wide variety of mineral salts and organic compounds, and can also be found naturally in the elemental or metallic form. The metal is a dull lustrous reddish-brown in colour, malleable, a good thermal conductor and an excellent electrical conductor. The metallic form is very stable to dry air at low temperatures but undergoes a slow reaction in moist air to produce a hydroxycarbonate or hydroxysulfate that forms a greenish-grey amorphous film over the surface which protects the underlying metal from further attack. The metal is sparingly soluble in water, in salt solutions and in mildly acidic solutions, but can be dissolved in nitric acid and sulfuric acid as well as in basic solutions of ammonium hydroxide, ammonium carbonate and cyanide in the presence of oxygen (Cotton & Wilkinson, 1989).

The electronic configuration of the metallic (Cu^0) form is $1s^2 2s^2 2p^6 3s^2 3p^6 3d^{10} 4p^1$. The common solution oxidation states are the cuprous (Cu(I) $3d^{10}$) or the cupric (Cu(II) $3d^9$) forms. The chemistry of the element, especially in biological systems, is profoundly affected by the electronic/oxidation state. The facile exchange between oxidation states endows the element with redox properties which may be of an essential or deleterious nature in biological systems.

The most important oxidation state in natural, aqueous environments is copper(II). Any copper(I) present is quickly oxidized by any oxidizing reagent present, or in a disproportionation reaction, unless it is stabilized by complex formation. The copper(II) ion binds preferentially via oxygen to inorganic ligands such as H_2O, OH^-, CO_3^{2-}, SO_4^{2-}, etc. and to organic ligands via phenolic and carboxylic groups (Cotton & Wilkinson, 1989). Thus, almost all of the copper in natural samples is complexed with organic compounds (Neubecker & Allen, 1983; Nor, 1987; Allen & Hansen, 1996).

Many cupric compounds and complexes are soluble in water and have a characteristic aqua–blue–green colour. The trivalent form of copper is found in only a few compounds and is a strong oxidizing agent (Cotton & Wilkinson, 1989). In environmental and mineral environments the divalent oxidation state readily adsorbs to a variety of hydrated metal oxides including those of iron, aluminium and manganese (Grant et al., 1990).

Identification, quantification and speciation of copper is described in sections 2.3 and 2.4 and the influences on the speciation in water and soil are described in section 2.4.1.

2.2 Physical and chemical properties

The physical and chemical properties of copper and some of its salts are summarized in Table 1.

2.3 Analytical methods

The wide range of copper species, inorganic and organic, has lead to the development of an array of sampling techniques and preparative and analytical methods to quantify the element in environmental and biological samples. The following sections offer a brief overview of these methodologies.

2.3.1 Sampling and sample preparation

Sampling and the subsequent work-up is highly dependent on the type of sample being analysed and the level of detail needed to evaluate it. Most of the techniques described below suffer at some

Table 1. Physical and chemical properties of copper and some of its salts[a]

	Copper	Copper(II) sulfate	Cuprous(I) oxide	Copper(II) hydroxide	Copper(II) chloride	Oxine-copper[b]
CAS registry number	7440-50-8	7758-98-7	1317-39-1	20427-59-2	7447-39-4	10280-28-6
Molecular formula	Cu	$CuSO_4$	Cu_2O	$Cu(OH)_2$	$CuCl_2$	$C_{18}H_{12}CuN_2O_2$
Relative molecular mass	63.55	159.6	141.3	97.56	134.45	351.9
Boiling point (°C)	2567	decomposes to CuO at 650 °C		decomposes at 140 °C	decomposes at 993 °C	
Melting point (°C)	1083.4	slightly decomposes at > 200 °C	1235	decomposes	620	decomposes at 270 °C
Vapour pressure (kPa)	1.33 at 1870 °C					
Water solubility	insoluble	143 g/litre at 0 °C	practically insoluble	2.9 mg/litre at 25 °C	706 g/litre	insoluble

[a] Lide & Frederikse (1993)
[b] Copper 8-hydroxyquinolinate.

level from the effects of the surrounding milieu or matrix. Qualitative analysis to determine the presence of copper in a sample, for instance, may or may not require consideration of the matrix, whereas quantitation of metals usually does. Quantitation of the various forms of copper requires a detailed evaluation of the matrix and the techniques being used.

2.3.1.1 Sampling

Owing to the abundance of copper in the environment, the collection of samples for copper analysis requires precautions to avoid accidental contamination. Most plastics and glassware are relatively free of copper contamination but care should be taken to avoid heavily pigmented plastics that could contain copper or other metals that might compromise the analysis. Interference by contaminating metals is more likely to be a problem in colorimetric analyses. Vessels to be used in the collection of samples for copper analysis should be cleaned of dust and debris and washed with a dilute metal-free mineral acid such as 0.1 mol/litre hydrochloric or nitric acid, rinsed copiously with clean distilled water and dried in a dust-free area. Copper is frequently and naturally found in industrial and household dusts (Kim & Fergusson, 1993) so care should be taken that the samples are not contaminated. Removal of copper from washing and rinsing water, and even distilled water, can be compromised by the use of copper plumbing and brass fixtures. Removal of metals and other ions can be accomplished through the use of ion-exchange resins.

2.3.1.2 Separation and concentration

It is not generally necessary that the metal itself be isolated before analysis, but frequently the metal or at least the inorganic portion of the sample must be concentrated. The requirement for concentration of the sample depends on the sensitivity of analytical method to be employed.

Particulates (dust, smoke, spray) are sampled from air on filters before analysis. Aqueous samples may need to be dried or concentrated using an ion-exchange procedure (Vermeiren et al., 1990; Chakrabarti et al., 1994).

Total copper (in water) includes all forms of copper irrespective of form, whether dissolved or bound. *Suspended copper* refers to

copper attached to suspended particles in water large enough to be filtered by a 0.45 μm membrane filter. *Dissolved copper* is defined operationally as all forms of copper which pass through a 0.45 μm membrane filter (ATSDR, 1990). Separation of dissolved and suspended forms of copper requires filtering. Special measures must be taken to avoid sample contamination when filtering. First, the membrane filter and filter holder must be acid cleaned. The filter must be discarded and the filter holder should be acid rinsed between samples and subsequently rinsed with metal-free water. Second, glass fibre filters must not be used. Third, the filter holder and membrane filter must be conditioned with the sample, i.e. an initial portion of the sample filtered and discarded. Lastly, if positive pressure filtration is used, the gas must be passed through a 0.2 μm in-line filter.

2.3.1.3 Sample preparation

Direct analysis of metals with little modification or preparation of the sample is desirable but frequently not achievable. Direct analysis of copper is appropriate when relatively concentrated samples are analysed (0.1–2 mg/litre or higher), provided they are very low in interfering inorganics and especially organic materials. More dilute samples can be concentrated as described above. Concentrated samples can be diluted with appropriate diluents, usually distilled water or dilute copper-free mineral acid solutions. Care should be taken to keep the pH near or below neutral to avoid the formation of insoluble copper hydroxides.

Sample preparation for the most widely utilized analytical techniques, or where the removal of the organic matrix is required, is generally achievable by means of a preceding open vessel oxidative degradation step involving nitric acid or acid mixtures such as aqua regia or sulfuric acid/hydrogen peroxide. (Perchloric acid is less frequently used because of its explosive nature.) A procedure using a mixture of nitric, perchloric and hydrofluoric acids was reported to give good recoveries of metals including cadmium, chromium, copper, manganese, nickel, lead and zinc in estuarine sediments (Bello et al., 1994). Recently, oxidative UV photolysis (Kolb et al., 1992) and microwave-assisted acid digestion in a closed vessel have become more popular in sample preparation for various sample matrices prior to elemental analyses. Microwave-assisted digestion has been

employed as a sample preparation procedure prior to the measurement of copper level in human bone (Baranowska et al., 1995), in duck eggs (Jeng & Yang, 1995), in sediments by anodic stripping voltametry (Olsen et al., 1994), in marine biological tissues such as mollusc, fish and crustacean by AAS (Baldwin et al., 1994), in steels and copper alloys by ICP-AES (Borszeki et al., 1994), and in plant materials (Matejovic & Durackova, 1994). The microwave digestion procedure is fast becoming the method of choice because sample preparation is rapid and the values of blanks are significantly lower than in the traditional wet and dry mineralization methods (Matejovic & Durackova, 1994). A fast and quantitative on-line microwave digestion/extraction of copper from different solid matrices, such as vegetables, powdery dietary products and sewage sludge, was developed using a flow injection-atomic absorption system (FI-AAS) (Delaguardia et al., 1993). A similar FI-AAS method for the determination of copper in whole blood was also reported by Burguera et al. (1993).

2.3.1.4 *"Clean" techniques for measurement of ultratrace copper levels*

Information provided by Shiller & Boyle (1987), Windom et al. (1991) and Hurley et al. (1996) has raised questions concerning the quality of data collected and reported for trace metals analysis over the past several decades. The concern is that insufficient care in sampling, sample preparation and analysis have resulted in samples being contaminated and the values reported in the sub-mg/litre range have questionable accuracy. It has been shown that many published literature values for surface waters are biased on the high side owing to contamination and/or matrix interferences. Matrix interferences commonly encountered in copper analyses are chemical, spectral, ionization and high dissolved solids. Copper determination by ICP emission spectroscopy (ICP-ES) can suffer from interference by iron, thallium and vanadium (US EPA, 1986). Copper determination by ICP-MS emission spectroscopy is susceptible to interference from chlorides, although procedures have been developed to overcome this interference in blood serum samples, for example (Lyon & Fell, 1990). Both ICP-ES and ICP-MS are excellent techniques for measuring copper if care is taken to eliminate interferences. "Clean" techniques (Prothro, 1993; US EPA, 1995) address the problem associated with making accurate and precise trace determinations of metals particularly

when attempting to lower detection limits and report microgram/litre and sub-microgram/litre concentrations. "Clean" techniques require special attention to be paid in seven areas:

1. use of "clean" techniques during collecting, handling, storing, preparing and analysing samples to avoid contamination
2. use of analytical methods that have sufficiently low detection limits
3. avoidance of interference in the quantification step
4. use of blanks to assess contamination
5. use of matrix spikes and certified reference materials (CRMs) to assess interference and contamination
6. use of replicates to assess precision
7. use of certified standards.

To achieve accurate and precise measurement of any particular sample, it is recommended that both the detection limit and the blank value should be less than one-tenth the sample concentration. This is a stringent requirement, but one that is especially important in measuring metals at concentrations near the method detection limit and at environmentally relevant concentrations. The methods employed to attain these goals seek to increase sensitivity, decrease contamination and decrease interference. The specific recommendations used to achieve these goals and address the seven items above are provided in Prothro (1993).

2.3.2 Detection and measurement

2.3.2.1 Gravimetric and colorimetric methods

Gravimetric and colorimetric methods were the earliest procedures used for the measurement of copper. Gravimetric methods are non-specific and may precipitate other cations including zinc, cadmium, cobalt and nickel. Useful spectrophotometric reagents for copper include cuprizone (biscyclohexanoneoxalydihydrazone) (Peterson & Bollier, 1955), bathrocuproinedisulfonic acid (2,9-dimethyl-4,7-diphenyl-1,10-phenanthrolinedisulfonic acid) (Zak, 1958), bathocuproine (dimethyl-4,7-diphenyl-1,10-phenanthroline) (Wharton & Rader, 1970) and more recently 1-(2-pyridylazo)-2-naphthol (Malvankar & Shinde, 1991), BPKQH (benzyl 2-pyridyl ketone 2-quinolylhydrazone (Garcia-Sanchez et al., 1990) and 2,2'-bichinchioninic acid (Brenner & Harris,

1995). The bathocuproine method can achieve a limit of detection of 2 μg Cu/litre in water samples.

Although colorimetric methods can suffer from lack of specificity, they are nevertheless useful, especially in laboratories where more sophisticated instrumentation is not available. Beyond a spectrophotometer and an analytical balance, no specialized equipment is required. In addition, the methods are, in general, simple, inexpensive, easily taught and rapidly carried out. Because of these advantages they should be considered in situations where extreme sensitivity is not essential.

2.3.2.2 *Atomic absorption, emission and mass spectrometry methods*

Atomic absorption spectrophotometric (AAS) methods are the most widely used for the determination of copper in various matrices. A dramatic increase in sensitivity over that obtained by flame AAS is obtained with GF-AAS. Increasingly more common is the use of emission methods in which the sample is introduced into a high temperature inductively coupled argon plasma (ICP) where the element is rapidly vaporized and ionized. The element is detected and quantified by atomic emission spectroscopy (ICP-AES).

A further increase in sensitivity is obtained through the coupling of the ICP to a mass spectrometer (ICP-MS). The attraction of the ICP methods is the ability to do multielemental analysis (Vollkopf & Barnes, 1995) which is the obvious advantage over other spectroscopic techniques. The ICP-MS technique has the additional advantage that isotopic information can be obtained, which is especially useful if stable isotopes of copper are used for bioavailability and other studies (Lyon et al., 1988, 1995, 1996). An isotope dilution ICP-MS method (Beary et al., 1994) reported precision of less than 0.15% for copper and cadmium in zinc ore and for copper and molybdenum in domestic sludge; others (Lu et al., 1993) reported a more conservative precision of less than 1% and a detection limit of 58 ng/litre for copper in a number of biological and environmental reference materials. The International Standards Organization have published procedures using AAS for the analysis of copper in water between 0.05 and 200 μg/litre (ISO, 1986). Detection limits are summarized in Table 2.

Table 2. Analytical methods for the detection of copper

Medium	Sample preparation	Method[a]	Detection limit	Reference
Air	filter collection on 0.8 µm membrane; acid digestion	ICP-AES	1 µg	ATSDR (1990)
	filter collection on 0.8 µm membrane; acid digestion	AAS	0.05 µg	ATSDR (1990)
Fresh water	acidify with 1:1 HNO_3 to a pH < 2	AAS	20 µg/litre	US EPA (1986)
	sample solutions should contain 0.5% HNO_3	GF-AAS	1 µg/litre	US EPA (1986)
	filter and acidity sample	ICP	2–10 µg/litre	US EPA (1986)
	filter and acidity sample	ICP-AES	6 µg/litre	ATSDR (1990)
	acid digestion with HNO_3, reflux and dilute with type 1 water	ICP-MS	0.01 µg/litre	US EPA (1994)
Sediment	acid digestion	AAS	1.0 µg/g	US EPA
	acid digestion	GF-AAS	0.05–0.20 µg/g	(1986)
	acid digestion	ICP	0.20–0.50 µg/g	US EPA
	acid digestion	ICP-MS	0.025–0.005 µg/g	(1986)
Tissue	acid digestion	AAS	0.5–1.0 µg/g	US EPA
	acid digestion	GF-AAS	0.05–0.20 µg/g	(1986)
	acid digestion	GF-AAS	0.25 µg/g wet weight	Lowe et al. (1985)
	acid digestion	ICP	0.04–0.1 µg/g	US EPA
	acid digestion	ICP-MS	0.025–0.05 µg/g	(1986)
	acid digestion	ICP-AES	0.2 µg/g tissue 1 µg/100 ml blood	NIOSH (1987)
Food	closed system digestion	ASV	0.32 µg/g	Holak (1983)

[a] See list of abbreviations on p. xxii.

2.3.2.3 Specialized methodologies

Many X-ray fluorescence (XRF) methods, which are nondestructive techniques, have been published for the determination of trace elements including copper. XRF has for a long time been used as a rapid and convenient method for trace element determination although its sensitivity is somewhat lower than anodic stripping voltametry (ASV) (Viksna et al., 1995). The technique can be used for a variety of sample types, such as human serum (Viksna et al., 1995), electrolyte purification solutions (Davidson et al., 1994), human kidney tumours (Hamilton et al., 1972) and contaminated soils (Wilson et al., 1995). Field instruments are available for scans of contaminated sites to estimate the metal in the surface layer of the soil. A proton-induced X-ray fluorescence technique (PIXE) was also reported for the measurement of trace elements in amniotic fluid (Napolitano et al., 1994).

Ion-selective electrode and potentiometric methods have been used for copper speciation in soil (Town & Powell, 1993), and in seawater (Román & Rivera, 1992; Soares et al., 1994). Voltammetric methods have comparable sensitivity to conventional AAS, but also offer speciation capability (Scarano et al., 1990; Chakrabarti et al., 1994; Cheng et al., 1994). Voltammetric/potentiometric analyses offer sensitivity in the parts per billion (µg/kg) range for copper and some other metals. Potentiometric analysis relies on the elements electrochemical properties. An attraction of potentiometric methods is their ability to help in the speciation of copper and limited multielement detection. ASV has been used to analyse copper in foods (Holak, 1983). Cathodic stripping voltametry (CSV) is an extremely sensitive method for copper in both seawater and fresh water, with a limit of detection of 0.005 µg/litre (Donat et al., 1994).

Some analytical methods for the detection of copper in different media are summarized in Table 2.

2.4 Speciation

Developing an objective assessment of the hazard that copper poses to humans and the environment depends on an intimate understanding of its bioavailability. Bioavailability, defined as the

extent to which the metal is taken up by an organism upon exposure, depends on the species of the metal or metallo complex and/or how easily it can be transformed to a more or less bioavailable species.

2.4.1 Speciation in water and sediments

In natural waters, only very small percentages of copper are present as the "free" aquo ion (Cu^{2+}); rather, most copper is adsorbed to suspended particles or complexed with various ligands (Florence & Batley, 1980). Inorganic ligands of greatest importance are hydroxide, carbonate and, in saline waters, chloride (Bodek et al., 1988). Binding of copper to fulvic and humic acids and to other organic compounds can be very strong, so that a large proportion of dissolved copper is often organically complexed (Neubecker et al., 1983; Coale & Bruland, 1988; Allen & Hansen, 1996). In air, copper is present in particulate form. In sediments and soils, most copper is also on or in particles, either as a constituent of mineral phases or adsorbed to oxide surfaces or organic matter; formation of copper sulfide can be particularly important in anoxic sediments (DiToro et al., 1990). Copper speciation in interstitial water can be affected by high concentrations of inorganic and organic ligands.

Speciation, the identification and quantitation of a metal in its various oxidation states, inorganic forms and organometallic complexes, is afforded through a wide variety of techniques (ICME, 1995).

2.4.1.1 Detection and quantification

a) *Electrochemical methods*

Electrochemical techniques, especially ASV, have been widely used to measure the "electrochemically labile" fraction of copper in water samples, with the assumption that the electrochemically labile fraction is an approximation of the bioavailable fraction of copper (Neubecker & Allen, 1983; Bruland et al., 1985; Buckley & van den Berg, 1986; Morrison & Florence, 1989; Florence et al., 1992; Donat et al., 1994). It has been shown that if the ASV measurement is carried out in a manner such that the copper complexing agents in the water sample affect only the efficiency of electrochemical deposition, but not the stripping process, then ASV-labile copper correlates very well with

bioavailable copper as measured by algal assay (Florence et al., 1992). Simple ASV analysis of a water sample at the natural pH where complexing agents affect both the deposition and stripping processes tends to underestimate the bioavailable fraction of copper (Zhang & Florence, 1987; Morrison & Florence, 1989).

Electrochemical titrations using ASV can provide information on the "complexing capacity" of a water sample, as well as quantitative data on the conditional formation constants of copper with the ligands present in the sample. Complexing capacity is defined as the total concentration of ligands, both organic and inorganic, in a water sample that will bind copper in nonlabile complexes (Donat et al., 1994).

b) *Equilibration methods*

Together with electrochemical methods, equilibration techniques are among the most popular and successful methods used for speciation studies. The equilibration methods mostly use ion-exchange resins or weak inorganic exchangers and complexing ligand. The equilibrium constant of both the resin and the complex has to be satisfied simultaneously. The distribution ratio for a fixed resin concentration is measured in the presence of a competing ligand with known metal equilibria, which determines the partition coefficient for the resin. Stability constants and ligand concentrations of unknown solutions can then be measured (Neubecker & Allen, 1983).

The total concentration of most biologically important trace metals including copper in seawater is in the range 10^{-10}–10^{-8} mol/litre and hence the concentration of any individual metal organic complex must be considerably lower. Characterization and identification of individual compounds at these concentrations in seawater by chemical techniques is very difficult, if not impossible. The methodology usually involves first extracting and concentrating the compounds from sample matrices on to a resin, followed by fractionation according to different chemical and physical properties. Since the compounds may not be volatile, the most useful technique is high performance liquid chromatography (HPLC); alternatively, the compounds can be made volatile by some derivatization steps then determined by gas liquid chromatography (GLC), or gas liquid chromatography mass spectrophotometry (GLC–MS). Thompson & Houk (1986) reported an HPLC-ICP-MS

method of multielemental analysis and speciation with a limit of detection of 4 ng of copper. Recently, the sensitivity for copper was increased by using an ion chromatography–ICP–MS (IC-ICP-MS) technique (McLaren et al., 1993). The aluminium hydroxide-cation exchange mini-column technique (Zhang & Florence, 1987) provides a rapid and simple method for determining bioavailable copper in both seawater and fresh water samples.

2.4.2 Speciation in biological matrices

The speciation of copper in tissue and blood samples has been studied (Florence & Batley, 1980; Brouwer et al., 1989; Florence et al., 1992). In particular, techniques have been developed for the separation and determination of caeruloplasmin in blood plasma (Lyon & Fell, 1990) and for metallothioneins in tissue samples (Florence et al., 1992).

3. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

3.1 Natural sources

Metal oxides, silicates and other materials are the building blocks of rocks forming the earth's crust and it is the weathering of these rocks that creates soils and sediment. Copper oxide, copper sulfide and other ores are among these components. Copper, along with other metals, is distributed through the environment by precipitation and resulting riverine flows which transport the particles. Depending on the flow dynamics, these particles settle out and form sedimentary deposits. Volcanic activity injects dust and particles into the atmosphere; they then settle out on soil and water surfaces. Wind is a significant factor in moving metal-laden soil particles around the land surface of the earth, which they can also reach from atmospheric sources by both wet (rain washout) and dry deposition. An important source of copper in aquatic sediments is from dead organisms which settle out and contribute both copper and organic material. This can be a significant source in the oceans, for example.

Copper has a natural abundance of approximately 60 mg/kg in the earth's crust and 2.5×10^{-4} mg/litre in the sea (Lide & Frederikse, 1993). It occurs naturally in many minerals such as cuprite (Cu_2O), malachite ($Cu_2CO_3.Cu(OH)_2$), azurite ($2CuCO_3.Cu(OH)_2$), chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S), and bornite (Cu_5FeS_4). Copper is also found naturally in its metal form (Tuddenham & Dougall, 1978). The copper content of ore deposits ranges from 0.5 to 5% by weight, whereas igneous rock contains 0.010% (Duby, 1980) and crystalline rock 0.0055% by weight. The most important sources of copper are chalcocite, chalcopyrite and malachite (Weant, 1985).

Figures from Cannon et al. (1978) indicate a range of 4–200 mg Cu/kg and a range of mean concentrations of 2–90 mg Cu/kg in igneous and sedimentary rocks. Nriagu (1989) estimated mean world-wide emissions of copper from natural sources as follows: windblown dusts, $0.9–15 \times 10^3$ tonnes; forest fires, $0.1–7.5 \times 10^3$ tonnes; volcanic particles, $0.9–18 \times 10^3$ tonnes; biogenic processes, $0.1–6.4 \times 10^3$ tonnes; sea salt spray, $0.2–6.9 \times 10^3$ tonnes.

Average background concentrations of copper in air in rural areas range from 5 to 50 ng/m^3. Copper levels in seawater of 0.15 µg/litre and in freshwater of 1.0–20 µg/litre are found in uncontaminated areas (Nriagu, 1979b). Background levels of copper in uncontaminated sediments range from 800 to 5000 mg/kg (dry weight) (Forstner & Wittmann, 1979). Copper levels in marine sediments range from 2 to 740 mg/kg (dry weight). Median copper concentrations in uncontaminated soil were reported to average 30 mg Cu/kg with a range of 2–250 mg/kg (Bowen, 1985). Detailed information on concentrations in the environment is presented in section 5.1. Copper is found as a natural component of foods eaten by humans and animals.

3.2 Anthropogenic sources

Anthropogenic sources of copper include emissions from mines, smelters and foundries producing or utilizing copper, zinc, silver, gold and lead. Environmental copper can also arise from the burning of coal for power generation and from municipal waste incinerators. A major release of copper to land comes from mine tailings and overburden from mining operations. Other anthropogenic sources of copper include its use as an antifouling agent in paints, agriculture (fertilizers, algicides, feed supplements) and animal and human excreta (animal manure and human sewage sludge). Copper is also intentionally released into some water bodies to control the growth of algae (Slooff et al., 1989; ATSDR, 1990).

Although it was estimated that 66% of copper emissions to the environment in 1983 were from anthropogenic sources (Nriagu, 1989), there is evidence that industrial emissions are decreasing owing to stringent controls developed in facilities manufacturing and using copper (Dann, 1994).

3.2.1 Production levels and processes

The mining and refining of copper takes place on all six continents. Mines in Chile, USA and Canada account for over 50% of the annual worldwide production of 11×10^6 tonnes of refined copper metal (ICSG, 1996). Other major areas for copper mining include Russia, Australia, Zambia, Indonesia, Peru, China and Poland. It is estimated that about 40% of the copper used worldwide (approximately

15×10^6 tonnes) comes from recycled metal (ATSDR, 1990). Release of airborne copper from smelters is currently one of the major sources of copper to the environment.

The majority of copper metal is produced by smelting of the copper sulfide ore followed by electrolytic refining (ATSDR, 1990). Some 10^6 tonnes were produced in Chile and North America using solvent extraction technology. The process involves extraction of copper from acidic leach solutions using organic reagents followed by electrolytic extraction. The principal sources of copper for this process are conventional mining of oxide ores in open pits, leaching of mine dump low-grade ore, and mill tailings and mine water run-off. Extraction of mine tailings and dumps in this way reduces the environmental impact of mine wastes by reducing the copper concentrations in these sources.

3.3 Copper use

The world uses approximately 15×10^6 tonnes of copper a year. Of this about one-third is derived from recycled metal, and the rest is supplied from the mining of ore bodies and refining of the extracted copper.

The unique combination of properties of copper, including durability, ductility, malleability and electrical and thermal conductivity, determine its uses in a vast range of applications. A summary of these uses in the USA, Western Europe and Japan is given in Table 3, compiled from Marco (1989).

Worldwide, the largest use of copper is in electrical wire and cable and other electronic applications, which can account for as much as 65% (9.75×10^6 tonnes) of total annual copper consumption. Rolled copper is also extensively used in architectural applications for roofing, rainwater goods and cladding, while rolled copper and brass are also used for vehicle radiators. Overall, the major industrialized countries consume over 1.5×10^6 tonnes of rolled product per year. Approximately 15% (2.25×10^6 tonnes) of copper is used annually in building and construction, including plumbing, architectural applications such as roofing, guttering and flashing, and in fixtures and fittings. The remaining 20% (3×10^6 tonnes) goes to transport equipment, air-conditioning and refrigeration as well as general and light engineering

Table 3. Copper consumption in 1988[a] (in thousands of tonnes)

Use	Building and construction	Electrical/ electronics	Industrial
Copper wire	0	4293	0
Copper rod	5	164	34
Copper sheet and strip	240	140	225
Copper tube	551	0	424
Alloy wire	7	9	65
Alloy rod	338	114	462
Alloy sheet and strip	66	123	443
Alloy tube	14	8	110
Castings	142	58	292
Totals	1363	4909	2055

[a] Based on figures from the USA, western Europe and Japan (about 75% of world consumption of 11 090 000 tonnes) (Marco, 1989)

uses such as machine parts, and process equipment, coinage, ordnance and consumer goods, such as domestic appliances as well as production of bronze and brass alloys.

Extruded brass is a raw material for the forging and machining sectors, and is turned into a wide range of components such as taps, valves and water fittings, and instrument and machine parts. Over 1.7×10^6 tonnes of extruded copper alloy products are consumed by the major industrialized countries annually.

Tubes in copper and copper alloys are widely and increasingly used for domestic plumbing and heating systems, air conditioning, refrigeration and industrial applications. Over 1.5×10^6 tonnes of tubes are consumed annually by the major industrialized countries.

A small percentage of copper production goes into the manufacture of copper compounds, particularly copper sulfate which is used primarily for industrial and agricultural purposes. In industry, copper sulfate is used as an activator in the froth flotation of sulfide ores,

production of chromated copper arsenate wood preservatives, electroplating, azo-dye manufacture, as a mordant for textile dyes, in petroleum refining and in the manufacture of other inorganic and organometallic compounds (ATSDR, 1990). Other copper compounds find uses as pigments, paints, dyes, glasses, catalysts and fungicides. Copper is finding increasing use as the active ingredient in antifouling paints. In this context it is also used in paints for operating theatres and other hospital facilities to reduce inadvertent contamination of surfaces and transmission of disease-causing organisms.

In agriculture, copper compounds, especially copper sulfate, are used as fungicides, pesticides, algicides, nutritional supplements in animal feeds, and fertilizers. Copper fungicides are used to treat foliage, seeds, wood, fabric and leather as a protectant against blights, downy mildews and rusts (ATSDR, 1990). One of the principle mixtures used to treat foliage for mildew and fungal infections is the Bordeaux mixture used to spray vines which typically contains 0.05–2% copper neutralized with soda lime (Pimentel & Marques, 1969). Copper sulfate is used throughout the world to kill and inhibit the growth of algae in municipal reservoirs, irrigation equipment and piping, swimming pools and industrial cooling systems. It is also used in animal feed additives and growth promoters, as well as for disease control in livestock and poultry (Grant et al., 1990).

Copper enjoys limited use in human and veterinary medicine, having been largely replaced by other compounds and treatments. Copper is, however, a major constituent of many of the metallic amalgams (e.g. mercury amalgams) used in dentistry. It is also used to prepare intrauterine devices (IUDs).

4. ENVIRONMENTAL TRANSPORT AND DISTRIBUTION

4.1 Transport and distribution between media

The information reviewed in this section describes the environmental fate of copper. The factors affecting the distribution of copper in air, water, sediment and soil are first described. This is followed by a review of the factors influencing the bioaccumulation of copper. This review is not intended to be exhaustive but rather to present selected representative papers.

4.1.1 Air

Copper is released to the atmosphere in the form of particulate matter or adsorbed to particulate matter. It is removed by gravitational settling (bulk deposition), dry deposition (inertial impaction characterized by a deposition velocity), washout by rain (attachment to droplets within clouds), and rainout (scrubbing action below clouds) (Schroeder et al., 1987). Removal rate and distance travelled from the source depend on source characteristics, particle size and wind velocity. Gravitational settling governs the removal of large particles (> 5 μm), whereas smaller particles are removed by other forms of dry and wet deposition. The relative importance of wet as compared to dry deposition generally increases with decreasing particle size (ATSDR, 1990).

Chakrabarti et al. (1993) analysed samples of rainwater (pH 5.3) and snow (pH 4.7) in Canada; the total copper concentrations were 30.3 μg/litre in the rainwater and 24.6 μg/litre in the snow. In the rainwater sample 98.3% of the copper was in the soluble phase (< 0.45 μm) and 1.7% in the particulate phase (> 0.45 μm) whereas in the snow sample 80.5% was found in the particulate phase and 4.8% in the soluble phase. Another snow sample (pH 3.9) was analysed and revealed a copper concentration of 5.7 μg/litre with 4.7 μg/litre in the soluble phase and 1.08 μg/litre in the particulate phase. Kinetic results suggested that the copper in the snow sample was probably bound to different sites having different bonding energies in polyfunctional complexing agents. Four different copper species having different

dissociation rate constants were observed (3.1×10^{-2}, 1.6×10^{-3}, 6.2×10^{-5} and 8.8×10^{-6} /s). Cheng et al. (1994) found that the distribution of copper species in rainwater collected in Ottawa, Canada, was very similar to that in the previously reported snow sample. The rainwater sample contained 7.10 μg Cu/litre of which 2.03 μg/litre was in the particulate phase and 5.07 μg/litre in the soluble phase (< 0.45 μm). The scavenging ratio of the copper concentration in precipitation (mg/litre) to air concentrations (μg/m^3) for large particles displays a seasonal variation reflecting the more effective scavenging of snow compared with rain (Chan et al., 1986).

There is large temporal and spatial variability in copper deposition. Schroeder et al. (1987) reviewed deposition rates and washout ratios for copper. Copper deposition rates in urban areas were estimated to be 0.119 and 0.164 kg Cu/ha per year for dry and wet deposition, respectively. Bulk deposition was reported to range from 0.002 to 3.01 kg Cu/ha per year. In rural areas bulk deposition was reported to range from 0.018 to 0.5 kg Cu/ha per year and wet deposition was 0.033 kg Cu/ha per year. The washout ratio is 114 000–612 000 (μg Cu/m^3 rain)/(μg Cu/m^3 air) [(140–751 μg Cu/kg rain)/(μg Cu/kg air)].

Ottley & Harrison (1993) calculated the dry deposition flux of copper to the North Sea to be 350 tonnes Cu/year. Migon et al. (1991) studied the input of copper through rainfall and dry deposition to the Ligurian Sea (Mediterranean) over a period of two years. The total flux was calculated to be 1.85 kg Cu/km^2 per year. A mean yearly atmospheric input for copper was calculated at 98 tonnes. Fergusson & Stewart (1992) estimated deposition flux for copper in the insoluble component of bulk deposition derived from Christchurch city, New Zealand. Copper fluxes followed approximately exponential decay curves away from the city. Deposition rates varied from 0.83 μg Cu/m^2 per day (a remote site) to 21 μg Cu/m^2 per day (an inner city site). In the city and nearby rural areas soil is not a major source of atmospheric copper, whereas at remote sites atmospheric copper is mostly soil-derived.

The atmospheric wet deposition of copper at Chesapeake Bay, USA, was examined during 1990 and 1991. The monthly integrated atmospheric fluxes exhibited a high degree of spatial and temporal variability. The arithmetically averaged annual wet flux was 260 μg

Cu/m^2 (Scudlark et al., 1994), and this was derived predominantly from anthropogenic sources. Wu et al. (1994) calculated the dry deposition flux for Chesapeake Bay to be 290–810 μm Cu/m^2 per year. Dry deposition fluxes for Lake Michigan were estimated at 690 and 800 μm Cu/m^2 per year.

Migon (1993) compared riverine and atmospheric inputs of copper with the Ligurian Sea (Mediterranean). Atmospheric inputs were found to be higher, with a ratio of 16.3 to 32.6.

Chan et al. (1986) reported that in southern Ontario, Canada during 1982, the mean concentration of copper in precipitation was 1.57 μg Cu/litre of which 1.36 mg Cu/m^2 was from wet deposition. The mean concentrations of copper in precipitation were 1.36 and 1.58 μg Cu/litre for central and northern Ontario, respectively. In both areas the annual wet deposition averaged 1.13 mg Cu/m^2.

Remoudaki et al. (1991) calculated the seasonal copper atmospheric deposition to the western Mediterranean. Atmospheric deposition of copper during the wet season ranged from 0.0004 to 0.0005 μg Cu/cm^2 per day and during the dry season 0.0007 to 0.0014 μg Cu/cm^2 per day.

Gorzelska (1989) analysed snowpack samples from 18 sites in the vicinity of Inuvik, Canada during 1985 and 1986. Copper concentrations ranged from 0.1 μg Cu/kg 20 km north of the town to 0.54 μg Cu/kg near a power plant. In all the samples the trace metals were enriched with respect to crustal material. Mass balance calculations have shown that most of the copper emitted by the local sources is transported outside the immediate vicinity of the town.

4.1.2 Water and sediment

Several processes influence the fate of copper in aquatic systems. These include complexation to inorganic and organic ligands, sorption to metal oxides, clays, and particulate organic material, bioaccumulation and exchange between sediment and water (Stiff, 1971; Callahan et al., 1979).

Much of the copper discharged to water is in particulate form and tends to settle out, precipitate out or be adsorbed by organic matter,

hydrous iron, manganese oxides and clay in the sediment or water column. Equilibrium is normally reached within 24 h. Copper discharged into a river leading into Chesapeake Bay contained 53 μg Cu/litre, of which 36 μg/litre was in the form of settleable solids (Helz et al., 1975). The concentration of copper 2–3 km downstream from the outfall had fallen to 7 μg/litre. Copper in particulate form includes precipitates, insoluble organic complexes and copper adsorbed to clay and other mineral solids (Stiff, 1971).

Owing to unacceptable past practices, Macquarie Harbour on the west coast of Tasmania, Australia contains dissolved copper levels as high as 560 μg/litre as a result of riverine transport in dissolved and particulate forms from the Mount Lyell copper mine (Carbon, 1996). Some 97×10^6 tonnes of mine tailings and 1.4×10^6 tonnes of slag were deposited into the Queen and King river system over a 78-year period before closure of the mine.

The copper(I) ion is unstable in aqueous solution, tending to disproportionate to copper(II) and copper metal unless a stabilizing ligand is present (Callahan et al., 1979). The only cuprous compounds stable in water are insoluble ones such as the sulfide, cyanide and fluoride. In its copper(II) state, copper forms coordination compounds or complexes with both inorganic and organic ligands. Ammonia and chloride ions are examples of species that form stable ligands with copper. Copper also forms stable complexes with organic ligands such as humic acids. In seawater, organic matter is generally the most important complexing agent. Samples collected from the surface waters (< 200 m) of the northeast Pacific revealed that over 99.7% of the total dissolved copper was associated with organically complexed forms. At depths of 1000 m approximately 50–70% of the copper was in the organically complexed form. Copper complexation gave rise to very low cupric ion activities in surface waters, around 1 pg Cu^{2+}/litre. The authors reported that two classes of copper-binding ligands were identified: an extremely strong ligand at low concentrations dominated in surface waters and a weaker class of ligand at higher concentrations was found throughout the water column (Coale & Bruland, 1988).

Tan et al. (1988) collected freshwater river samples from the Linggi river basin, Malaysia. Samples were separated into colloidal fractions and soluble fractions. Soluble fractions were classified

according to the lability of the copper forms in the water. Categories range from very labile (e.g. free metal ion) to nonlabile (e.g. colloidally bound metal). In this study 18–70% of the dissolved copper was moderately labile and 13–30% was slowly labile.

Copper in the fresh and estuarine waters of the Cochin estuary, India, was found to be extensively associated with organic colloidal matter. The relationship between exchangeable and total particulate copper did not show a significant correlation during the study, emphasizing the role of lattice-incorporated copper as distinct from particulate scavenged/adsorbed exchangeable copper (Shibu et al., 1990).

A detailed study of the Tamar estuary, United Kingdom, revealed a decrease in the α-coefficient for complexation of Cu^{2+} by natural organic ligands (log α_{Cul}) from 10.8 to 8.3 with increasing salinity, demonstrating that major cations compete with copper for the complexing sites. The free Cu^{2+} concentrations were very low ($16.2 < pCu(II) < 18.2$) throughout the estuary even though the total dissolved copper concentrations were high (up to 300 nmol/litre), probably because of complexation to dissolved organic complex (Van den Berg et al., 1990).

Giesy et al. (1986) isolated dissolved organic carbon from nine surface waters in the southeastern USA and found that the binding of copper by humate occurs with different strengths at a number of sites, the binding strength at the sites varying by two orders of magnitude, dependent on the ratio of copper to total organic ligand.

Organic compounds form complexes with 94 -98% of dissolved copper in the surface waters of the North Sea. In all samples strong copper-chelating compounds were found at concentrations of 4–10 μg Cu/litre (60–150 nmol/litre). The major inorganic complexes in the seawater samples were $CuCO_3^0$ (60%), $CuOH^+$ (16%) and $Cu(OH)_2^0$ (16%) (Van den Berg, 1984).

Mackey & Higgins (1988) found that the strong copper-complexing capacity of seawater can vary by more than three orders of magnitude. Copper-complexing capacity was related to the phytoplankton biomass. High values were associated with high

phytoplankton mass, whereas when the biomass was low the copper-complexing capacity was also low. The authors found that in nutrient-limiting, oligotrophic waters of low average productivity the copper-complexing capacity was variable.

Midorikawa et al. (1992) identified three classes of natural organic ligands in coastal seawater classified by differences in their complexing abilities for copper.

Gardner & Ravenscroft (1991) studied the behaviour of copper complexation in rivers and estuaries of northeast England. They found that copper speciation in rivers and estuaries is dominated by organic complexation. The authors found a mixture of ligands of different affinities for copper in natural waters. The complexation of copper discharged to rivers and estuaries occurred very rapidly. Complexation capacities were consistently in the range 10–25 μg Cu/litre (150–400 nmol/litre). The copper-complexing capacity of Linggi river water (Malaysia) was in the range 26–74 μg Cu/litre (410–1160 nmol/litre) (Tan et al., 1988).

Sharma & Millero (1988) measured the oxidation of copper(I) in air-saturated solutions of seawater as a function of pH (5.3–8.6), temperature (5–45 °C) and salinity (5–44‰). The rate of reaction increased with pH and temperature, and decreased with salinity (ionic strength). The results indicate that the rates are controlled by the concentration of Mg^{2+}, Ca^{2+}, Cl^- and HCO_3^- through complex formation and ligand exchange.

Bradley & Cox (1988) found that 80% of the measurable copper in standard river sediment SRM 1645 was in the organic fraction. In Yamuna river sediments, India, copper is mainly associated with the organic matter owing to its high complexing tendency for organic matter. A high percentage of copper is also found in the residual fraction, and much lower concentrations are associated with the carbonate and iron–manganese oxide phases (Gadh et al., 1993).

Calmano et al. (1993) studied the mobilization of copper from contaminated sediments. The dominant mobilizing factor was pH with mobilization increasing with increasing acidity. At pH values of < 4.5 there was a strong influence of pH on mobilization. At identical pH

values the mobilized portions of copper from the oxic sediment are tenfold higher than those from anoxic sediment.

Samanidou & Fytianos (1990) estimated a mobilization of 10–15% of copper due to NTA and EDTA in two rivers in northern Greece, with no consideration of the biodegradation of metal complexes. Samanidou et al. (1991) estimated that humic substances (~2–3 mg/litre) were able to cause the long-term release of 70–80% of copper in the same rivers. In experimental studies copper was remobilized by synthetic complexing agents more readily than other metals tested (cadmium, lead, manganese and chromium).

4.1.3 Soil

In the terrestrial environment, a number of important factors influence the fate of copper in the soil. These include the nature of the soil itself, its pH, the type and distribution of organic matter, the soil redox potential, the presence of oxides, the base status of the soil and its cation exchange capacity (CEC), the rate of litter decomposition and the proportions of clay to silt to sand particles. The residence time of copper in the soil is also a function of overall climate and of the vegetation present at a site.

Most copper deposited on soil from the atmosphere, from agricultural applications and from sewage sludge amendments is strongly adsorbed to the upper few centimetres of the soil. It is especially bound to the organic matter, as well as being adsorbed by carbonate minerals and hydrous iron and manganese oxides. Copper binds more strongly than most other metals and is less influenced by pH as a result. The greatest amount of leaching of copper occurs from sandy soils, compared with clays and peats, whereas acidic conditions favour copper leaching to the groundwater from the soil.

Lehmann & Harter (1984) studied the kinetics of copper desorption from the A horizon of Paxton soil (surface soil), USA, following addition of copper at rates ranging from 100 to 500 mg/kg. When 500 mg Cu/kg is added to this soil, about 94% is adsorbed within 15 min. The copper appears to be preferentially adsorbed to high energy sites. It appears that this soil is capable of retaining about 100 mg Cu/kg on high-energy bonding sites. If the copper is present

in excess of the high energy sites, the surplus fills low-energy sites. This more loosely bonded fraction continues to react for several hours. After 1 day this latter process reaches equilibrium, although the soil continues to adsorb copper very slowly from solution for up to 4 days.

Assaad & Nielsen (1984) studied the adsorption of copper in three Danish soil types (two orthic luvisols and a eutric fluvisol). The Langmuir adsorption equation was found to be the best to describe copper adsorption in these soils. Copper adsorption increased with increasing soil pH (pH 4.91–8.48) and decreased with increasing temperature (5–25 °C).

Petruzzelli et al. (1988) found that fly ash (10%) and humic acid (1%) increased the adsorption of copper (up to 100 µg/ml) in histosol. The addition of sewage sludge to a sandy loam soil increased the sorption of copper solutions of differing concentrations (0.1–1.5 µmol Cu/cm^3). The authors suggested that new adsorbing sites become available on the solid phase of the soil following "low metal" sludge addition (Petruzzelli et al., 1994).

King (1988) incubated 13 soil types (10 mineral and 3 organic) collected from the southeastern USA with 70 mg Cu/kg for 6 days. The amount of copper adsorbed ranged from 36% to 100%. Removal of copper from solution was much higher in surface soils than in subsurface sandy soils. Nonexchangeable copper was relatively high (up to 100%) in all but some of the acid subsoils. In the B and C horizons 96% of the variation in sorbed copper was explained by pH, whereas copper in the A horizon (surface soil) was unaffected by pH. The soil/water partition coefficient for copper was > 64 for mineral soils and 403 for organic soils.

Elliott et al. (1986) studied pH-dependent adsorption of copper, cadmium, zinc and lead on to four soils with differing chemical properties. Copper and lead were more strongly retained under acidic conditions (pH 5.0) than cadmium and zinc. Adsorption increased with pH (pH 3–5). The removal of organic matter from the soils substantially reduced the adsorption of copper.

Sanders & McGrath (1988) studied the extent of copper complex formation by soluble organic matter extracted from an organic soil, a

clay and two sandy loams. Copper was extensively complexed in these solutions. The percentage of copper existing as Cu^{2+} fell as the pH increased, and also fell as the total copper concentration decreased. Weight for weight, organic matter from the sandy loams was most effective at forming complexes with copper within the experimental pH range (pH 4–7) followed by the organic soil and then the clay.

Allard et al. (1991) studied the distribution of copper within an illitic clay formation beneath an old (~150 years) deposit of sulfidic mine tailings. The adsorption in the lower pH range had little impact on the mobility of copper: at pH levels in excess of 5, copper is immobilized. The results suggest that transport of copper originating from the tailings is diffusion controlled.

Tyler & McBride (1982) studied the relative mobility of copper added to several mineral and organic soils and the simultaneous desorption and leaching of metals determined by eluting soil columns with 0.01 mol/litre calcium chloride. Copper was eluted much more slowly and in much smaller quantities than zinc, cadmium or nickel.

Berggren (1992) studied the factors affecting the mobilization of copper in spruce, beech and birch forest soil profiles (podzols and cambisols) at two sites in Sweden. At a depth of 15 cm almost all of the copper was found to be organically bound. The results also indicate that organically-complexed copper constituted the predominant copper form in soil solutions at 50 cm despite the relatively low dissolved organic carbon (3–14 mg/litre) and the highly aluminium-saturated organic compounds.

Strain et al. (1984) studied the leaching of copper by simulated "acid" rain (pH 2.8–4.2) applied in rainwater to soil from Swedish spruce forest polluted by a brass mill. Leaching of copper increased considerably when water at pH < 3.4 was applied to the soil.

Campanella et al. (1989) found that UV (mercury lamp) irradiation of urban sludge resulted in an increased mobility of copper eluted with sulfuric acid; this was attributed to degradation of organic matter through radical reactions which provoked the formation of smaller molecules acting as more soluble metal carriers.

Wong et al. (1993) found that a copper(II)-accumulating bacterial strain (*Pseudomonas putida* II-11) isolated from electroplating effluent removed a significantly high amount of copper(II) from growth medium and buffer. The adsorption was pH dependent with a maximum at pH 8.0.

Groudev & Groudeva (1993) studied the microflora of four industrial copper dump leaching operations. It was found that copper solubilization depended mainly on the amount and activity of the mesophyllic acidophilic chemolithotrophic bacteria which occurred in the ore dumps.

4.1.4 Sewage sludge inputs to land

Land treatment is increasingly being utilized as a method of waste disposal for sewage effluent and sludge. The intent is to combine the benefits of fertilizer effects and organic additions to soils, with safe land disposal of the large quantities of domestic sewage being generated (Brown et al., 1983; Juste & Mench, 1992; Henry & Harrison, 1992). Sewage effluent and sludges vary greatly in their content of metals and especially when domestic sewage is not separated from industrial sources the metal levels can be high (e.g. for chromium, copper, zinc, nickel, cadmium) and can pose potential hazards as a result of metal accumulation if applied to land at high rates over the long term. There are a number of sources of copper in sewage effluent and sludge including human excreta, from the corrosion of copper pipes in domestic water supplies and from direct additions from industrial processes. In view of the recent interest in the sustainability of agricultural land focus has been on the potential of land treatment to cause elevated and toxic levels in the soils. Present national and regional guidelines are aimed at protecting such amended land into the future (Table 4).

Copper concentrations in sewage sludge vary greatly. For example, Hedberg et al. (1996) quote copper concentrations from 0 to 16 000 mg/kg per day sludge for Finland, with a median value of 214 mg Cu/kg. In nine different sewage districts in Norway the levels in sludge varied from 100 to 500 mg Cu/kg d.s. For this Norwegian data set, there was a relationship between the copper content in the sewage sludge and the pH of the drinking-water. The average copper

Table 4. Directives for maximum allowed metal concentrations in sewage sludge used as a soil improvement agent in agriculture (From: Hedberg et al., 1996)

Country/ area	Maximum allowed metal concentration (mg/kg dry weight)			
	Copper	Zinc	Lead	Cadmium
EU[a]	1000–1750	2500–4000	750–1200	20–40
Denmark	1000	4000	120	0.8
Germany	800	2500	900	10
Finland	600	1500	100	1.5
France	1000	3000	800	20
Netherlands	75	300	100	1.25
Norway[a]	1000–1500	1500–3000	100–300	4–10
Sweden	600	800	100	2
USA (EPA)	1500–4300	2800	300–840	89

[a] The higher level is valid for application on greenlands

content in the sludge was 140 mg Cu/kg d.s. for those drinking-water plants with pH adjustments (pH increased to 8–8.5) while the average copper content in the sewage sludge which had received water without pH adjustments was 320 mg Cu/kg d.s. Attempts to reduce the corrosivity of piped water supplies can lead to changes in the copper (and iron) in sewage sludge.

Copper, like other metals applied to land by sludge or effluent amendments, is rather strongly adsorbed in the upper surfaces, especially by organic matter, for prolonged periods. It is already organically bound and, upon release by respiratory breakdown, is then re-absorbed. Juste & Mench (1992) examined the long-term effects of sewage sludge applications (10 years or more in duration) on metal distribution in the soil profile as well as crop responses and metal uptake from field trials in the EC and the USA. In almost all cases, sludge-borne metals appeared to remain in the zone of sludge incorporation to soils (0–15 cm). Mass balances on metal recoveries from soil additions ranged from 30% to 90%. Lateral soil movement

was the main explanation of the progressive disappearance of metal from experimental plots. Copper was a good deal less bioavailable to crops from sludge amendments than cadmium, nickel and zinc, but somewhat more mobile and bioavailable than lead.

In forest soils the retentivity of copper in the profile may be even greater from sludge amendments than in agriculture systems. For example, Zabowski & Zasoski (1987) equilibrated three soil horizons (A, B2 and C) of an acidic forest soil with copper solutions in the presence and absence of municipal sewage sludge leachate. Copper binding to the soils in each of the three horizons was greater than that of cadmium or zinc. Sludge leachate reduced copper adsorption in all three horizons.

In the great majority of sludge metal studies done to date, although copper is a constituent of the sludge, it is very rarely the element which imposes the limits for addition of sludges or sewage effluent to land.

4.1.5 Biodegradation and abiotic degradation

Copper is transformed in the environment to forms that are either more or less bioavailable, depending upon the physical and chemical conditions present in the environment of interest. For information on the speciation of copper, see section 2.4.

4.2 Bioaccumulation

Bioaccumulation is defined as the net uptake of copper by microorganisms, plants or animals from their surrounding environment (water, sediment, soil and diet). The species of copper present in environmental media and its associated bioavailability, together with differences in plant and animal uptake and excretion rates, determine the extent of bioaccumulation. For aquatic organisms bioconcentration refers specifically to water.

4.2.1 Microorganisms

Sahoo et al. (1992) found that a bacterial (*Bacillus circulans*) biomass of 1.48–1.52 g/litre (dry weight) removed 80% of copper in a 495 mg Cu/litre solution. A reduction of the pH was detrimental to the accumulating capacity of the bacteria.

Bengtsson et al. (1983) grew the hyphomycete (fungus) *Verticillium bulbillosum* in agar containing 15, 45 or 150 mg Cu/litre for one week. Mean copper concentrations in the mycelium were, respectively, 1296, 2608 and 3245 mg/kg for the three exposure concentrations.

4.2.2 Aquatic plants

Bioaccumulation factors have been calculated for over 20 species of marine macroalgae showing maximum values up to 27 000, depending on the exposure concentration (Bryan & Hummerstone, 1973; Phillips, 1977; Malea et al., 1994; Correa et al., 1996).

Hall et al. (1979) found that a nontolerant strain of the brown alga *Ectocarpus siliculosus* exposed to various copper concentrations (up to 250 µg/litre) displayed higher accumulation values than did a tolerant strain. At 72 h incubation, the tolerant strain accumulated mean copper values of 20 mg/kg (wet weight) with no added copper and 234 mg/kg at 250 µg Cu/litre in the medium (Hall, 1981). The same strain incubated for 14 days displayed accumulation values of 13 mg/kg with no added copper and 1075 mg/kg at 250 µg Cu/litre in the medium. Reed & Moffat (1983) exposed the green alga *Enteromorpha compressa* to copper concentrations of up to 610 µg/litre (9.6 µmol/litre) for 6 days. Copper accumulation was linearly dependent on the exposure concentration and the pattern was similar in both the tolerant and non-tolerant strains. Mean maximum concentrations in the algae were 22.2 mg Cu/kg (0.35 µmol/g) (fresh weight) for the nontolerant strain and 25.4 mg Cu/kg (0.4 µmol/g) for the tolerant strain. Equilibrium was not reached within the experimental time period.

Mersch et al. (1993) maintained the aquatic moss *Rhynchostegium riparoides* in water containing copper levels ranging from 4.5 to 50 µg/litre for 27 days. Accumulation was rapid and reached a plateau after 18 days. At the end of the 14-day depuration phase the moss had lost 50% of the accumulated copper. Claveri et al. (1994) studied the uptake of copper (5–342 µg/litre) by *R. riparoides* for periods of up to 168 h. The accumulation of copper occurred predominantly during the initial 96 h and had reached equilibrium within 168 h. Copper concentrations in the mosses ranged from 30 to 2500 mg/kg (dry

weight). During the 10 day depuration period there was a rapid decrease in copper levels during the first 72 h after which copper concentrations in the mosses approached equilibrium values ranging from 32 to 700 mg/kg (dry weight).

Sinha & Chandra (1990) studied the accumulation of copper (0.05–5.0 mg/litre) by the aquatic plant *Bacopa monnieri* for 168 days. Accumulation was directly related to the exposure concentration. Copper concentrations in shoots ranged from 20 to 721 mg/kg (dry weight) and in roots from 195 to 3821 mg/kg.

The uptake of copper by duckweed (*Lemna minor*) and water velvet (*Azolla pinnata*) was investigated by Jain et al. (1989). Plants were grown in copper solutions of 1, 2, 4 or 8 mg/litre under static renewal conditions for 14 days. Copper concentrations in the plants ranged from 979 to 6714 mg/kg (dry weight) for duckweed, and from 1159 to 7725 mg/kg for water velvet. Uptake rate was highest at the lower exposure concentrations; concentration factors ranged from 51 to 60 for duckweed, and from 58 to 66 for water velvet. Dirilgen & Inel (1994) grew duckweed (*Lemna minor*) in Jacob nutrient medium at copper concentrations ranging from 0.23 to 2.03 mg/litre for 7 days. Bioconcentration factors, based on copper content of plants on a dry weight basis, were 1447, 444 and 314 at copper concentrations of 0.23, 1.03 and 2.03 mg/litre, respectively.

Kay et al. (1984) exposed water hyacinths (*Eichhornia crassipes*) to copper (0.5–5.0 mg/litre) for 6 weeks. At the highest copper concentration levels in leaves, stems roots and dead tissue were 321, 710, 8160 and 5151 mg/kg (dry weight) respectively; bioconcentration factors ranged from 64 to 1632. Nor & Cheng (1986) grew water hyacinths in 2 mg/litre copper solutions. Fulvic acid (10–50 mg/litre) did not affect the uptake of copper by *Eichornia*; however, humic acid (20 and 50 mg/litre) strongly inhibited copper uptake. In the absence of ligands *Eichornia* accumulated 204 and 2451 mg/kg (dry weight) from copper solutions of 1 and 10 mg/litre, respectively.

4.2.3 Aquatic invertebrates

Hansen et al. (1995) exposed the marine demosponge *Halichondria panicea* to dissolved copper concentrations ranging from

0.45 (control) to 1000 µg/litre for 14 days. The sponge accumulated copper in direct proportion to the concentration of the dissolved metal in the surrounding medium. Final body copper concentrations were 236 and 818 mg/kg (dry weight) at exposure concentrations of 300 and 1000 µg dissolved Cu/litre, respectively. There was no significant loss of copper during an 8 day depuration period. The authors proposed this species as a suitable biomonitoring organism.

Elliott et al. (1985) found that the marine mussel *Mytilus edulis* exposed either continually, or in a 2 day cycle, to copper (10 µg/litre) exhibited a linear accumulation over a 40 day period. Mussels exposed under cycled conditions showed a lower rate of accumulation. Copper accumulation was not in direct proportion to the time exposed to the elevated concentration. The presence of cadmium reduced the accumulation factor by 50%.

Holwerda (1991) exposed freshwater clams (*Anodonta cygnea*) to copper (47 µg/litre) for 6.5 weeks. An accumulation factor of 55 was calculated for the exposure period. Crecelius et al. (1982) exposed clams (*Macoma inquinata*) and shrimps (*Pandalus danae*) to copper concentrations ranging from 5 to 30 µg/litre for one month. Body burdens ranged from 25 to 97 mg Cu/kg (dry weight) for clams and from 146 to 322 mg Cu/kg for shrimps. Ageing of the solutions prior to exposure reduced the bioavailability of copper. In a static system with added sediment more than 50% of the added Cu^{2+} became bound to the organic fraction of the sediment and was unavailable to suspension-feeding clams (*Protothaca staminea*); however, deposit-feeding clams (*Macoma inquinata*) placed in the sediment doubled their copper body burden within 2 months.

Biological half-lives for depuration of copper from "green" oysters (*Crassostrea gigas*) and mussels (*Mytilus smarangdium*) from a copper-contaminated area, and "normal" oysters were 11.6, 6.4 and 25.1 days, respectively (Han et al., 1993).

Rainbow & White (1989) exposed decapods (*Palaemon elegans*), amphipods (*Echinogammarus pirloti*) and barnacles (*Elminius modestus*) to copper at concentrations ranging from 31.62 to 3162 µg/litre for 28 days. Whole-body copper levels (129.3 mg/kg) are regulated in the decapods at exposures up to and including

100 µg/litre and at higher exposures there is net accumulation. In amphipods and barnacles there was net accumulation of copper at all exposures with no apparent regulation of copper levels.

Weeks & Rainbow (1991) exposed the talitrid amphipods *Orchestia gammarellus* and *O. mediterranea* to copper concentrations ranging from 31.6 to 3162 µg/litre for 21 days. Mean rates of copper accumulation (measured as net accumulation of total copper) ranged from 0.9 to 77.0 µg/g per day for *O. gammarellus* in a dose-related manner; rates of accumulation in *O. mediterranea* ranged from 1.19 to 28.1 µg/g per day showing an increase with copper exposure at concentrations ≥ 100 µg/litre. Weeks & Rainbow (1993) fed the talitrid amphipods *O. gammarellus* and *O. mediterranea* on discs of algae treated with copper (16.3–2070 mg/kg) for 21 days. *O. gammarellus* accumulated whole-body copper concentrations ranging from 104 to 163 mg/kg; haemolymph concentrations ranged from 525 to 677 mg/kg (dry weight). Rates of accumulation ranged from 0.52 to 4.71 µg/g per day, increasing with increasing copper exposure. The rates of accumulation for *O. mediterranea* remained fairly constant at all exposure concentrations (0.28–0.37 µg/g per day) except the highest (1.61 µg/g per day). It was concluded that for *O. gammarellus* accumulation of copper from food was a more important route than accumulation of copper from solution. *O. mediterranea* was unable to satisfy its copper requirements from a food source but was able to do so from solution.

Weeks et al. (1993) exposed shore crabs (*Carcinus maenus*) to 750 µg Cu/litre for up to 7 days at various salinities. Copper accumulated in the gills and midgut gland but not in muscle. The accumulation of copper in gill tissue was positively correlated with salinity.

Ozoh (1994) exposed ragworms (*Hediste diversicolor*) to a copper concentration of 200 µg/litre for up to 15 days. At 12 °C, low salinity (7.5‰) increased the availability of copper to the worms and more copper was accumulated, copper concentrations ranging from 83.27 to 183.12 mg/kg (dry weight). Increasing salinities of 15.25 and 30.5‰ reduced the accumulation of copper. At 17 and 22 °C more copper was accumulated than at 12 °C, with copper concentrations ranging from 58.7 to 784 mg/kg. The addition of sediment to the test system reduced the accumulation of copper by the worms (Ozoh, 1992b).

Zia & Alikhan (1989) found that crayfish (*Cambarus bartoni*) accumulated copper concentrations ranging from 130 to 296 mg/kg after exposure to copper concentrations ranging from 125 to 500 µg/litre for 4 weeks. Copper was predominantly accumulated in the gills and hepatopancreas.

Winner (1984) exposed *Daphnia magna* to copper (30 µg/litre) for 7 days; during this period daphnids accumulated whole-body copper residues of 70.7 mg/kg (dry weight). The addition of 0.75 mg humic acid/litre had no significant effect on the accumulation of copper.

Giesy et al. (1983) found that the presence of organic matter decreased the accumulation of copper by the softwater cladoceran *Simocephalus serrulatus*. When bioconcentration factors (BCF) were calculated using Cu^{2+} the BCFs were similar for the different water types tested, while when based on total copper concentrations they varied greatly owing to varying amounts of organic matter. The authors concluded that most of the copper accumulated by this species was Cu^{2+} or the labile aquatic forms and that a decrease in Cu^{2+} due to binding of copper by organic matter reduced accumulation.

Vogt & Quinitio (1994) exposed juvenile giant tiger prawns (*Penaeus monodon*) to 1 mg Cu/litre for 10 days. Copper deposition was investigated by histochemistry and electron microscopy. Copper granules were accumulated in large quantities in the hepatopancreas tubules, the amount and size of the granules increasing along the tubules in relation to the cells' age. The granules were released by discharge of senescent hepatopancreas cells and were added to the faeces.

Timmermans & Walker (1989) exposed fourth instar larvae of the midge *Chironomus riparius* to copper (50 or 100 µg/litre). Larvae accumulated copper with increasing levels of exposure, but very small amounts were recovered in pupae or imagines. Average body burdens were approximately 425 and 750 ng copper, respectively, for the two exposures.

Dodge & Theis (1979) reported that copper (85 or 325 µg/litre) was accumulated from solutions by midge larvae (*Chironomus tentans*) in which the dominant aqueous forms were free Cu^{2+} ion and a copper

hydroxy complex reaching concentrations in excess of 200 mg/kg (dry weight). No significant uptake was observed when copper–glycine and copper–NTA complexes were dominant.

4.2.4 Fish

Peres & Pihan (1991b) exposed carp (*Cyprinus carpio*) for up to 3 weeks to copper concentrations of 20, 40 and 120 µg/litre at water hardnesses of 50, 100 and 300 mg $CaCO_3$/litre, respectively. Accumulation in gills after 3 weeks was 53, 58 and 78 mg/kg dry weight for the three exposure conditions, compared to 13 mg/kg initially.

Daramola & Oladimeji (1989) exposed the freshwater fish *Clarius anguillaris* and *Oreochromis niloticus* to copper for 8 weeks. For *C. anguillaris*, whole body accumulation was 15.7, 21.8 and 31.2 µg Cu/g dry weight for exposure concentrations of 27, 55 and 110 µg Cu/litre, compared to 6.9 µg Cu/g in control fish. For *O. niloticus*, accumulation was 34.7, 36.1 and 81.0 at exposures of 0.05, 0.10 and 0.20 µg Cu/litre, respectively, compared to 17.6 µg Cu/g in controls.

Playle et al. (1992) studied the accumulation of copper (16 µg/litre) on the gill of fathead minnow (*Pimephales promelas*) exposed for 2–3 h. The addition of Ca^{2+} (2100 or 4200 µeq/litre) reduced gill copper accumulation during exposures at pH 4.8 but not at pH 6.3. EDTA eliminated copper deposition at both pH levels when equimolar with copper, but reduced copper deposition by 50% when half equimolar at pH 4.8. The authors concluded that copper accumulation on the fish gills was reduced by Ca^{2+} and H^+ competition at the gill surface, and by EDTA complexation of copper in the ambient water.

Buckley et al. (1982) exposed coho salmon (*Oncorhynchus kisutch*) to copper at concentrations of 70 and 140 µg/litre for 15 weeks. Copper accumulation in liver was greatly elevated, averaging approximately 180 and 320 µg Cu/g dry weight versus 60 µg Cu/g in control fish in the latter half of the experiment. Gill concentrations were also significantly elevated, averaging 5.6 µg Cu/g and 9.5 µg Cu/g compared to 3.2 µg Cu/g in controls. Copper concentrations in plasma were not significantly elevated by copper exposure except during the first day, while concentrations in kidney were only slightly

elevated (6.6, 7.2 and 9.4 μg Cu/g dry weight for controls, low and high exposures, respectively).

Lanno et al. (1985) fed rainbow trout (*Oncorhynchus mykiss*) diets containing various levels of copper. For an 8 week exposure, copper concentrations in liver ranged from 127 μg/g dry weight for a diet containing 8.5 mg/kg dry weight to 3200 μg Cu/g for a diet of 3100 mg Cu/kg. For a 24 week exposure, accumulation in liver ranged from 295 μg Cu/g for a diet of 8.5 μg Cu/g to 1640 μg Cu/g for a diet of 660 μg Cu/g, while concentrations in kidney ranged only from 8.5 to 21.8 μg Cu/g.

Mount et al. (1994) fed rainbow trout (*Oncorhynchus mykiss*) on a brine shrimp (*Artemia* sp.) diet containing 9.4, 440, 830 or 1000 mg Cu/kg (dry weight) for up to 60 days. After 35 days whole-body copper concentrations were 5.9, 36, 43.5 and 57.5 mg Cu/kg (dry weight) for the control and three doses, respectively, but after 60 days copper levels had fallen to 3.6, 19.6, 22.4 and 27.7 mg Cu/kg. In a second experiment fish were fed diets containing copper concentrations ranging from 7.8 to 320 mg Cu/kg. Whole-body copper concentrations ranged from 2.7 to 35.8 mg Cu/kg after 35 days, and from 2.3 to 8.8 mg Cu/kg after 60 days.

4.2.5 Terrestrial plants

Terrestrial plants respond in a number of ways to copper in the soils on which they grow. Rooted species are subject to exposures which vary seasonally and over the plants' lifetime. Perennial and especially long-lived species may experience wide changes in exposure over time. Species differ both in their requirements and in their tolerances for copper. Indeed, some terrestrial species are well known and used in mineral prospecting as copper indicators. These include both mosses and higher plants. Others are hyperaccumulators (Brooks, 1977; Baker & Brooks, 1989; Brooks et al., 1992). Among the metal accumulators, a number of species from widely different plant families can accumulate from 2000 to 14 000 μg Cu/g (dry weight) in foliage, compared with 20–40 μg Cu/g (dry weight) in other species (Baker & Brooks, 1989).

In Austria, the average copper level in soils was 17 μg/g and that in vegetation 12 μg/g; for Belgium it averaged 17 μg/g for soil and

17 μg/g for vegetation; in Finland, 4.3 μg/g for soil and 6.1 μg/g for vegetation; and for Germany 22 μg/g for soil and 24.5 μg/g for vegetation (Angelone & Bini, 1992).

In studies of copper tolerant and sensitive strains (varieties) of the forage grass (*Festuca rubra*) Wong et al. (1994) showed that copper concentrations in hydroponic solution of 50 μg/g allowed growth of a tolerant variety whereas even 5 μg Cu/g inhibited a sensitive strain. Root copper concentrations reached 750 μg/g in the tolerant strain exposed to 1 μg Cu/g, whereas in the sensitive strain they were about 390 μg/g at the same exposure. In contrast, in the shoots of these same plants exposed to 1 μg Cu/g the tolerant plants contained 18 μg Cu/g and the sensitive plants 10 μg Cu/g. Higher root than shoot concentrations of copper are normal in terrestrial plants.

In contrast to the situation for aquatic biota, copper levels in soils can vary over a wide range of concentrations and plant genetic tolerances allow an equally wide range of responses to these copper exposures. Copper levels in foliage can be below the soil concentrations over which they grow or can be very much higher in accumulator species.

4.2.6 Terrestrial invertebrates

Moser & Wieser (1979) fed snails (*Helix pomatia*) on a diet containing 230 or 1390 mg Cu/kg for 3 weeks. Animals exposed during the summer accumulated copper concentrations ranging from 76 mg/kg (dry weight) (buccal mass and oesophagus) to 238 mg Cu/kg (intestine). Copper contents of midgut gland and foot were 44.7 and 56.0 μg/kg (dry weight) respectively. In snails exposed during the winter months much higher concentrations were accumulated, ranging from 106 mg Cu/kg in the buccal mass and oesophagus to 1621 mg Cu/kg in the intestine. In short-term (2–10 days) feeding experiments with lettuce containing 1390 mg Cu/kg, about 97% of the metal ingested remained in the snail. Berger & Dallinger (1989) fed terrestrial snails (*Arianta arbustorum*) on copper-enriched agar at concentrations of 209 mg Cu/kg or 723 mg Cu/kg (dry weight). The highest concentrations of copper following exposure to the lower concentration for 21 days were in the midgut (492 mg Cu/kg). The copper concentration of the faeces increased continuously during the

experiment but the highest value recorded at 69.5 mg Cu/kg was only one-third of the concentration in the food. In a 14-day copper balance study utilizing the higher dose (723 mg/kg) the mean rate of copper uptake was 6 µg/day. The main site of copper storage seemed to be the foot/mantle tissues where 49% of the ingested copper was found. The efficiency of copper assimilation always exceeded 95%. Dallinger & Wieser (1984) maintained snails (*Helix pomatia*) on a diet of lettuce enriched with 533.8 mg Cu/kg for 32 days. Copper contents of foot (0.579 g dry weight), midgut gland (0.326 g dry weight) and posterior gland (0.057 g dry weight) were 90.1, 42.7 and 15.0 µg after 32 days; copper contents in foot and midgut gland had fallen to 39.6 and 23.1 µg after 38–48 days on a "clean" diet. Copper was distributed more evenly in the organs of the snail than the other metals investigated (lead, zinc and cadmium); the midgut gland did not play such a dominant role in the storage of copper.

Dallinger & Wieser (1977) exposed three species of isopods to copper concentrations of 340 and 5200 mg/kg (dry weight) in food (birch litter) for 14 days. When feeding on natural litter with a low concentration (20 mg Cu/kg) all three species lost more copper through their faeces than they ingested. When fed artificially enriched litter the efficiency of assimilation increased, so that at the highest concentration tested between 80% and 99% of the ingested copper was assimilated. Isopods are capable of digesting even tightly bound copper during one passage of food through the gut. However, they are unable to resorb more copper than they lose unless the food is enriched with soluble copper or the rate of food passage through the gut is slowed down.

4.2.7 Terrestrial mammals

Dodds-Smith et al. (1992a) maintained shrews (*Sorex araneus*) on a diet containing copper at an intake of 2.13 mg/day for 12 weeks. Mean whole-body copper concentrations were 23.6 mg/kg (dry weight) in males and 64.8 mg/kg in females; mean total body burden was 64.7 µg Cu in males and 150.1 µg Cu in females. Mean copper concentrations were 31.0 and 23.4 mg/kg in kidneys of males and females, and 192.5 and 820.5 mg/kg in livers of males and females, respectively (Dodds-Smith et al., 1992b).

5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 Environmental levels

There is a very large amount of information on the levels of total copper in the various environmental compartments but little information on speciation. Therefore, an attempt has been made to summarize those values related to temporal or geographical trends, polluted sites and known sources of copper.

The largest release of copper is to *land*; the major sources of release are mining operations, agriculture, solid waste, and sludge from sewage treatment works. Mining and milling contribute most of the solid wastes. Copper is released to *water* as a result of natural weathering of soil, discharges from industries and sewage treatment plants, and from antifouling paints. Copper compounds may also be intentionally applied to water to kill algae. Copper is emitted to the *air* naturally from windblown dust and volcanoes; however, anthropogenic sources contribute more to modern atmospheric levels from activities such as primary copper smelters, ore processing facilities and incineration (ATSDR, 1990).

5.1.1 Air

Hong et al. (1996) measured copper concentrations in Greenland ice samples. The results revealed that anthropogenic sources of atmospheric copper first occurred in the Bronze Age, and that peaks of pollution occurred 2000 years ago due to the Romans and 900 years ago due to the Sung dynasty in China, before rapidly rising over the last century with some evidence of decline in recent years.

The concentrations of copper in air depend on the proximity of the site to major sources such as smelters, power plants, and incinerators. Average concentrations are in the range 5–50 ng Cu/m^3 in rural areas and 30–200 ng Cu/m^3 in urban locations (Nriagu, 1979b). Evans et al. (1984) reported on the US EPA's national surveillance network for the years 1977, 1978 and 1979. Copper levels in air were 133, 138 and 96 ng/m^3, respectively, for urban samples and 120, 179 and 76 ng/m^3

for non-urban samples. In the study 10 769 urban and 1402 non-urban air samples collected for 24 h were analysed. The maximum urban and non-urban copper concentrations were 4625 and 4003 ng/m^3, respectively.

Atmospheric copper concentrations at the South Pole were found to range from 25 to 64 pg/m^3 with a mean value of 36 pg/m^3 (Zoller et al., 1974). Copper concentrations in Atlantic aerosols were collected during 1980–1982. Mean concentrations ranged from 1.0 to 4.5 ng/m^3 for the North Atlantic and from 0.29 to 0.31 ng/m^3 for the South Atlantic. In remote areas of the Atlantic, where the influence of continental sources is less, oceanic copper can make up over half of the total copper in the aerosol (Chester & Murphy, 1986).

Sweet et al. (1993) analysed airborne particulate matter in southeast Chicago and East St Louis, USA. Copper concentrations ranged from < 0.1 to 1610 ng/m^3 in fine particles (< 1–2.5 μm), and from < 0.1 to 224 ng/m^3 in coarse particles (2.5- 10 μm). Concentrations were found to be higher in samples from St Louis; these higher levels of copper in both fine and coarse fractions occurred in winds from the direction of several nonferrous metal smelters.

Anderson et al. (1988) analysed atmospheric aerosols collected in Chandler, Arizona, USA in 1982. Several major copper smelters are located approximately 120 km southeast of the sampling point. The most abundant copper-bearing particle (particles containing > 0.5% copper), representing 74% of the total, was associated with sulfur, 16% was associated with silicon and 4% was associated with chloride. Germani et al. (1981) reported that mean copper levels in particulate matter were found to be 2800 and 6800 ng Cu/m^3 in the plumes of two copper smelters in Arizona, USA. Mean concentrations ranging from 2000 to 9500 ng Cu/m^3 were reported for the first 8 km of plumes from five copper smelters (Small et al., 1981). Atmospheric particulate aerosol samples were collected at sites along the normal plume pathway at distances ranging from 2.5 to 8.0 km from a copper smelter (western Poland). Copper concentrations were inversely correlated to distance with levels of 165, 89 and 51 ng Cu/m^3 (2.6, 1.4 and 0.8 nmol/m^3) at distances of 2.5, 5.0 and 8.0 km, respectively (Zwozdziak et al., 1985).

Romo-Kröger & Llona (1993) analysed aerosols in the Chilean central Los Andes mountain range at varying distances from a copper mine. Copper concentrations in fine (< 0.4 μm) particles ranged from 414 ng/m^3 (5 km from the mine) to 22 ng/m^3 at > 25 km from the mine. A similar correlation between distance from the mine and copper levels was found for coarse (> 8.0 μm) samples although levels were lower, ranging from 40 to 101 ng Cu/m^3. Romo-Kröger et al. (1994) found that copper levels were related to mining operations. Sampling at 13 km from the mine revealed copper concentrations of 66 and 131 ng/m^3 for fine (< 2.5 μm) and coarse (2.5–15 μm) particles, respectively, during mining operations. Sampling during strike periods gave levels of 22 and 50 ng Cu/m^3, respectively.

Johnson et al. (1987) reported elevated levels of copper in fog water 3 km downwind of a refuse incinerator in Switzerland. Highest copper concentrations were associated with lower pHs. The maximum concentration was 673 μg Cu/litre (10.6 μmol/litre) at pH 1.94, with levels > 127 μg Cu/litre being associated with pH values < 3.6.

The annual average concentrations of copper in aerosols < 10 μm in the Netherlands varied between 11 and 25 ng/m^3. None of the eight sites was directly affected by industrial sources (Slooff et al., 1989).

5.1.2 *Water and sediment*

Copper is widely distributed in water because it is a naturally occurring element. Nriagu (1979b) reported average copper levels in seawater ranging from 0.15 μg/litre in open ocean to 1.0 μg/litre in polluted near-shore waters; levels in fresh water were 1.0–20 μg/litre. Other reports indicate that copper concentrations in seawater are highly variable, ranging from 0.005 μg/litre in the Black Sea (Haraldsson & Westerlund, 1988) to 40 μg/litre in estuaries in southwest Spain (Cabrera et al., 1987). Additional variation in copper concentrations is related to depth and the area in the ocean examined. Surface concentration in the North Pacific Ocean drops from 0.1 μg Cu/litre (1.2 nmol/kg) in the California Current to 0.03–0.04 μg Cu/litre (0.4–0.5 nmol/kg) in the central oceanic region, and increases to 0.24 μg Cu/litre (3 nmol/kg) in deep waters (Boyle et al., 1977; Bruland, 1980). In the North Atlantic Ocean surface waters display values of copper from 0.07 μg/litre (1.1 nmol/kg) to 0.11 μg/litre

(1.7 nmol/kg), whereas concentration of the metal increases to 0.13–0.26 µg/litre (2–4 nmol/kg) in deep waters (Moore, 1978). Similarly, in the Ligurian Sea, Italy, Fabiano et al. (1988) reported 3.57–16.6 µg dissolved Cu/litre in the surface layer (0–50 m) and 0.7–72 µg/litre in deeper waters (200–2000 m). Bryan & Langston (1992) reported dissolved copper concentrations of up to 600 µg/litre for Restronguet creek, a branch of the Fal estuary, United Kingdom, which receives acidic drainage from past and present mining activity.

Bubb & Lester (1994) found mean copper concentrations in total and soluble (filter size 0.2 µm) river water for the river Stour, United Kingdom, to be 5.8 (3.0–19.5) and 2.2 (1.0–5.5) µg/litre, respectively. Background levels were 1.0 µg Cu/litre derived from an upper catchment control site. Fourfold increases in copper concentrations were apparent downstream of a sewage treatment works.

Dissolved copper was monitored for 11 months in four recreational marinas, a large harbour, two major river systems and a heavily used shipping canal in Chesapeake Bay, USA. Mean copper concentrations were 9.1, 13.2, 17.8 and 18.2 µg/litre for the four marinas, 7.9 µg/litre for the harbour, 6.4 and 11.9 µg/litre for the two river systems and 9.6 µg/litre for the shipping canal. Copper concentrations ranged from < 10–80 µg/litre for the marinas to 10–14 µg/litre for the harbour and 10–20 µg/litre for the river systems and the shipping canal. The authors concluded that the likely source of the highest copper concentrations was from antifouling paints used on boats in the marinas (Hall et al., 1988). An evaluation of dissolved copper concentrations at three sampling stations in 1989 showed that mean concentrations from biweekly sampling for four months were 2.7, 7.8 and 10 µg Cu/litre. Copper concentrations decreased with distance from marinas, and at all three stations were significantly lower in 1989 than in 1988 (Hall et al., 1992).

Parrish & Uchrin (1990) sampled Lakes Bay, near Atlantic City, USA during the summer of 1986. Dry weather concentrations of copper were found to be typical of those found in natural waters, but higher levels were recorded during storm events. Significant amounts of copper were found to originate from a major stormwater sewer which discharges into the bay. Total copper in runoff from a car park near Portland, Oregon, USA varied among different storm events over

a wide range of concentrations (< 2–33 µg/litre). Copper levels in a detention pond ranged from 5 to 12 µg/litre. Copper was found to be deposited in pond sediments in a small highly concentrated plume (up to 130 mg/kg) extending from the runoff inlet pipe (Mesuere & Fish, 1989).

Hurley et al. (1996) measured the concentration of copper and several other metals in 11 tributaries (rivers) feeding Lake Michigan, USA using low-level techniques. They reported dissolved and total copper concentrations ranging from 0.2 to 2.0 and 0.4 to 5.5 µg/litre, respectively.

Shiller & Boyle (1987) measured dissolved concentrations of copper in the lower Mississippi river, USA seven times. The Mississippi was chosen because it is the most heavily industrialized of the 10 largest rivers in the world. The authors concluded that the levels of copper and several other metals do not appear to be significantly higher than in several other less industrialized and disturbed rivers. Dissolved copper concentrations ranged from 1.16 to 1.96 µg/litre. Samples from the Yangtze, Amazon and Orinoco rivers were analysed for comparison. Dissolved concentrations of 1.24, 1.52 and 1.20 µg/litre were determined, similar to levels in the Mississippi river.

Ouseph (1992) reported that dissolved and particulate copper concentrations in the unpolluted zone of the river Periyar, India, were 0.8–10.0 µg/litre and 48–140 mg/kg, respectively, in 1985–1986. The Cochin estuary is subjected to various types of effluents from the Eloor and Chitrapuzha industrial belts. Levels in the estuary ranged from 2.2 to 22.2 µg/litre for dissolved copper and from 44 to 298 mg/kg for particulate copper. Copper concentrations showed high seasonal variations, with the lowest levels being detected during the monsoon season.

Filipek et al. (1987) found that dissolved copper concentrations reflected the acidity of waters affected by acid mine drainage of West Squaw Creek, California, USA. At pH > 5, copper concentrations were generally below the detection limit (< 0.01 mg/litre). Dissolved copper concentrations ranged from 0.12 to 13.5 µg/litre at pH 3–4, and at pH 2.4 a concentration of 190 µg/litre was found. Håkansson et al. (1989) found that the transfer of copper from the aqueous to the solid

particulate phase is significant at pH 3–3.5 and increases with pH. Copper concentrations in suspended solids were 2.7, 2.0 and 0.5 mg/kg at pH levels of 4.5, 5.4 and 6.5, respectively, in a drainage stream for a mine waste deposit. Camusso et al. (1989) monitored seasonal variations in copper in suspended particulate matter in the north basin of the acidic (pH 4.4) Lake Orta, Italy, between 1985 and 1987. Copper in the lake occurred mainly in the dissolved form (94%) and levels are still high (32–34 µg/litre) because of past industrial activity.

Sediment is an important sink and reservoir for copper. Background levels of copper in natural river sediments range from 16 to 5000 mg/kg (dry weight) (Förstner & Wittmann, 1981). Copper levels in marine sediments range from 2 to 740 mg/kg (dry weight) (Nriagu, 1979b). Bryan & Langston (1992) reported that sediment copper levels in United Kingdom estuaries range from 10 to > 2000 mg/kg (dry weight), the highest values being for Restronguet creek which receives acidic drainage from mining activity. In the creek, adsorption of most of the dissolved copper by flocculated oxides of iron and associated humic substances during estuarine mixing leads to very high sediment concentrations.

Bubb et al. (1991) found that copper loadings for fluvial sediments from the river Yare, United Kingdom, ranged from 5 to 375 mg/kg. Levels displayed the profile of a pollution plume originating from a point source. A peak located at 1–2 km from a sewage treatment works outlet was recorded. Bubb & Lester (1994) found copper concentrations at 24.2 and 39.0 mg/kg above and below a sewage treatment works, respectively. Background levels from a control site were 6.17 mg Cu/kg.

Palanques & Díaz (1994) found that the surface sediments of the continental shelf off Barcelona, Spain, are greatly influenced by anthropogenic contamination of heavy metals discharged by the littoral sewers and the Besos river. Copper concentrations ranged from 300 to 400 mg/kg at the mouth of the Besos river and declined at increasing distances from the shoreline.

A large gold and copper mining project began in 1984 on the Ok Tedi river, a tributary of the Fly river, Papua New Guinea. Baker et al. (1990) analysed suspended sediment samples from the Torres Strait

near the mouth of the Fly river system in 1989. Mean copper concentrations ranged from 1.4 to 13.3 µg/kg. The highest levels of copper were found at stations closest to the Fly river. Sediments of the Ok Tedi river are enriched with copper. Approximately 60% of the input has a particle size of < 100 µm and is transported as a suspended load throughout the entire length of the river (> 1000 km). Copper concentrations in the fraction < 2 µm reaches levels of 6000 mg/kg (Salomons & Eagle, 1990). Mean copper concentrations in the surficial sediments of the Fly river delta and the Torres Strait were 28 and 8.2 mg/kg, respectively (Baker & Harris, 1991).

Copper contamination of sediment samples in northern Sweden was correlated with distance from the Ronnskar smelter. Concentrations ranged from 1556 mg Cu/kg at a distance of 3 km to 37 mg Cu/kg at 80 km (Johnson et al., 1992). Ünlü & Gümgüm (1993) analysed sediment samples from the Tigris river, Turkey, in the vicinity of the Ergani copper plant. Copper concentrations were 641 mg/kg 5 km upstream of the plant, 3433 mg/kg at the outflow and around 900 mg/kg downstream.

5.1.3 Soil

Median total copper concentrations in uncontaminated soil were reported to be 30 mg/kg (range 2–250 mg/kg) (Bowen, 1985). Shacklette & Boerngen (1984) analysed soil samples from various locations in the USA, finding that copper concentrations ranged from < 1 to 700 mg/kg with an average of 25 mg/kg. Kabata-Pendias & Pendias (1984) reviewed the worldwide literature on copper in uncontaminated surface soils and report mean concentrations ranging from 6 to 80 mg Cu/kg (dry weight). Much higher levels were associated with mining activity, metal-processing industries and fertilizer and fungicide application.

Copper can accumulate in soils from the long-term application of fertilizers or fungicides. Reuther & Smith (1952) analysed soils from mature Florida citrus groves and found that copper oxide levels in the topsoil increased with grove age. Copper oxide levels of 247 and 93 mg/kg (dry weight) were measured at depths of 0–8 cm and 8–15 cm, respectively. At depths of > 15 cm copper oxide levels of ≤ 18 mg/kg were measured. Copper oxide levels in adjacent untreated soil ranged from 1 to 2 mg/kg. Christie & Beattie (1989) reported an

accumulation of copper in soil from the application of pig slurry (50–200 m^3/ha per year). EDTA-extractable copper concentrations of up to 85.2 mg/kg were recorded; levels in control soils ranged from 4.4 to 5.4 mg/kg. Paoletti et al. (1988) found that in Italy vineyard soil to which copper-containing fungicide had been applied contained mean copper concentrations of 89.8 mg/kg (dry weight). Soils from other locations contained mean levels ranging from 44.0 to 52.1 mg/kg. Holmgren et al. (1993) analysed surface soil samples from agricultural regions throughout the USA. Copper concentrations ranged from 0.3 to 495 mg/kg (dry weight). Copper levels were higher in the organic soil areas of Florida, Oregon and the Great Lakes, reflecting the use of copper fertilizers and fungicides.

Fjeldstad et al. (1988) found that levels of copper in surface peat showed a negative correlation with distance from a nickel smelting factory in Kristiansand, Norway. Dumontet et al. (1990) monitored copper in acidic peat located along two transects from a smelter plant in the Noranda region of Quebec, Canada and found that copper concentrations in surface samples (0–15 cm) ranged from 5525 mg/kg at a distance of 1 km to 28 mg/kg at 42.5 km. The majority of the deposited copper remained in the upper 15 cm of the soil profile. Soil samples taken in the vicinity of a copper smelter at Legnica in southern Poland contained copper levels of 7400 mg/kg (Helios Rybicka et al., 1994). Wu & Bradshaw (1972) reported that soil copper levels in the vicinity of a metal refinery (southwest Lancashire, United Kingdom) established in 1900 contained total copper concentrations ranging from 1930 to 4830 mg/kg. Hunter et al. (1987a) reported mean surface soil copper concentrations of 15.1, 543 and 11 000 mg/kg at a control site, 1 km from a copper refinery (Merseyside, United Kingdom) and at the refinery, respectively. Beyer et al. (1985) monitored soils 10 km upwind and 2 km downwind of zinc smelters in eastern Pennsylvania, USA. Copper concentrations ranged from 12 to 34 mg/kg and from 9.9 to 440 mg/kg (dry weight) for the two sites, respectively. Almost all of the copper contamination was held at the surface of the mineral soil.

5.1.4 Biota

5.1.4.1 Aquatic

The levels of copper in marine algae vary from 0.64 µg/g in *Laminaria religiosa* from Japan (Suzuki et al., 1987) to 407 µg/g in

Jania rubens from Antikyra Gulf, Greece (Malea et al., 1994). An important source of variation in the copper content in algae is the part of the plant analysed, generally being higher in older parts than in fast growing, younger apices.

Freshwater mussels (*Unio pictorum*) in the area of a sailing boat harbour (Lake Balaton, Hungary) contained significantly higher levels of copper than those from open water areas. Mean gill and adductor muscle copper concentrations were, respectively, 203 and 221 mg/kg (dry weight) in the harbour and < 20 mg/kg in open water (V-Balogh, 1988). Batley et al. (1992) analysed Sydney rock oysters (*Saccostrea commercialis*) from the Georges river, New South Wales, Australia. Mean copper concentrations ranged from 12 to 95 mg/kg (wet weight) in 1988 and from 19 to 89 mg/kg in 1991, and the authors state that overall copper concentrations in oysters have fallen since the banning of tributyltin. Claisse & Alzieu (1993) found an increase in copper concentrations in oysters collected between 1979 and 1991 in the bay of Arcachon, France. Annual mean copper concentrations have increased from 48.3–81.1 mg/kg (dry weight) in 1979 to 74.6–135 mg/kg in 1991. Data collected from 1977 to 1990 by the California mussel watch programme were analysed for long-term trends in copper. Copper showed a steady increase over time at 5 of the 20 sampling stations. The authors suggest that the increases in copper may be related to increased vessel traffic and the increased use of copolymer copper antifouling paints (Stephenson & Leonard, 1994).

Rainbow et al. (1989) monitored the copper concentrations in several species of talitrid amphipod at several sites in the United Kingdom. *Orchestia gammarellus* was found to be the most suitable biomonitor of copper in British coastal waters. Weeks (1992a) found the talitrid amphipod *Platorchetsia platensis* to be a good indicator species in Danish waters. Samples with significantly higher copper burdens, for example, 110 mg Cu/kg (dry weight) compared to 32 mg Cu/kg, were associated with local sources of metal enrichment, due to anthropogenic inputs (antifouling paint leachates) or geological conditions. Negligible quantities of copper were found in cast exuvia of talitrid amphipods during the moult cycle (Weeks et al., 1992b). Moore et al. (1991) found the beach-hopper (*Orchestia gammarellus*) to be a very convenient and sensitive biomonitoring species for copper levels along the North Sea coasts. Typical background concentrations

were approximately 70 mg Cu/kg (dry weight); samples with higher concentrations (up to 218 mg Cu/kg) were associated with local sources of contamination such as antifouling paints or the metal-rich mineralogy.

Alikhan et al. (1990) measured the concentration of copper in crayfish (*Cambarus bartoni*) trapped from increasing distances, up to 150 km from a nickel–copper smelter (Canada). Their results indicate that the concentrations in the crayfish decreased with increasing distance from the source; the highest concentration (1986 µg Cu/g) was measured in the hepatopancreas.

Schmitt & Brumbaugh (1990) analysed freshwater fish from throughout the USA in 1984–1985. A mean copper concentration of 0.65 mg/kg (wet weight) and a maximum copper level of 23.1 mg/kg were recorded. No significant change in the mean concentration of copper was found when compared with monitoring results from 1976.

Lee & Stuebing (1990) analysed liver tissue from river toads (*Bufo juxtasper*) near a copper mine in east Malaysia. Mean copper concentrations in toads downstream of the mine and from a control area were 438 mg/kg (dry weight) and 46 mg/kg, respectively. Copper levels of 117 and 273 mg/kg were recorded in toads collected from areas known to be rich in minerals.

5.1.4.2 Terrestrial

Stewart et al. (1991) sampled tree ring wood from kahikatea trees in urban Christchurch and the west coast of South Island, New Zealand. For the urban ring wood cores copper levels showed an elevation over baseline levels with an approximately threefold increase beginning around 1940. This was probably due to increased industrial emissions.

Kalac et al. (1996) measured the concentrations of copper in edible mushrooms in the vicinity of mercury and copper smelters in eastern Slovakia. Copper concentrations up to 236 mg/kg and 231 mg/kg (dry weight) were measured in *Lepiota procera* and *Lepisia nuda*, respectively.

The metalliferous hillocks of the Shaba Province in southwest Zaire have soil copper concentrations of up to 30 g/kg (Malaisse et al., 1979). The region supports an extremely unusual endemic flora, composed mainly of herbs and grasses, that can tolerate concentrations of copper in excess of 1% in the soil. Terrestrial higher plants which accumulate copper concentrations in excess of 1000 mg/kg (0.1%) (dry matter) are known as "hyperaccumulators" (Brooks et al., 1977). Brooks et al. (1980) reported hyperaccumulation of copper in 24 taxa from the Shaban region. The most unusual of these is *Aeollanthus biformifolium* which can contain as much as 13.7 g/kg (1.37%) (dry weight) in the whole plant (Malaisse et al., 1978).

The first workers to present data indicating hyperaccumulation of copper were Duvigneau & Denaeyer-De Smet (1963) who reported values of 1200, 1660 and 1960 mg Cu/kg (dry weight) for *Ascolepis metallorum*, *Silene cobalticola* and *Haumaniastrum robertii*, respectively.

The labiate (mint family) *Becium homblei* occurs on copper deposits in Zaire, Zimbabwe and Zambia. Reilly (1967) and Reilly & Reilly (1973) described *B. homblei* as a cuprophile, tolerant to > 70 g Cu/kg (dry weight) in soil, and accumulating up to 17% of copper in the leaves, organically bound to the cell walls. They also noted that some other species of *Becium* in the same area had no special ability to accumulate copper.

Hunter et al. (1987a) reported annual mean copper concentrations in the dominant plant species growing near a metal refinery in the United Kingdom (*Agrostis stolonifera*, *Festuca rubra*, *Equisetum arvense* and *Tussilago farfara*). Mean copper concentrations ranged from 7.6 to 18.6, 22.8 to 25.8 and 73.3 to 260 mg/kg (dry weight) at a control site, 1 km from a metal refinery and at the refinery respectively. Vegetation levels of copper showed marked seasonal variations at contaminated sites with peak values during the winter months. The increased levels were due to a combination of root absorption and accumulation of particles on external leaf surfaces. Copper concentrations in grasshoppers (*Chorthippus brunneus*) ranged from 37.5 mg/kg (dry weight) at a control site to 380 mg/kg at the refinery (Hunter et al., 1987c). Hunter et al. (1987b) analysed invertebrates from both contaminated and semi-contaminated grasslands in the

vicinity of a major copper refinery. All species showed significant elevations of total body copper concentrations relative to controls. Highest concentrations were found in isopoda species. Detritivorous soil macrofauna showed accumulation of copper (2–4 times) with respect to concentrations in refinery site organic surface soil and plant litter. Herbivorous invertebrates also showed body : diet concentration factors of 2–4 times for copper.

Ferns growing in the vicinity of ore smelters at Sudbury, Ontario, Canada, contained copper concentrations ranging from 27.2 to 73.0 µg/g (dry weight). Plants collected from control sites contained concentrations ranging from 7.4 to 11.5 mg Cu/kg (Burns & Parker, 1988). Analysis of lowbush blueberry (*Vaccinium angustifolium*) at sampling sites ranging from 6.5 to 74 km from Sudbury smelting operations revealed a significant relationship between copper concentrations and distance from the smelter (Bagatto et al., 1993). Alikhan (1993) analysed terrestrial isopods (*Porcellio spinicornis*) 2 km downwind of a primary smelting works (nickel) in Ontario, Canada. Mean copper concentrations in the isopods were 1137 mg/kg (dry weight) for the contaminated site and 685 mg/kg for a control site. Leaf litter contained approximately 12 times more copper at the contaminated site than at the control site.

Morgan & Morgan (1988) analysed earthworms (*Lumbricus rubellus* and *Dendrodrilus rubidus*) from both contaminated (the vicinity of disused nonferrous metalliferous mines) and non-contaminated sites in Wales. There were significant positive correlations between total copper concentrations in the earthworms and in the soil. Copper concentrations in earthworms ranged from 8 and 9 mg/kg (dry weight) at uncontaminated sites to 104 and 34 mg/kg at contaminated sites for the two species.

Ash & Lee (1980) analysed earthworms from roadside verges in the United Kingdom and found a relationship between traffic density and copper burden. Mean copper concentrations ranged from 3.9 to 8.9 mg/kg (dry weight) for heavy traffic, 2.3 to 6.6 mg/kg for intermediate traffic and 0.2 to 0.83 mg/kg for low levels of traffic. However, for the more contaminated sites other industrial sources of copper could not be ruled out.

Wieser et al. (1976) found two species of isopods (*Tracheoniscus rathkei* and *Oniscus asellus*) to be good indicator species for copper. Total copper concentrations in isopods ranged from 74 mg/kg (dry weight) for a spruce forest to 538 mg/kg for an overgrown slag heap of an old copper mine in the Tirol region of Austria. Hopkin et al. (1993) proposed the isopod *Porcellio scaber* as an ideal candidate for biomonitoring the bioavailability of metals to soil and leaf litter invertebrates. The authors provide a table of concentration ranges for this species related to degrees of contamination. For example, isopod copper concentrations of < 250 mg/kg (dry weight) would be classified as uncontaminated with medium contamination at 400–600 mg/kg and high contamination at 600–1000 mg/kg. Hopkin et al. (1986) analysed hepatopancreas and whole body of woodlice (*Porcellio scaber*) collected from 89 sites in southwest England. The main source of copper pollution was centred on Avonmouth, the site of a primary zinc, lead and cadmium smelting works. The correlation coefficients between the concentrations of copper in woodlice and soil, and between woodlice and leaf litter, were positive and statistically significant.

Rose & Parker (1983) reported concentrations of copper in tissues of ruffed grouse from a site near a copper–nickel smelter and a control, uncontaminated site near Sudbury, Ontario, Canada. Mean copper concentrations in kidney, liver and breast muscle ranged from 11.7 to 24.6, 12.6 to 16.3 and 1.5 to 2.3 mg/kg (dry weight), respectively. Their results indicate no difference between the two sites.

Hunter & Johnson (1982) analysed small mammals in the vicinity of a copper refinery in the United Kingdom. Liver concentrations were significantly elevated at the refinery in wood mouse (*Apodemus sylvaticus*) (23.7 mg Cu/kg dry weight) and common shrew (*Sorex araneus*) (56.1 mg Cu/kg) but not in short-tailed vole (*Microtus agrestis*) (13.5 mg Cu/kg). However, even these significant accumulations were rather limited bearing in mind the soil copper levels of 2000–3000 mg/kg (dry weight) at the refinery site. At reference sites copper concentrations in whole-body samples of small mammals ranged from 8 to 13 mg/kg (dry weight) (Smith & Rongstad, 1982; Beyer et al., 1985).

5.2 General population exposure

5.2.1 Air

Pulmonary exposure occurs through the inhalation of dusts, fumes, smoke and sprays that contain copper.

Exposure to copper by inhalation is determined by air concentrations, particulate size and the respiratory rate. Concentrations of copper determined in over 3800 samples of ambient air at up to 29 sites in Canada over the period 1984–1993 averaged 0.014 μg/m^3. The maximum value was 0.418 μg Cu/m^3, detected in 66% of samples (Dann, 1994). In the USA, air levels of copper vary between 96 and 138 ng/m^3 in urban samples and 76 and 176 ng/m^3 in non-urban settings (see section 5.1.1), though levels as high as 4629 ng/m^3 have also been recorded.

Based on data collected in the province of Ontario, Canada, copper levels in ambient air have decreased over 70% in the last 10 years, though some of this decrease is likely attributable to variations in sampling and analytical methods (OMME, 1992).

Estimated mean intake, based on these data (22 m^3 air/day) (ICRP, 1974) and the mean Canadian values, are less than 0.28 μg/day.

5.2.2 Food and beverages

The actual concentration of copper in food and beverages from various countries varies widely depending upon the food product, the growing conditions (soil, use of fertilizers high in copper, water, use of copper fungicides) and the type of processing used; in particular, pH levels and the use of copper vessels (Tanner et al., 1979; Muller et al., 1996).

In some countries, it has been customary to prepare milk by boiling it in copper vessels. Levels of copper in such milk have been reported as up to about 60 mg/litre (Muller et al., 1996). Studies have shown that copper binds predominantly to casein, which is the main constituent of milk protein. In acidic pH (as in gastric juice) casein liberates most of this bound copper as a copper ion, making it available for rapid absorption (O'Neill & Tanner, 1989). Calculations reveal that

whereas total breast feeding would supply up to 0.9 µmol Cu/kg per day (60 µg/kg per day), feeding similar amounts of brassy milk would supply up to 14.6 µmol Cu/kg per day (930 µg/kg per day) or 10–20 times the physiological intake per kg body weight per day. Traditional "tinning" of copper and brass vessels protects from such contamination by copper, yet it is a procedure often neglected because of cost and effort.

Copper is widely distributed in foods, with organ meats (e.g. liver) and seafood having the highest concentrations (10–100 mg/kg) and dairy products having relatively low levels (Table 5). High levels of copper have also been identified in wheat bran, beans and seeds, based on a recent, detailed investigation (Jorhem & Sundstrom, 1993). Baseline values have been reported as 0.2–0.3 µg Cu/litre for mother's milk and 0.7–1.1 µg Cu/kg for infant formula (Richmond et al., 1993). Chocolate may contain more than 5 mg Cu/kg. Values quoted for tea and coffee are highly variable but may exceed 10 mg Cu/kg (dry weight) (Slooff et al., 1989; ATSDR, 1990). In general most other foods contain much less than 10 mg Cu/kg.

Copper levels in common foodstuffs and beverages have been determined in many countries, including the USA (Pennington et al., 1986), Australia (NFA, 1992) and the Netherlands (Slooff et al., 1989). Copper levels in representative foodstuffs in these three countries are given in Table 5. From these market basket surveys, average daily intakes have been calculated (Pennington et al., 1986, 1989; Slooff et al., 1989; NFA, 1992), or actual dietary surveys have been conducted to determine the daily intake from food and beverages (Pettersson & Sandström, 1995).

Representative mean total daily intakes of copper from foods and beverages in several countries are given in Table 6. As shown, the total daily intake of copper in adults varies between 0.9 and 2.2 mg. Intake in children has been estimated to be 0.6–0.8 mg/day (0.07–0.1 mg/kg body weight per day).

In relation to the intake of copper in food, the WHO (1996) noted the insufficiency of global data and concluded that:

> "The scarcity of adequately planned studies is again evident, with insufficient data from Africa, the Eastern Mediterranean

Table 5. Levels of copper in foodstuffs (mg/kg wet weight)[a]

Food stuff		Mean	Minimum	Maximum	*n*
Meat					
	beef	0.8, 1.1	0.74	1.6	39
	pork	0.9, 1.4	0.44	7.22	150
	lamb	1.6	1.1	1.9	24
Liver					
	beef	39	8.8	87	7
	pork	9.0	0.9	29	126
	lamb	97	28	195	32
Kidney					
	beef	3.7	2.8	4.2	6
	pork	6.1	2.9	15	75
Fruit					
	apples	0.25	0.21	0.31	6
	pears	0.81	0.48	2.7	24
	bananas	0.95, 0.96	0.70	1.2	12
Vegetables					
	potatoes	0.72, 0.96	0.26	2.2	40
	carrots	0.40, 0.61	0.26	0.95	30
	lettuce	0.47, 0.72	0.20	1.4	40
	tomatoes	0.36, 0.55	0.29	1.1	26
Fish					
	cod	0.19	0.12	0.28	5
	tuna	0.64	0.48	0.80	9
Wheat					
	flour	1.5	0.95	2.9	56
	bread (white)	1.5	0.89	2.2	32
Milk					
	cow	0.06	trace	0.14	31
	human	0.54	0.22	0.90	28
Cocoa powder		36.4	33.0	410	9

[a] Adapted from Jorhem & Sundstrom (1993) for Sweden and NFA (1993) for Australia

Table 6. Estimated average dietary intake of copper in various countries

Country	Method of sampling [a]	Intake of copper (mg/day)	Reference
Australia	MB (adult male)	1.9	NFA (1992)
	MB (adult female)	2.2	
	MB (2 years)	0.8	
Denmark	DD	1.2	Bro et al. (1990)
Finland	TD[b]	2.00	Kumpulainen et al. (1987)
Germany	DD	0.95	Anke (1991)
The Netherlands[c]	MB	1.5	Slooff et al. (1989)
Norway	DD	1.0	Pettersson & Sandström (1995)
Sweden	MB	1.20	Becker & Kumpulainen (1991)
United Kingdom	TD (adult male)	1.63	Gregory et al. (1990)
	TD (adult female)	1.23	
	TD (1.5–4.5 years)	0.5	Gregory et al. (1995)
USA[c]	MB (6–11 months)	0.47	Pennington et al. (1986)
	MB (2 years)	0.58	
	MB (adult male)	1.24	
	MB (adult female)	0.94	

[a] MB = market basket survey; TD = total diet study; DD = duplicate diet study
[b] Total diet from food record
[c] In calculations of dietary intake of copper the USA and the Netherlands consider water as part of the diet

and South-East Asia. The apparently higher proportion of European studies suggesting undesirably low population mean intakes of copper needs to be investigated more closely to determine whether it is a truly characteristic feature of diets of the eastern German communities from which these particular samples were drawn. Before it is concluded that intakes of copper are likely to be reasonably adequate in the Americas, the western Pacific fringe and the remainder of Europe, it must be strongly emphasized that none of the surveys covered were representative of those socially and nutritionally disadvantaged communities in which food preferences lead to the consumption of diets providing as little copper as those reported to induce clinical signs of deficiency elsewhere" (WHO, 1996).

A summary of preliminary data from a global literature survey of dietary intakes by IAEA has been published (WHO, 1996). When all the IAEA data are considered, approximately 10% of reported mean intakes are below the proposed minimum basal mean value for copper in adult males (1.2 mg/day) and approximately 25% are below the corresponding minimum normative mean population intake (1.4 mg/day). Intakes five times higher than the basal minimum mean are observed in some population groups, but these are still well below the upper limit of the safe range of mean population intake (12 mg Cu/day for men) and there is no evidence from the IAEA database that the copper intake from diets for young children is sufficiently high to cause concern in the communities studied.

5.2.3 Drinking-water

5.2.3.1 Organoleptic characteristics

The taste of copper in drinking-water has been described as metallic, bitter and persistent. Taste thresholds have been reported between 0.8 and 5 mg Cu/litre, depending on the purity of the water (Cohen et al., 1960; Béguin-Bruhin et al., 1983). Concentrations of copper greater than 5 mg/litre may render water unpalatable although individuals can adapt to such levels (Scheinberg & Sternlieb, 1994). Aesthetic considerations relating to copper levels in drinking-water include blue or green staining of plumbing fixtures, hair and laundry.

5.2.3.2 Copper concentrations in drinking-water

Levels of copper in surface waters used for the production of drinking-water are presented in section 5.1.2. Copper is also introduced into drinking-water during distribution, owing to leaching from plumbing fixtures and copper piping. Leaching is dependent upon a number of factors, including pH, temperature, hardness, carbon dioxide content of the water, the length of time in contact with the pipe or fixture and the age of the piping (Schock & Neff, 1988; Alam & Sadiq, 1989). Some of these factors cannot easily be controlled; in particular, hard waters with high buffering capacity cannot have the pH raised sufficiently to moderate copper solvency (Dieter et al., 1991). It is thus insufficient to ascribe all problems of copper solvency to soft, acidic waters with low buffering capacity and nonadjusted pH.

In distributed water from 70 municipalities across Canada, median concentrations of copper ranged from ≤ 0.02 mg/litre to 0.75 mg/litre. In about 20% of the distributed water supplies, the level of copper was significantly higher than the corresponding treated water samples. Furthermore, the increase was higher in those areas where the water was soft and corrosive (Meranger et al., 1979).

In the USA, 85% of fully flushed tap water samples had copper levels below 0.06 mg/litre and 98% were below 0.46 mg/litre. Less than 1% exceeded 1 mg Cu/litre and the maximum level measured was 2.37 mg Cu/litre (US EPA, 1991).

The difference between samples of running water and those where water was standing for some time is evident from studies in several countries. Murphy (1993) measured copper levels in drinking-water fountains in 50 schools in New Jersey, USA. Median levels in first-draw water (0.26 mg Cu/litre) decreased significantly after 10 min of flushing (0.068 mg Cu/litre), but increased by lunchtime to 0.12 mg Cu/litre after normal use of fountains. In Canada, copper levels in running water from private wells were extremely low, but 53% of the samples from standing water exceeded 1 mg Cu/litre (Maessen et al., 1985). In a study in one US city (Seattle), mean copper levels in running and standing water were reported as 0.16 and 0.45 mg/litre, respectively, with 24% of standing water samples exceeding 1.0 mg/litre (Dangel, 1975). In the Netherlands, values between 0.2

and 3.8 mg Cu/litre were reported in water standing 16 h. This compares to the level of 3.0 mg/litre in water standing 16 h, which is the maximum permissible level for copper in drinking-water in the Netherlands (Slooff et al., 1989). These same authors report average copper levels between 0.04 and 0.69 mg/litre in other municipalities.

Pettersson & Sandström (1995) reported that in a study of 400 children aged 9–21 months the daily intake of copper from drinking-water ranged between 0.01 and 3.2 mg, with a mean of 0.3 mg. The study was conducted in two cities where it was suspected that levels of copper in drinking-water were high. In these cities, the mean copper levels in standing water were 0.7 mg/litre with a 90th percentile of 2.1; in water for consumption, the mean was 0.6 mg/litre with a 90th percentile of 1.6 mg/litre.

From the data available, and assuming a daily intake of drinking-water of 1.4 litres (IPCS, 1994), daily intakes of copper from drinking-water by adults will vary between less than 0.01 mg to over a few mg per day, with highest intake in areas with corrosive water using copper piping.

5.2.4 Miscellaneous exposures

In addition to airborne copper and copper in foods and beverages, the general population may be exposed to this metal from a variety of other sources. It is extremely difficult to quantify such exposures and in most cases they make only a minor contribution to the daily intake of copper by the general population when compared to the major source of copper which is food and drinking-water (1–3 mg Cu/day). Intake of dietary supplements containing copper will also contribute to total exposure.

In a study of the metal content of tobacco, the copper content in cigarette tobacco was found to vary between 9 and 66 μg Cu/g with a mean value of 15.6 μg Cu/g (Mussalo-Rauhamaa et al., 1986). Approximately 0.2% of this copper was detected in mainstream smoke (about 0.05 μg Cu/cigarette). This would result in a daily exposure of about 1 μg Cu from 20 cigarettes (Mussalo-Rauhamaa et al., 1986).

Dermal exposure to copper can result from the use of consumer products containing copper pigments, through the use of copper as an algicide in swimming pools and the use of copper jewellery. No quantitative exposure levels could be found.

Excluding the use of copper IUDs, the use of copper in medical applications has been replaced with other treatment regimens. However, in rare cases, notably the treatment of burns with copper sulfate, increased copper absorption has occurred with resulting toxicities observed (Eldad et al., 1995). The use of copper IUDs may result in exposure to as much as 80 µg Cu per day (Kjaer et al., 1993) with decreasing levels after the first few weeks after insertion.

Copper is a component of many amalgams used in dentistry, including mercury amalgams. The loss of copper from these sources has been reported as minimal (Johansson & Moberg, 1991; Lussi et al., 1992).

5.3 Occupational exposures

There is a wide range of industrial activities in which workers can be exposed to copper and copper compounds. Copper exposures in occupational settings are to particulates to which the metal or metal compound is adsorbed or to metal fumes (aerosols).

In the mining industry, workers (miners and millers) are exposed to dusts both from rocks and from the ore itself, containing 0.05–5% of copper (Weant, 1985). Multiple exposures occur, as the ore may contain high levels of nickel, arsenic and silica (McLaughlin et al., 1992). Exposure to copper fumes and to a lesser extent dusts is a feature of smelting operations but can occur through brassing, welding, cutting or polishing of copper and brass and in joinery shops where preserved woods are used. Other occupations in which exposures to copper and compounds occur are agriculture (fungicides), wood working, textiles, munitions and pyrotechnics, electrical, paint, paper and tyre manufacturing (Fisher, 1992).

Very little published data could be found on copper concentrations in air within occupational settings. Although dust and fume levels may be measured regularly, they are normally reported in terms of

concentrations of other elements of greater toxicological significance (e.g. arsenic, lead, acid mist). The bias towards reporting these contaminants explains the difficulty of relating any health effects noted in these environments to copper. Most countries have set exposure standards for copper containing dust in the range 0.5–1 mg Cu/m^3 and for copper fumes between 0.1 and 0.2 mg Cu/m^3 (ILO, 1991).

Some sense of the relationship between air copper and serum copper levels can be obtained from a study of copper milling and sanding operations in which exposures were reported as 0.01 and 0.68 mg Cu/m^3, respectively: plasma copper levels in these workers ranged from 660 to 1260 µg Cu/litre, all below the upper level reported for adults of 1300 µg Cu/litre (NIOSH, 1981a). In another study (NIOSH, 1981b), personal sampling of smelter workers in the blast and converter furnaces and in the sampling area had a mean copper fume concentration of 0.39 Cu/m^3 with a range from 0.12 to 0.99 mg Cu/m^3, while personal samples for workers exposed to copper dust during the cleaning of waste heat boilers and mertz furnace tear-down had average exposures ranging from 1.2 to 17.6 mg Cu/m^3. Serum copper values in these workers were unrelated to occupational exposure levels. Particle size distribution for the dust exposures were not given, which may partly explain the lack of a relationship. Exposures during welding of brassware ranged from 0.027 to 0.89 µg Cu/m^3 with a mean of 0.36 µg Cu/m^3 (Rastogi et al., 1992).

5.4 Total human intake of copper from all environmental pathways

For healthy, non-occupationally-exposed humans the major route of exposure to copper is oral. As shown in Table 6, the total daily intake of copper in adults ranges between 0.9 and 2.2 mg. A majority of studies have found intakes to be at the lower end of that range. The variation reflects different dietary habits as well as different agricultural and food processing practices used worldwide. In some cases, drinking-water may make a substantial additional contribution to the total daily intake of copper, particularly in households where corrosive waters have stood in copper pipes. In areas without copper piping copper intake from drinking-water will seldom exceed 0.1 mg/day, although intakes greater than a few mg per day can result from corrosive water distributed through copper pipes. In general,

total daily oral intakes of copper will be between 1 and 2 mg/day, although they may occasionally exceed 5 mg/day.

All other intakes of copper (inhalation and dermal) are insignificant in comparison to the oral route. Inhalation adds 0.3–2.0 μg/day from dusts and smoke. Even women using copper IUDs will be exposed to only 80 μg or less of copper per day in addition to their oral intake of between 1 and 3 mg.

6. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Copper is an essential trace element involved in a variety of critical metabolic processes. However, as with other essential trace elements such as iron and zinc, excessive exposure may be toxic. All mammals have metabolic mechanisms that maintain homoeostasis (a balance between metabolic requirements and prevention against toxic accumulation). Special populations with genetic defects or abnormalities in the metabolism of copper may be sensitive to levels of exposure that are nontoxic to persons without these defects. This chapter provides an overview of the metabolic mechanisms that provide copper homoeostasis in mammalian systems.

An organism, or cells within an organism, will seek to maintain copper levels within a range that avoids both deficiency and excess. The mechanisms for absorption and storage of copper are relatively little studied but include biological chelators, specific receptors, sequestering peptides and proteins and uptake pumps. Likewise, the defence mechanisms to prevent or limit copper toxicity include extracellular chelators, sequestering peptides and proteins, export pumps and disposal of the metal into vesicles. Many of the peptides and proteins that are involved in these events have been characterized and their metabolic roles investigated. The regulation of copper metabolism is not fully understood, although a great deal is being learned from simple model systems.

Critical to the metabolism of copper is the chemical behaviour of the element and its complexes because this behaviour controls its interaction with other elements in processes such as absorption, transport, distribution and toxicity. The general metabolism of copper is described in the following sections. The bulk of the studies related here are derived from animal and other model systems. Where appropriate, sections will highlight human studies.

6.1 Essentiality

The essentiality of copper was not recognized until 1928 when Hart et al. (1928) showed copper to be essential for erythropoiesis in rats fed a milk-based diet. He was able to correct the anaemia by the

addition to the diet of ash from animal or vegetable sources. He went on to demonstrate that the hydrogen sulfide precipitate from the ash, containing copper sulfide, was responsible for the recovery. Similar findings in humans established the basis for essentiality (Mills, 1930; Josephs, 1931).

Copper is also essential for the utilization of iron in the formation of haemoglobin (Friberg et al., 1979) and in the maturation of neutrophils (Percival, 1995).

The essentiality of copper arises from its specific incorporation into a large number of enzymatic and structural proteins. The role of copper in oxidation/reduction enzyme activities is a consequence of its ability to function as an electron transfer intermediate. Thus copper is present in enzymes involved in cellular respiration, free radical defence, neurotransmitter function, connective tissue biosynthesis and cellular iron metabolism. In some of them, copper is required as a cofactor, e.g. superoxidase dismutase 1 (SOD1), cytochrome oxidase and ceruloplasmin. Moreover, the oxidase activities of ceruloplasmin and SOD1 have been shown to specifically require copper. In other cases, copper appears to be involved as an allosteric component of enzymes, conferring an appropriate structure for their catalytic activity. No other element can substitute into these proteins to provide the redox properties that copper provides. These enzymes serve critical functions in their respective organisms (Hartmann & Evenson, 1992; Linder & Hazegh-Azam, 1996). An illustrative selected list of the enzymes that rely on the redox properties of copper for catalysis is shown in Table 7.

Copper plays an important role in the activation and repression of gene transcription. Studies of copper-regulated transcription in yeast have advanced the identification of the mechanisms of action of copper-regulated transcription factors in eukaryotes. ACE1 (Dameron et al., 1991) and AMT (Zhou & Theil, 1991) are homologous copper-DNA binding proteins that regulate the synthesis of the metallothionein message through specific fungal promoter elements in, respectively, *Saccharomyces cerevisiae* and *C. glabrata*. The *S. cerevisiae* SOD is also regulated by ACE1 (Gralla et al., 1991; Carry et al., 1991). Metal responsive elements (MREs), 13–15 base pair repeats, have been found in the metallothionein promoters of all higher eukaryotes, but the

Table 7. Copper metalloenzymes and proteins[a]

Enzyme	Function
Amino acid oxidase	amino acid metabolism
Ascorbate oxidase	terminal oxidase in plants
Azurin	electron transfer
Benzylamine oxidase	oxidation of amines
Ceramide galactosyl transferase	myelin synthesis
Ceruloplasmin	copper transport, oxidation
Cytochrome c oxidase	terminal oxidase in animals
Diamine oxidase	amine metabolism
Dopamine-β-hydroxylase	norepinephrine (noradrenalin) synthesis
Galactose oxidase	carbohydrate metabolism
Haemerythrin	oxygen transport
Haemocyanin	oxygen transport
Indole 2,3-dioxygenase	amine metabolism
Laccase	terminal oxidase, plants
Lysyl oxidase	collagen, elastin cross-linking
Plastocyanin	electron transfer in plants
Polyphenyl oxidase	quinone biosynthesis
Prostaglandin reductase	prostaglandin biosynthesis
Rusticyanin	electron transfer in fungi
Stellacyanin	electron transfer in fungi
Superoxide dismutase	superoxide radical destruction, dismutation
Tyrosinase	amino acid metabolism, pigment formation
Uricase	nucleic acid metabolism
Spermine oxidase	amine metabolism
Tryptophan 2,3-dioxygenase	amino acid metabolism
Monoamine oxidase[a]	neurotransmitter synthesis

[a] Linder & Hazegh-Azam (1996)

metal-regulated transcription factors have not been characterized. Mac1 has been found to regulate the transcription of FRE1 (encoding a plama membrane protein associated with both Cu(II) and Fe(III) reduction) and CTT1 (encoding the cytosolic catalase) (Jungmann et al., 1993).

Despite the obvious differences in physical form, at a metabolic/biochemical level animals have very similar molecular requirements for copper. The deficiencies, therefore, are very similar to those described for copper deficiencies in humans. The copper-dependent enzyme lysyl oxidase, for instance, has been associated with connective tissue disorders involving cardiovascular lesions, bone formation and eggshell development. Cardiovascular lesions associated with copper deficiencies have been found in mice (Rowe et al., 1977), rats (Petering et al., 1986), rabbits (Hunt & Carlton, 1965; Hunt et al., 1970), pigs (Ganezer et al., 1976; Schoenemann et al., 1990), and cattle (Mills et al., 1976). In chickens and mice the lesions have been linked to decreases in lysyl oxidase (Rowe et al., 1977). Similarly rats (Alfaro & Heaton, 1973), cattle (Mills et al., 1976) and chicks (Rucker et al., 1969) manifest bone formation defects in copper deficiencies. Copper-deficient hens lay eggs with weak or no shells as a result of the failure of lysyl oxidase in the oviduct (Harris et al., 1980). Animals also show evidence of hair discolouration and brittleness and flaccid skin, as seen in humans (Blakley & Hamilton, 1985).

6.2 Homoeostasis

6.2.1 Cellular basis of homoeostasis

An interpretation of the intracellular homoeostasis of copper in an human hepatocyte (the pathway and regulation of the importation, utilization, detoxification and export of copper) is illustrated in Fig. 1. Copper itself has a major role in the regulation of the mechanisms that control its cellular homoeostasis.

Copper as Cu(II) entering into hepatocytes is initially reduced and complexed by glutathione, prior to binding and induction of metallothionein (Freedman, 1989). Alternatively, copper entering the cell may be exported by a copper ATPase translocase.

Metallothionein, the main intracellular copper-binding protein, is a protein with 62 amino acids and two domains, rich in cysteine (30%), which can bind up to 12 Cu(I) atoms. The metallothioneins are involved in the detoxification and possibly storage of excess copper (Bremner, 1987). All metallothioneins are transcriptionally regulated by metals, except two newly isolated metallothioneins that may have

Human Copper Metabolism

(Courtesy J.L. Gollan)

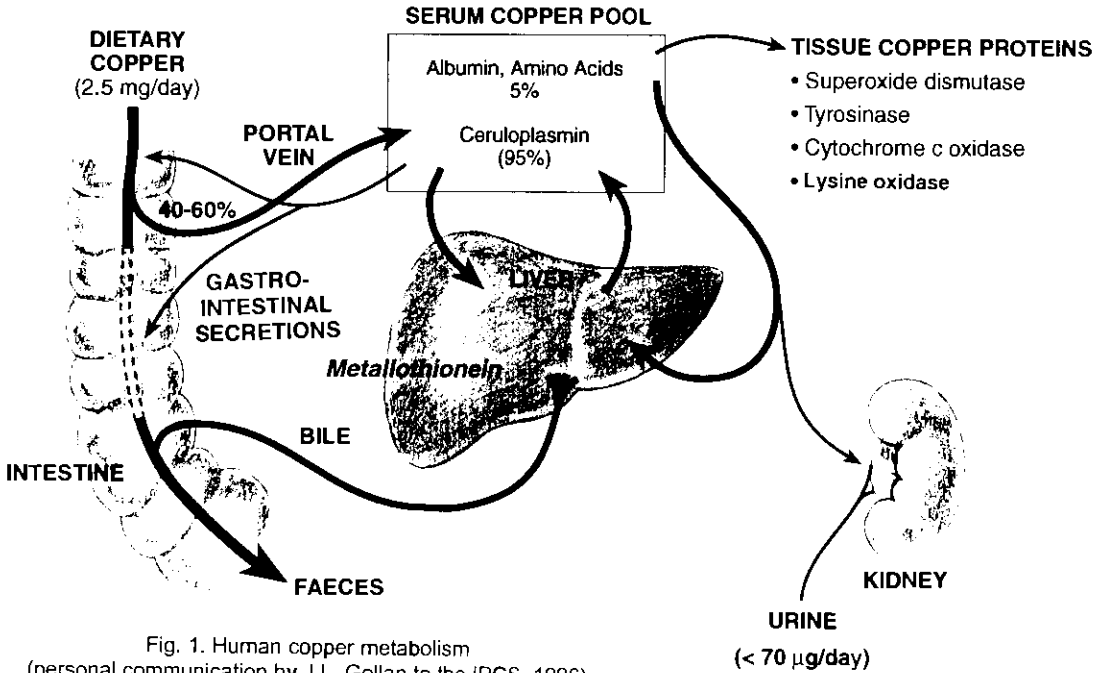

Fig. 1. Human copper metabolism
(personal communication by J.L. Gollan to the IPCS, 1996)

specialized functions (Hammer, 1986; Palmiter, 1993; Palmiter et al., 1993). A wide variety of metals have been shown to induce the synthesis of metallothioneins. The mammalian transcription factor is a complex of proteins activated by a wide range of metals (Palmiter, 1993). When copper binds to the transcription factor complex, its affinity for metal regulatory elements in the promoter of the metallothionein gene is enhanced. The resulting increased level of metallothionein sequesters the excess copper, preventing toxicity.

Copper ions are exported from liver cells by a P-type copper ATP translocase (Cox, 1995). The copper translocases in liver are located in the Golgi, endoplasmic reticulum and plasma membrane and are responsible for copper transport. A mutation of this gene is responsible for Wilson disease. Copper is poorly incorporated into ceruloplasmin when the translocase is defective (Cox, 1995). Metal ions are also sequestered into lysosomes, especially in conditions of copper overload (Mohan et al., 1995).

6.2.2 *Absorption in animals and humans*

Foods rather than water contribute virtually all of the copper consumed, and the copper content of different foods varies considerably. Absorption of copper occurs primarily through the gastrointestinal tract although small amounts can be incorporated through inhalation and skin contact. The intestinal absorption process is affected by numerous physiological and dietary factors as described in section 6.4.

Radioisotope studies in experimental animals suggest that copper is absorbed from the stomach to some extent, but that the major site of absorption is the duodenum (Van Campen & Mitchell, 1965). The pH of the stomach is such that many weak copper complexes will dissociate. Enzymatic degradation of proteins and dietary fibres should also make the metal more available. It also appears likely that low molecular weight substances (e.g. amino acids) in gastrointestinal secretions such as saliva, gastric and pancreatic juice, bind copper and thereby maintain the metal in solution in the alkaline milieu of the upper small intestine (Gollan & Dellor, 1973). Moreover, it has been suggested that copper is primarily absorbed in the form of amino acid complexes (Marceau et al., 1970). Limited absorption of copper also

occurs at the distal part of the small intestine. Absorption of copper across the brush border into the cells of the intestinal mucosa and its subsequent transfer across the basolateral membrane into interstitial fluid and blood occur by different mechanisms. Transfer across the mucosal barrier probably occurs by non-energy-dependent diffusion. With the levels of copper normally ingested, transfer of copper across the basolateral membrane appears to be rate-limiting and is mediated by a saturable, energy-dependent mechanism. At higher intakes, additional diffusional or carrier-mediated systems in the basolateral membrane come into play, and it seems likely that these are the sites where competition for absorption between copper and other transition metal ions takes place (Linder, 1991).

Turnlund et al. (1989) have used stable isotope methodology to study copper absorption in adults. Diets were labelled extrinsically with ^{65}Cu and isotope mass ratios were analysed in the diets and stools by thermal ionization mass spectrometry. Copper absorption was dependent on the amount of copper in the diet; when a low copper diet (0.78 mg Cu/day) was given, absorption was 55.6%, whereas it was 36.3% from the same diet with copper added to an adequate level (1.68 mg Cu/day) and 12.4% from the same diet but with high copper content (7.53 mg Cu/day). Thus, it appears that copper absorption in adults is saturable and that the percentage absorbed decreases with the level of dietary copper. However, total retention of copper increased with the level of dietary copper. Balances were positive even at the lower copper level studied, suggesting that copper intakes of approximately 0.8 mg/day are adequate to sustain balance.

Early balance studies in preterm infants by Cavell & Widdowson (1964) and Dauncey et al. (1977) showed negative balances of copper for several months after birth. Most of the copper was found in the stool, suggesting ineffective absorption or poor retention mechanisms. Negative copper balance was also found in 40% of infants studied by Tyrala (1986) despite feeding a formula with a copper concentration of 2.1 mg/litre. More recent studies in "healthy" preterm infants fed modern artificial formula or unpasteurized human milk using combined chemical balance and stable isotope tracer (^{65}Cu) determinations indicate that they absorb sufficient copper to meet the requirements imposed by growth. Twelve infants fed preterm human milk absorbed 40–60% of intake while 33 receiving premature formula absorbed only

15%. The absolute retention of copper in infants fed human milk (40–50 μg/kg per day) approached the expected retention based on *in utero* accretion data. This study demonstrates that infants respond to a higher copper intake in a similar way to adults, by increasing fecal losses and decreasing percentage absorption (Ehrenkranz et al., 1989).

A portion of the absorbed copper is lost during the turnover of the intestinal cells and is subsequently lost in the faeces. Copper absorbed into the intestinal endothelial cells can be sequestered by metallothionein or may pass into the portal circulation. Metallothionein may be an intermediate through which all or part of the absorbed copper passes in route to the circulation (Felix et al., 1990). Most of the copper transfer across the serosal membrane appears to be done by the copper translocase. This mechanism operates in animals and humans, and homologous proteins have been identified in yeasts (Rad et al., 1994) and bacteria (Odermatt et al., 1993; Solioz et al., 1994). Intestinal metallothionein may be acting as a temporary metal-storage protein and be involved in the detoxification of excess copper.

Pulmonary absorption occurs through the inhalation of dusts, fumes, smoke and sprays. Persistent exposure to copper in sprays, such as Bordeaux mixture, can lead to increased absorption and accumulation (Pimentel & Marques, 1969; Pimentel & Menezes, 1975; Viren & Silvers, 1994).

Topical use of copper compounds, as treatment for or prevention of microbial infections, can lead to increased copper absorption (Eldad et al., 1995).

6.2.3 Transport, distribution and storage

The liver is the major organ for the distribution of copper in mammals. The liver sequesters the newly absorbed copper, routing it through the blood to other tissues (Owen, 1965; Evans, 1973; Marceau & Aspin, 1973a; Sternlieb, 1980). In blood, copper is distributed into a nonexchangeable red cell pool, a plasma pool associated with proteins, and a labile pool of low molecular weight complexes. In humans, approximately 80–90% of the plasma copper is tightly bound ceruloplasmin while the rest is bound to albumin and amino acids.

In rats, ingested copper (^{64}Cu) appears first in the blood complexed to albumin; a small portion of newly absorbed copper was later shown to complex with amino acids in the serum (Neumann & Sass-Kortsak, 1967). Albumin is a 68 kDa protein, found in serum and in the interstitial spaces, which has copper binding sites. Approximately 50% of the copper in whole blood is in erythrocyte SOD and small peptide complexes. Erythrocyte copper does not play a role in the transport of newly absorbed copper from the gut to the liver (Gubler et al., 1953). Ceruloplasmin does not have a role in transport of copper from gut to the liver, which is principally carried out by albumin and amino acid complexes. Recently *in vivo* NMR analysis of whole blood has confirmed in humans that copper in the portal circulation is bound to albumin (Bligh et al., 1992) adding weight to the earlier studies (Beam & Kunkel, 1964).

Transport from the liver to peripheral tissues is one of the most widely debated issues in the field of copper metabolism, but it is thought to involve ceruloplasmin, albumin, transcuprien or amino acids. Metallothionein has been suggested to play an important role in the transport of copper in fetal blood. Its concentration is elevated in the plasma and there appears to be little copper bound to ceruloplasmin and albumin (Bremner, 1987). The proposal that metallothionein is involved in the fetal copper transport has been questioned, as mouse mutants lacking metallothionein develop normally (Michalska & Choo, 1993; Masters et al., 1994).

Transport of copper from the liver to the peripheral tissues is presumed to require either ceruloplasmin or serum albumin. The available studies can neither exclude or prove the possibility that one of these proteins is an obligatory copper transporter (Linder et al., 1998). The peripheral tissues of humans with little or no ceruloplasmin are not copper deficient (Frommer, 1981). Radioisotope studies (Owen, 1965; Marceau & Aspin, 1973a,b), in which an isotope of copper (^{64}Cu or ^{67}Cu) is used to trace the transfer of copper from one metabolic pool to another, are more supportive of ceruloplasmin's role in copper transport. Its role is also supported by nutritional studies (DiSilvestro & Harris, 1981; Harris & DiSilvestro, 1981) and combined isotopic and nutritional studies (Dameron & Harris, 1987a,b; Percival & Harris, 1990, 1991; Steinkuhler et al., 1991). The conflicting observations could be reconciled if there is redundancy in

the transport process, as might be expected for a critical process like the delivery of copper.

Receptors for ceruloplasmin have been tentatively identified in the plasma membrane fractions of chick aorta and heart (Stevens et al., 1984), rat erythrocytes (Stern & Frieden, 1993), rat liver (Kataoka & Tavassoli, 1984; Tavassoli et al., 1986; Omoto & Tavassoli, 1990) and rat brain (Mash et al., 1990). Membrane receptors for ceruloplasmin have also been described in human erythrocytes (Barnes & Frieden, 1984) and leukocytes (Kataoka & Tavassoli, 1985), and K562 cells (Percival & Harris, 1988, 1990). The studies by Percival & Harris (1990) imply that the copper may be removed from ceruloplasmin after reduction and that the protein may not be internalized.

A carrier-mediated facilitated diffusion system for uptake of copper complexes, amino acids and small peptides, into rat hypothalamns has been identified (Hartter & Barnea, 1988). The system has a broad ligand specificity with respect to amino acids (histidine, cysteine, threonine, glycine) and polypeptides (Gly-His-Lys, glutathione) but will not transport albumin-bound copper.

Absorbed copper is primarily incorporated into the soluble fraction of the liver and is associated with three main liver fractions in the cytosol: a high molecular weight pool that has not been completely identified, a 30 000 kDa pool which appears to be SOD and a 10 000 kDa pool composed mostly of metallothionein. In chicks and other animals, newly absorbed copper appears to be initially incorporated into SOD and metallothionein (Balthrop et al., 1982), the amount incorporated into each varying with the amount of copper absorbed and the route of administration (Prins & van den Hamer, 1981). Some of the copper that enters the liver is not retained in or does not enter the protein fractions and is instead excreted through the bile. Copper bound to metallothionein may be targeted for excretion through the bile, but may be used in the synthesis of other copper proteins (Bremner, 1987). The role of metallothionein in the cellular detoxification of copper, and possible roles for this protein in the uptake, storage and transport of copper, have been reviewed by Bremner (1987).

The liver synthesizes and regulates the plasma levels of ceruloplasmin, the major copper-binding protein in serum and

cerebrospinal fluid. Some other tissues also synthesize ceruloplasmin, or isoforms produced from alternative splice sites (Yang et al., 1990).

Ceruloplasmin (ferroxidase) is a 160 kDa, blue, heavily glycosylated, α_2-globulin, with 6–8 tightly bound Cu(II) atoms (Owen, 1982). It is an acute-phase plasma protein, increasing in concentration in a variety of non-specific diseases. It also has ferroxidase activity and facilitates the oxidation of Fe^{2+} to Fe^{3+} (Frieden & Hsieh, 1976).

Copper-deficient diets lower total liver copper, metallothionein copper (Balthrop, 1982), and copper–zinc SOD activity (Dreosti & Record, 1978; Bettger et al., 1978). Synthesis of fully active ceruloplasmin by the liver is decreased or eliminated in copper-deficient animals (Owen, 1965; Harris & DiSilvestro, 1981) and in humans with Wilson disease. In contrast, deficient diets can lower the copper enzyme levels in some tissues even when the tissue copper level is constant. Aortic lysyl oxidase, an extracellular enzyme, decreases in chicks on a copper-deficient diet (Harris et al., 1974), even though the tissue copper level does not decrease (Balthrop et al., 1982).

Copper balance and tissue distribution in typical adult humans is summarized in Fig. 1. Liver copper content accounts for close to 20%; this is the only true storage site that can be mobilized in case of negative copper balance. Muscle accounts for nearly 40% of total body copper and brain close to 20%. Connective tissue, blood and kidney each accounts for 8%.

The fetus is fully dependent on copper uptake from the maternal circulation. The transport of copper through the placenta is mediated by a specific carrier copper transport from ceruloplasmin (McArdle & Erlich, 1991; Lee et al., 1993). Other copper-binding complexes such as albumin, or histidine-bound copper, can also contribute to the fetal supply (Wirth & Linder, 1985). The fetus accumulates copper at a mean rate of close to 50 µg/kg per day, principally over the later half of pregnancy; over half of the copper is stored in the liver, mainly in the form of metallothionein (Widdowson et al., 1974). The increase in fetal liver store is due to both increased liver size and higher concentration per unit of liver weight. The brain is the second site for copper in fetal life; by the end of gestation the fetus will have accumulated close to 15 mg of copper, of which 9 mg will be in the

liver. After birth the concentration of copper in the liver drops during the initial months of life, reaching adult levels by 6 months. Copper saturation of metallothionein is high during the first 6 months of life (up to 50%), dropping quickly thereafter (Klein et al., 1991). Biliary secretion is extremely low *in utero* and rises progressively postnatally.

Pregnancy is associated with increase copper retention: this may be due in part to decreased biliary excretion induced by hormonal changes typical of pregnancy. Serum copper and ceruloplasmin rise significantly during the last trimester (McArdle, 1995). Maternal plasma copper concentrations during the latter half of gestation are 5–7 times higher than levels measured in the cord blood.

6.2.4 Excretion

Bile constitutes the major route of excretion of liver copper in mammals, and thus represents the most important homoeostatic mechanism determining the hepatocellular levels of the metal (Cousins, 1985; Winge & Mehra, 1990). Approximately 80% of the copper leaving the liver is excreted via the bile (Winge & Mehra, 1990). The urinary excretion of copper is quantitatively unimportant and only 30–60 μg of copper is eliminated through this route per day in adult human (Harris, 1991).

Several pathways have been proposed to explain copper transport into the bile (Kressner et al., 1984). Kinetic studies using radioisotopes of copper have revealed that the intracellular source of copper to be excreted in the bile is in a different compartment from the copper destined for incorporation into ceruloplasmin (Dunn et al., 1991). The existence of at least two transcellular pathways via the hepatocytes has been proposed. Copper transport into bile takes place in association with the biliary excretion of glutathione (Freedman et al., 1989). It has been suggested that glutathione is involved in the final step of copper excretion from the hepatocyte into the bile (Alexander & Aaseth, 1980). The coordinated release of copper and lysosomal enzymes into the bile of normal and copper-loaded rats suggests that biliary copper may be largely derived from lysosomes (Gross et al., 1989) and thus biliary copper excretion may be related also to the hepatocellular content of metallothionein.

Copper is found bound to a range of unidentified components of both high and low molecular weight, which may consist of protein, micelles, bile salts, peptides and amino acids, depending on the species and on the degree of copper loading (Bremner, 1987). However, none of the major forms can be related to copper complexes identified in the liver, although small amounts of ceruloplasmin, metallothionein and glutathione or their degradation products may be present (Sato & Bremner, 1984; Bremner et al., 1987).

In rats, net biliary copper excretion is relatively low in the first week of life and is independent of metallothionein and glutathione secretion. Excretion increases significantly as glutathione output increases (Mohan et al., 1995). Studies with human hepatic and gallbladder bile have documented the presence of a major high molecular weight glycoprotein, which avidly binds copper (Gollan & Dellor, 1973). A low molecular weight component(s) is also present in both rat and human bile (Gollan & Dellor, 1973). Both the high and low molecular weight components await characterization. Copper bound to the macromolecular component in bile undergoes minimal intestinal reabsorption. Thus, biliary copper does not appear to undergo significant enterohepatic circulation (Gollan & Dellor, 1973), with most being recovered in the faeces (Winge & Mehra, 1990).

In sheep, biliary excretion of copper does not represent the major elimination pathway. However, this route of copper excretion can be enhanced by the administration of tetrathiomolybdate (Winge & Mehra, 1990). In addition to an elevation in biliary excretion of copper, the hepatic copper levels are also reduced in treated sheep (Gooneratne et al., 1989). The limited biliary excretion of copper in sheep may partly account for the susceptibility of sheep to copper-associated toxicity (Winge & Mehra, 1990).

Animals that tolerate copper well exhibit an enhanced biliary excretion of copper. Copper-loaded rats, with hepatic copper levels up to 8-fold greater than controls, have shown a 10-fold increase in biliary copper output (Gross et al., 1989). Biliary obstruction induced by deliberate ligation or pathological lesions, or due to a particular metabolic state of the animal, leads to significant hepatic copper retention as well as some increase in urinary copper excretion (Gross et al., 1989). Retention of hepatic copper also occurs in pregnant rats correlating with diminishing biliary excretion (Winge & Mehra, 1990).

At least three genetic disorders associated with defective hepatobiliary copper transport and accumulation of copper in the liver have been described: Wilson disease (hepatolenticular degeneration) in human and copper toxicosis in Bedlington terriers and Long-Evans cinnamon rats (Sternlieb, 1980; Schilsky & Sternlieb, 1993; Mori et al., 1994). These disorders are characterized by a decreased biliary copper excretion, but differ from each other in the hepatic distribution of the retained copper.

Minimal amounts of copper are lost in human sweat. The loss is not believed to be sufficient to disturb the normal copper balance (Turnlund et al., 1990).

6.3 Methods of studying homoeostasis

The purpose of this section is to highlight appropriate clinical and biochemical methods that can be used to assess the copper status of laboratory animals and humans. The goal is not to provide a compendium of methods and analytical techniques but to offer an overview of how to conduct these studies.

6.3.1 Analytical methods

A detailed discussion of analytical methods for the determination of copper in solids and dilute liquids is given in chapter 2 of this monograph and in WHO (1996). In general, solid samples require an acid digestion prior to flame AAS. Low concentration samples require more sensitive methods such as GF-AAS. Radioactive copper isotopes ^{64}Cu and ^{67}Cu (chapter 2) have been widely used in experimental animals and cell culture studies to follow the uptake and distribution of the metal (Petris et al., 1996). The short half-lives of these isotopes and safety considerations make them less suitable for human studies. The stable isotope ^{65}Cu is now widely available and relatively inexpensive. Determination of the enrichment of the ^{65}Cu/^{63}Cu ratio in human body fluids and excreta after a bolus dose of ^{65}Cu can be measured either by thermal ionization mass spectrometry (TIMS) (Turnlund et al., 1989) or by ICP-MS (Lyon & Fell, 1990; Lyon et al., 1995, 1996).

6.3.2 Intake

The principle purpose of dietary intake analysis is to determine the adequacy of copper supply and bioavailability for the general population or sub-populations. Dietary analysis requires the determination of copper in food and liquids that are consumed.

6.3.3 Diet

The preferred procedure for assessment of copper intake is the use of "duplicate diet studies" in which a duplicate portion of all food normally consumed by the test subject is collected, and the total copper content determined. A secondary method is to estimate the copper intake through dietary surveys using food composition from tables. Descriptions of methods for dietary assessment of the trace elements have been published by WHO (1996).

There is a need for standardized sampling and analytical procedures for the determination of dietary copper. There is also a great need for standardized sampling and analytical procedures for the analysis of copper in drinking-water. Where appropriate, the copper content of foods such as infant formulae prepared using drinking-water should also be measured.

6.3.4 Balance studies

The difference between the total copper input (diet and water) and the total output (faeces and urine) is the *copper balance*. Balance data provide an estimate of whether the body is losing or gaining copper. Copper balance can be used to estimate the amount required to prevent deficit, since a negative balance in the long run will give rise to clinical signs of deficit; conversely, a positive balance, except during growth, will give rise to potential problems once reserves are replete. In order to achieve copper balance children require 0.1–0.15 mg Cu/kg body weight per day; adults need 0.02 0.05 mg Cu/kg body weight (1–3 mg/day). In general the percentage of copper absorbed from the intestinal tract decreases as copper intake increases.

Estimation of copper excretion is primarily made by the determination of fecal copper loss. Healthy subjects are in equilibrium; that is, dietary intake equals fecal copper output (see Fig. 1 on page

78). The duration of faecal collection should be at least 3–5 days for children and appropriate inert markers should be used to ensure completeness of collection. Longer periods may be necessary for adequate balance studies in adult humans. Fecal output represents both the copper that is not absorbed from the gut and also any excreted through the bile.

Urinary copper is a minor pathway for excretion (see Fig. 1) but should be measured to assure completeness of any balance study. Urinary copper is increased when renal tubular function is compromised. It can also be increased in copper overload (O'Donohue et al., 1993). Sequential measurement of urinary copper excretion can be used to monitor chelation therapy in Wilson disease.

The balance data from chemically defined diets are used to develop an understanding of the bioavailability and percentage retention using different copper intakes. Such data can be used to estimate the amount of copper required to prevent deficit and give some information on the functional and clinical effects of excess intakes. Some balance studies are summarized in Table 8.

The use of copper tracers, radioisotopes and stable isotopes provides kinetic information to complement the balance studies. The results from such studies can be mathematically modelled to provide estimates of whole body and specific tissue compartments, such as liver stores. True absorption and endogenous losses can be directly measured from the copper isotope ratios in stool and diet (Turnlund et al., 1991).

The reference interval for serum copper for normal adult males is in the range 800–1200 µg/litre (WHO, 1996). Values for women are about 10% higher. Serum copper is reduced in moderate to severe symptomatic copper deficiency. However, serum copper concentration is not a sensitive marker of recent onset of deficiency (Milne et al., 1990; Turnlund et al., 1990; Milne & Johnson, 1993). Other conditions which modify these laboratory parameters include inflammation or infection, neoplasms and anticonvulsant or oestrogen therapy (Solomons, 1979; Fischer et al., 1990; Jain & Mohan, 1991; Nielsen et al., 1992; Milne & Johnson, 1993).

Table 8. Daily copper intake and copper balance studies

Subjects	Methods	Results	Reference
4 patients aged between 0.36 and 1.53 years	metabolic balances were performed on subjects who had been on a comminuted chicken diet mixed with a trace element supplement for at least 3 weeks	mean copper total excretion and retention were 1.39 and 0.34 µmol/kg per day at a mean copper intake of 1.73 µmol/kg per day (110 µg/kg body weight per day) increasing to 1.72 and 0.51 µmol/kg per day, respectively, at a mean copper intake of 2.23 µmol/kg per day (142 µg/kg body weight per day)	Thorn et al. (1978)
11 girls, 12.5–14.2 years	the effect of feeding two different levels of zinc (11.32 mg and 11.64 mg/day) on copper balance was determined during a 30-day period	copper excretion in the feces was significantly increased when subjects consumed the diet with the higher level of zinc. The copper fecal losses and apparent retention of the girls when fed 11.64 mg of zinc daily were 30.60 ± 6.50 ng/day and -0.97 ± 6.09 mg/day, respectively. The corresponding figures for girls when fed 11.32 mg/day of zinc were 27.99 ± 1.67 ng/day and 1.40 ± 1.56 mg/day, respectively	Greger et al. (1978)
11 men aged 22–35 years	subjects were confined to a metabolic research unit for 90 days to determine the effect of the level of dietary copper on absorption and retention	absorption and retention averaged 36.3 ± 1.3% and 0.17 mg/day, respectively, with an adequate-copper diet (1.68 mg/day). Absorption averaged 55.6 ± 0.9% and retention averaged -0.316 mg/day for 6 days and 0.093 mg/day for the next 36 days of a low-copper diet (0.785 mg/day). Absorption averaged 12.4 ± 0.9% with a high-copper diet (7.53 mg/day) and retention was strongly positive at first, decreasing linearly with time. In conclusion: copper absorption is strongly dependent on dietary copper level and copper balance can be achieved by most young men from a diet of 0.8 mg of copper daily	Turnlund et al. (1989)

Table 8 (contd).

10 obese men	balance studies were conducted over 40 days. Two diets providing, 400 kcal (1.7 MJ) and 100 g of protein daily were administered; to five subjects, a collagen diet that was severely deficient in both zinc and copper, and another five subjects, a soy diet that provided a marginal intake of zinc and an adequate intake of copper	the mean daily intakes of zinc and copper in the soy group were 6.81 and 3.1 mg/day, respectively, and in the collagen group these figures were 0.32 and 0.54 mg/day, respectively. Copper balances were determined during eight 5-day periods. During each period copper balance was markedly positive in the soy-diet group and negative in the collagen-diet group	Lowy et al. (1986)
24 men aged 21–57 years	subjects received one of two diets low in copper (1.03 mg per day and 2850 kcal, 12 MJ) and containing 20% of the calories as either fructose or cornstarch	apparent copper balance was significantly greater when the subjects consumed the fructose diet (copper intake 1.11 ± 0.02 mg, balance 0.17 ± 0.08 mg) as compared to the starch diet (copper intake 0.94 ± 0.04 mg, balance -0.08 ± 0.08 mg)	Reiser et al. (1985)

In copper-deficient infants, it is mainly the ceruloplasmin-bound fraction of serum copper that is decreased (Holtzman et al., 1970). The non-ceruloplasmin fraction of serum copper is much less affected and is more rapidly restored when copper supplementation is initiated. Apo-ceruloplasmin cannot be detected in human serum during copper deficiency, suggesting that even if the apo-form may accumulate in the liver (Holtzman et al., 1970), ceruloplasmin is not released until the holo-form can be formed. However, even if apo-ceruloplasmin cannot be detected in its completely unsaturated form, low ceruloplasmin enzyme activity, concomitant with normal immunoreactive ceruloplasmin levels, has been observed in copper-deficient human adults. In fact, it has been suggested that the ratio between ceruloplasmin oxidase activity and its mass concentration determined by immunological methods may be used as an indicator of copper status (Milne & Johnson, 1993). Recent studies by one group, in which the enzymatic activity and concentration of ceruloplasmin have been measured, show that in copper deficiency there is a reduction of enzymatic activity of ceruloplasmin and the ceruloplasmin protein concentration is conserved (Johnson & Murphy, 1988). Therefore, the enzymatic activity/concentration ceruloplasmin ratio may be a better indicator of copper status, with the additional advantage that it is not influenced by factors such as hormones and gender (Vohra et al., 1965).

Plasma copper will be elevated (up to three times the upper reference value) in acute copper toxicity. In such circumstances, signs of intravascular haemolysis may be present. However, in chronic copper overload, plasma copper and ceruloplamin concentrations are not elevated (O'Donohue et al., 1993).

SOD is a copper-containing enzyme found in the cytosol of virtually all cells, including the erythrocyte. Reduced SOD activity has been demonstrated in copper-deficient animals and in humans (Uauy et al., 1985). This decrease is proportional to the magnitude of the deficiency of this mineral (Harris & Percival, 1991). Studies in humans have shown decreased activity of erythrocyte SOD in copper-deficient patients or in subjects receiving a low copper intake (Disilvestro & Harris, 1981; Van der Berg & Beynen, 1992). SOD activity was restored to a normal level when the subjects' diet or drinking-water was supplemented with copper (Vohra et al., 1965; Van der Berg & Beynen, 1992).

It has also been shown in humans that cytochrome c oxidase activity of leukocyte and platelets is reduced in copper deficiency (Johnson & Murphy, 1988). This decrease occurs before the appearance of a reduction of SOD activity (Johnson & Murphy, 1988). If confirmed, this finding suggests that cytochrome c oxidase activity in leukocytes or platelets could be a sensitive indicator of copper status. Although there is no single specific indicator of copper deficiency (WHO, 1996), evidence of deficiency can be based on observing the rate of disappearance of copper-dependent enzymic activities and their subsequent return to normal levels with copper supplementation. Deficiency studies are very valuable because specific proteins can be singled out and studied with little interference from other cuproenzymes. For instance, extracellular lysyl oxidase, intracellular SOD and mitochondrial cytochrome oxidase can be assayed, and changes over time following copper repletion experiments can be used to trace the movement of copper through the cellular compartments. To be a sensitive tool in nutritional studies, an enzyme must respond reversibly to a copper deficiency, be easily quantitated and have a short half-life so the change in activity can be measured rapidly. Unfortunately, the copper enzymes used in many studies are difficult to quantitate, hard to purify and have long half-lives. The sensitivity of deficiency studies can be enhanced by using copper isotopes to label the target proteins, which can then be identified and quantitated enzymatically, immunochemically or by both procedures. The major requirement in such experiments is that the turnover, synthesis or activation of the enzyme must be rapid so the isotope can be incorporated into the target protein and measured in a reasonably short period of time.

Excessive copper accumulation in the liver can be determined by needle biopsy. This requires an adequate sample taken under controlled conditions in order to avoid contamination. Analysis must be carried out in a specialized laboratory. This is the preferred method for measurement of copper excess and should be included in the evaluation of children and adults with liver disease of unknown aetiology. The reference value for liver copper is 20–40 µg/g (dry weight) but is significantly higher in the newborn. Nonspecific copper accumulation occurs in a variety of cholestatic liver disease without a specific pathological effect. Liver copper in excess of 250 µg/g (dry weight) in the presence of other biochemical and clinical evidence is indicative

of Wilson disease, ICC or ICT (see chapter 8). Copper accumulation in other tissues can be assessed only by postmortem analysis.

6.4 Biochemical basis of copper toxicity

The requirement for copper in various organs or systems within the body is effectively regulated by homoeostatic control mechanisms. Toxicity is likely to occur only when such homoeostatic control within any particular compartment is overwhelmed and/or basic cellular defence or repair mechanisms are impaired.

The essentiality and potential toxicity of copper in biological systems relies basically on the specific electron configuration, particularly of the outer electron shells. Accordingly, the cuprous (Cu^{1}) ion is highly polarizable and binds mainly to nitrogen- and sulfur-containing ligands by sharing their electronic orbitals. Cupric (Cu^{2+}) ions, on the other hand, are able to form both coordination complexes with oxygen-containing ligands and partly covalent bonds with nitrogen- and sulfur-containing centres. Therefore, copper has to be considered fairly reactive and able to bind strongly to many types of electron-rich structures. The affinity of copper ions towards a particular ligand, however, is also influenced by the polarizability of the ligand itself (Nriagu, 1979).

Toxicity of copper may arise when excess copper provokes the following adverse reactions:

- Structural impairment of essential metal binding sites by displacement of metals resulting, for example, in membrane changes such as depolarization and impairment of receptors or transporter molecules (Alt et al., 1990).

- Functional impairment by binding of copper to crucial sites in such macromolecules as DNA or enzymes particularly containing sulfhydryls, carboxylates or imidazoles (Alt et al., 1990). This will lead to direct protein damage, or oxidative DNA changes leading to various functional changes, because of the large number of enzymes dependent upon copper and the possible misreading of genetic codes.

- Cellular injury due to the production of oxyradicals by the Fenton reaction (Goldstein & Czapsky, 1986):

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{\cdot} + OH^{-}$$

The excessive production of such radicals will initiate a cascade of oxidation–reduction reactions (oxidative stress) finally leading to the loss of cellular integrity. The causes of injury considered include increased cytosolic calcium levels, ATP depletion, thiol oxidation, lipid peroxidation, DNA damage and critical damage to organelles such as mitochondria and lysosomes.

Threshold levels for copper toxicity have not yet been established, although the main intracellular binding site for copper, metallothionein, appears to become saturated with copper before the occurrence of any toxic effects. Metallothionein also has been suggested to act as an intracellular antioxidant, thereby protecting cells by the direct scavenging of reactive oxygen species. *In vitro* metallothionein exhibits a very high reaction constant for hydroxyl radicals (Thornalley & Vasak, 1985) and according to recent experiments, mouse cells lacking metallothionein were more sensitive to oxidative stress (Liu et al., 1995).

6.5 Interactions with other dietary components

The absorption of copper is inhibited by the presence of some other essential and nonessential trace metals (e.g. zinc, iron, molybdenum, lead and cadmium) (WHO, 1996). The absorption of copper is also influenced by a number of other dietary and endogenous factors. Easily digested proteins may enhance copper absorption; for example, proteins in human milk are more easily digested than proteins in cow's milk and lend to enhance copper absorption. Citrate, phosphate and glutamate all form complexes with copper that facilitate absorption. Phytate, dietary cellulose fibre and ascorbic acid decrease copper absorption (Cousins, 1985).

6.5.1 Protein and amino acids

Animal protein enhances copper absorption (Turnlund et al., 1983). Copper absorption was higher from an animal protein diet

(41%) than from a plant protein diet (34%). Different milk proteins have been shown to have varying effects on copper status: whey protein had a negative effect on copper absorption (Lynch & Strain, 1990). Soy protein isolates, as used in infant formula, reduce copper bioavailability (Lo et al., 1984; Greger & Mulvaney, 1985). Specific amino acids are known to form complexes with divalent cations such as copper. Histidine chelates copper with a greater affinity than it does zinc (Ashmead et al., 1985). Copper accumulation in the mucosal tissue was higher when an excess of histidine to copper and zinc was used (Wapnir & Balkman, 1992). It is possible that a copper–histidine complex may be an effective way to provide bioavailable copper. In contrast, cysteine has an inhibitory effect on copper utilization (Robbins & Baker, 1980; Baker & Czarnecki-Maulden, 1987). This effect on copper absorption is evident at both deficient and excess copper levels in the diet (Aoyagi & Baker, 1994).

6.5.2 Phytate and fibre

Turnlund et al. (1984) used stable isotopes to study the effect of copper on the absorption of phytate and α-cellulose in young men. They found no effect of either component in human subjects and suggested that high levels of phytate or fibre do not decrease copper absorption. The authors proposed that zinc–phytate complexes precipitate at the pH of the gastrointestinal tract, whereas copper–phytate complexes do not. Since phytate in the soluble copper–phytate complex can easily be replaced by other chelators, such as amino acids (Jacobsen & Slotfeldt-Ellingsen, 1983), there may be no inhibitory effect of phytate on copper absorption. A study on cereal products supports this hypothesis (Lyon, 1984); zinc solubilized from cereal by the addition of acid precipitated completely when the pH was raised to 7, whereas copper remained in solution.

6.5.3 Ascorbic acid

Van den Berg & Beynen (1992) suggested that the primary effect of high dietary ascorbic acid was to reduce intestinal absorption of copper, but that it also increased hepatic uptake and biliary excretion of ^{64}Cu. The effect of ascorbic acid on copper metabolism was more pronounced in copper-deficient than in copper-adequate animals.

Finley & Cerklewski (1983) found decreased ceruloplasmin oxidase activity and lower serum copper in young adult men after 64 days of 1500 mg ascorbic acid/day (values were determined after the vitamin was discontinued). However, this effect could be independent of lower copper absorption, as Jacob et al. (1987) found no difference in copper absorption in young men given different levels of ascorbic acid. Ascorbic acid may promote the dissociation of copper from ceruloplasmin, thus lowering its oxidase activity. This was supported by the finding that immunological quantitation of ceruloplasmin showed no change in apoprotein levels. A clinical study on low birth weight (LBW) infants fed formula supplemented with ascorbic acid (50 mg/day) did not show any negative effects on copper balance (Stack et al., 1990). However, the LBW infants were largely in negative copper balance and thus may have been copper deficient. It is possible that ascorbic acid under these conditions may not exert overall negative effects on copper utilization as observed in copper-deficient rats (Van den Berg et al., 1994).

6.5.4 Zinc

High levels of dietary zinc have a negative effect on copper absorption. Since supplemental zinc is often used in infants, children and pregnant women in order to avoid possible zinc deficiency, the possible interference with copper absorption needs to be considered. High doses of zinc (40–50 mg/day) have been used successfully to treat patients with Wilson disease (Brewer et al., 1983; Hoogenraad & van den Hamer, 1983). Zinc limits the amount of copper absorbed (Lyons et al., 1995), possibly by increasing intestinal metallothionein concentrations and, therefore, slowing the progression of the disease (Fischer et al., 1983; Oestreicher & Cousins, 1985). However, high intakes of zinc should be viewed with some concern since copper deficiency may be induced. Conversely, copper supplementation may interfere with zinc absorption (Salim et al., 1986).

Human subjects fed diets with different zinc copper ratios have not exhibited a significant effect on copper absorption. August et al. (1989) used a stable isotope of copper to study copper absorption in young adults and elderly subjects. They used zinc copper ratios of 2 : 1, 5 : 1 and 15 : 1, finding no significant effects of these ratios on copper absorption.

6.5.5 Iron

Copper absorption may also be affected by high levels of dietary iron. Haschke et al. (1986) studied the effect of two levels of iron fortification of infant formula on copper balance in full-term infants. They found that the higher level of iron (10.8 mg/litre) resulted in lower copper balance than when the lower iron level was used (1.8 mg/litre). Barclay et al. (1991) have shown reduced SOD levels in premature infants given iron supplements. Earlier studies in experimental animals had shown a reduction in liver copper concentrations when dietary iron was increased 10-fold (Smith & Bidlack, 1980). However, modest supplements of iron did not appear to affect serum copper levels in older infants (Yip et al., 1985). Several studies suggest that high dietary iron only affects copper absorption when copper status is low or marginal (Cohen et al., 1985a,b; Johnson & Murphy, 1988).

High intakes of iron and ascorbate may act together to adversely affect copper status. Johnson & Murphy (1988) found that high iron with ascorbic acid caused severe anaemia in copper-deficient rats and decreased plasma ceruloplasmin by 44% in copper-adequate rats. Since iron and ascorbate are commonly used together in nutritional supplements for humans, the possibility of a negative effect on copper metabolism should be considered.

6.5.6 Carbohydrates

In rats, dietary fructose worsens the effects of copper deficiency (Fields et al., 1984; Reiser et al., 1985) in that fecal and urinary excretion of copper are elevated when the rats are fed fructose as compared to starch. Data from humans do not support these findings (Reiser et al., 1985; Holbrook et al., 1989).

6.5.7 Infant diets

Studies on full term infants fed on breast or cow's milk formula suggest that copper is better absorbed from human milk than from a cow's milk formula (Dörner et al., 1989). Studies using stable isotopes of copper support this finding (Ehrenkranz et al., 1989). Studies in suckling rats have revealed slightly higher copper bioavailability (estimated from uptake of ^{64}Cu by 6 h post-dosing) from human milk

than from cow's milk formula (Lonnerdal et al., 1985). A more recent study, using the same rat pup model, evaluated several varieties of infant formula (Lonnerdal et al., 1994). In general, copper absorption was relatively high from milk formulae but lower from soy formulae. The lower copper bioavailability from cow's milk combined with its low copper content most likely explains the copper deficiency found in some premature infants fed cow's milk formulae.

6.5.8 Other interactions (molybdenum, manganese, selenium)

Dietary molybdenum, in the presence of sulfate, forms insoluble complexes with copper thereby decreasing the availability of copper for absorption. Thus, high levels of molybdenum in the diet may induce or aggravate copper deficiency (Ladefoged & Sturup, 1995). The addition of copper to diets of rats decreases tissue manganese levels, suggesting that copper impairs manganese absorption. Manganese absorption is greatest in animals that are deficient in copper and manganese (Johnson & Korynta, 1992). Research efforts on copper–selenium interactions have not been revealing, except for showing the complementarity in antioxidant protection of copper SOD and selenium-containing glutathione peroxidase (Fischer et al., 1992; Olin et al., 1994).

7. EFFECTS ON LABORATORY MAMMALS AND *IN VITRO* TEST SYSTEMS

The effects of exposure of experimental animals to common inorganic salts of copper have been summarized in Tables 9–12. These studies represent the better-quality and better-documented studies in each toxicological area. Studies in which the compound was administered by injection have generally not been included, owing to their uncertain relevance to environmental or occupational exposures. The results of such studies have, however, been included in the table, when no information was available for more relevant routes of exposure.

In this section and the associated tables, information on dosage with respect to body weight was obtained from the original papers wherever possible. When doses were not expressed in this way by the investigators and could not be calculated from the data provided, approximate doses have been estimated based on data presented in standard sources (IAT, 1963; FDO, 1965; Gold et al., 1984).

7.1 Single exposure

7.1.1 Oral

The acute oral toxicity of various copper salts is summarized in Table 9. A wide range of LD_{50} values has been reported, with the most soluble salts (e.g. copper(II) sulfate and copper(II) chloride) generally being more acutely toxic than those with lower solubility (e.g. copper(II) hydroxide and copper(I) oxide). From the available information on copper(II) sulfate, rats appear to be less susceptible to copper than domestic animals; this pattern is also evident in studies involving repeated exposure (section 7.2). In the various acute studies, as the lethal oral dose is approached, signs of copper toxicity include excessive salivation, vomiting, diarrhoea, gastric haemorrhage, increased heart rate, hypotension, haemolytic crisis, convulsions and paralysis.

7.1.2 Dermal

In the only dermal studies identified, LD_{50} values of > 1124 and > 2058 mg Cu/kg body weight per day were reported, the first for

Table 9. Toxicity of copper compounds after a single oral exposure

Salt	Species	LD_{50} value (mg/kg body weight)	Equivalent copper dose (mg Cu/kg body weight)	Reference
Copper(II) acetate	rat	595	208	NIOSH (1993)
	rat	710[a]	226	Smyth et al. (1969)
	mouse	1600[a] (lethal dose)	509	Schafer & Bowles (1985)
Copper(II) carbonate	rat	159	82	Lehman (1951)
	mouse	320 (lethal dose)	165	Schafer & Bowles (1985)
Copper(II) carbonate hydroxide	rat (male)	1350	388	Hasegawa et al. (1989)
	rat (female)	1495	430	
	rabbit	317	91	NIPHEP (1989)
Copper(II) chloride	rat	140	66	Lehman (1951)
	mouse	190	90	NIPHEP (1989)
	guinea-pig	32	15	NIPHEP (1989)
Copper(II) hydroxide	rat	1000	651	Pesticide Manual (1991)
Copper(II) nitrate	rat	940*b*	247	Smyth et al. (1969)
Copper(I) oxide	rat	470	417	Smyth et al. (1969)
Copper(II) oxychloride	rat	700–800	417–476	Tomlin (1994)
	rat	1440	857	NIPHEP (1989)
Copper(II) sulfate	rat	300	120	Lehman (1951)
	rat	960[c]	244	Smyth et al. (1969)
	mouse	50 (LD_{100})	20	Venugopal & Luckey (1978)
	rabbit	125	50	Eden & Green (1939)

a Monohydrate
b Trihydrate
c Pentahydrate

rats exposed to copper(II) oxysulfate (NIOSH, 1993) and the second for rabbits exposed to copper(II) hydroxide (Tomlin, 1994).

7.1.3 Inhalation

The LC_{50} value for inhalation exposure of rabbits to copper(II) hydroxide (physical form and duration unspecified) was > 1303 mg Cu/m^3 (Tomlin, 1994). Intratracheal instillation in rats of copper(II) oxide at 222 mg Cu/kg body weight was lethal (NIOSH, 1993).

Guinea-pigs exposed to copper(II) oxide aerosol at 1.6 mg/m^3 (1.3 mg Cu/m^3, as particles with a count median diameter approximately 0.03 μm) for 1 h showed significant reductions ($P < 0.05$) in tidal volume, minute volume and lung compliance, both during and after exposure, while respiratory frequency was slightly but not significantly increased (Chen et al., 1991).

In two studies involving the intratracheal instillation in rats of copper(II) oxide (Hirano et al., 1993) or copper(II) sulfate pentahydrate (Hirano et al., 1990) at doses of up to 0.1 or 0.05 mg Cu/rat, respectively (roughly 0.36 or 0.18 mg Cu/kg body weight), acute inflammatory changes were evident in the lungs from 0.018 mg Cu/kg body weight with the soluble sulfate salt and from 0.073 mg Cu/kg body weight with the insoluble oxide.

7.2 Short-term exposure

There have been numerous studies of the effects of short-term exposure to copper compounds. In rats exposed by the oral route to approximately 30–50 mg Cu/kg body weight per day as copper(II) sulfate, the most common compound-related effects observed have included those on the liver, kidney and lungs, as well as alterations in haematology (particularly anaemia) and in blood biochemistry. Effects are qualitatively similar with other copper compounds, and in other species. However, pigs and especially sheep are more susceptible to the toxic effects of copper compounds; exposure of sheep to doses of 1.5–7.5 mg Cu/kg body weight per day in diet as copper(II) sulfate or copper(II) acetate was associated with progressive liver damage, followed by a haemolytic crisis and ultimately death. In inhalation studies, morphological changes were induced in the tracheal epithelium

and in the alveoli by short-term inhalation of 0.06 mg Cu/m^3 copper(II) sulfate in mice, but not in hamsters.

7.2.1 Oral

The most comprehensive studies of short-term toxicity in rats and mice were conducted by Hébert et al. (1993). In a 15-day feeding study in rats involving the administration of up to 16 000 mg/kg copper(II) sulfate pentahydrate in the diet (estimated intakes up to 305 mg Cu/kg body weight per day), weight gain was reduced from 194 mg Cu/kg body weight per day, but there were no other overt signs of toxicity. Effects on the forestomach were evident from 45 mg Cu/kg body weight per day, on the kidneys from 93 mg Cu/kg body weight per day, and on the liver and bone marrow from 194 mg Cu/kg body weight per day. The NOEL in this study was 23 mg Cu/kg body weight per day (Hébert et al., 1993). When the same investigators administered copper(II) sulfate to rats in the drinking-water for 15 days at up to 30 000 mg/kg (estimated intakes up to 97 mg Cu/kg body weight per day), the various clinical signs of toxicity and deaths that were evident from around 31 mg Cu/kg body weight per day were attributed to dehydration, as a result of the poor palatability of the drinking-water. The NOEL in females was 26 mg Cu/kg body weight per day, while in males there was evidence of kidney damage from the lowest dose tested of 10 mg Cu/kg body weight per day (Hébert et al., 1993). (Effects on gastric mucosa have only been observed in rodent studies in which copper(II) sulfate was administered in the diet, and not in the drinking-water studies. It is likely that these effects are due to irritation, particularly as copper(II) sulfate may dissociate to form sulfuric acid in the stomach.)

From the evidence of one 15-day feeding study (Hébert et al., 1993), mice appear to be less sensitive than rats to the toxic effects of copper. When copper(II) sulfate pentahydrate was administered at up to 16 000 mg/kg in the feed, weight gain was reduced only in females at the top dose (estimated intake 781 mg Cu/kg body weight per day), while the only effects observed on microscopic examination of the liver, kidneys and forestomach were hyperplasia and hyperkeratosis in the forestomach from 197 (males) or 216 (females) mg Cu/kg body weight per day. The NOEL in this study was 92 mg Cu/kg body weight per day in males and 104 mg Cu/kg body weight per day in females.

In the equivalent drinking-water study, the findings (reduced water consumption, body weight, clinical signs at doses of 58–62 mg Cu/kg body weight per day and higher) were again, as in the rats, thought to be confounded by dehydration of the treated animals (Hébert et al., 1993).

Other studies summarized in more extensive reviews on copper (Slooff et al., 1989; ATSDR, 1990) have deficiencies in design and/or level of experimental details and results, which make it impossible to utilize in any dose–response evaluation. They are, therefore, not considered here.

7.2.2 *Inhalation*

7.2.2.1 *Copper(II) sulfate*

When unspecified numbers of mice and hamsters were exposed by inhalation to copper(II) sulfate aerosol at 0.06 mg Cu/m^3 for 3 h/day, 5 days/week for 1 or 2 weeks, the tracheal epithelium and the alveoli of mice were altered in appearance, whereas hamsters showed no treatment-related effects on the tracheal epithelium or on ciliary activity (Drummond et al., 1986).

7.2.2.2 *Copper chloride*

In an inhalation study, repeated exposure of rabbits (group sizes not specified) to copper(II) chloride aerosol at 0.6 ± 0.3 mg Cu/m^3 for 6 h/day, 5 days/week for 4–6 weeks did not produce any histological lesions in the lungs, and alveolar macrophage activity appeared to be unaffected despite some morphological changes (Johansson et al., 1983, 1984; Lundborg & Camner, 1984).

7.3 Repeated exposure: subchronic toxicity

There are a limited number of studies of the subchronic toxicity of copper compounds to animals. In comprehensive studies in rats, there were histopathological effects on the forestomach and indications of anaemia at 34 mg Cu/kg body weight per day as copper(II) sulfate in diet. Higher doses elicited degenerative changes in the liver and kidney in rats in this and several other studies, with recovery observed in some of these. As was observed in the short-term studies

(section 7.2), mice are markedly less sensitive than rats to the toxicity of copper(II) sulfate. Other copper compounds have not been well studied, although exposure of rats to approximately 10 mg Cu/kg body weight per day as copper(I) chloride induced transient reductions in the activities of glutathione *S*-transferases, and the same dose as copper(II) carbonate increased systolic blood pressure and haemoglobin levels.

7.3.1 Oral

7.3.1.1 Copper(II) sulfate

The critical study is that of Hébert et al. (1993) which is described here. Details of other experiments of repeated long-term exposures of copper are given in Table 10.

In comprehensive 90-day studies in both rats and mice (Hébert et al., 1993), in which copper(II) sulfate pentahydrate was administered in the feed at up to 8000 mg/kg in rats (up to 138 mg Cu/kg body weight per day) and up to 16 000 mg/kg in mice (up to around 1000 mg Cu/kg body weight per day), there were no overt signs of toxicity other than a dose-related reduction in growth (statistically significant in male and female rats from 67 and 138 mg Cu/kg body weight per day, respectively, and in male and female mice from 97 and 267 mg Cu/kg body weight per day). Microscopic examination of the tissues revealed hyperplasia and hyperkeratosis in the forestomach in both species (from 34 mg Cu/kg body weight per day in rats and from 187–267 mg Cu/kg body weight per day in mice), and liver and kidney effects in the rats only (from 67 mg Cu/kg body weight per day). In the rats, iron levels were reduced in the spleen, and haematological changes indicative of microcytic anaemia were observed at 34 mg Cu/kg body weight per day and higher. The NOEL was 17 mg Cu/kg body weight per day in rats, and 44 and 126 mg Cu/kg body weight per day in male and female mice, respectively. The liver and kidney effects observed in the rats in this study included inflammation of the liver and degeneration of the kidney tubule epithelium, and were similar to those found at higher doses (> 100 mg Cu/kg body weight per day) in more limited studies in rats (Haywood, 1980, 1985; Haywood & Loughran, 1985).

Table 10. Toxicity of copper after repeated oral doses

Species	Protocol	Results	Effect level	Reference
Copper(II) sulfate Rats (F344/N, groups of 10 males and 10 females, additional groups of 10 males & 10 females for clinical pathology studies at intermediate time points)	copper sulfate pentahydrate given in the feed for 92 days at levels of 0, 500, 1000, 2000, 4000 and 8000 mg/kg diet. Estimated intakes were 0, 8, 17, 34, 67 or 138 mg Cu/kg body weight per day. Comprehensive microscopic examinations carried out at the top dose level, in the controls, and in the animals that died early. Liver, kidney and forestomach examinations were carried out to establish a NOEL. Intermediate haematology and clinical chemistry evaluations carried out on days 5 and 21, and urinalysis on day 19. These tests also carried out at termination of the study	Survival was unaffected. Body weight gain was significantly depressed in the males at 4000 mg copper sulfate/kg diet ($P < 0.05$) and in both sexes at 8000 mg copper sulfate/kg diet ($P \leq 0.01$). Average feed consumption was reduced in both sexes at 8000 mg copper sulfate/kg diet. There were no other clinical signs of toxicity in the treated rats Gross and microscopic lesions of the forestomach (hyperplasia and hyperkeratosis of the limiting ridge) were seen at 2000 mg copper sulfate/kg diet and above. Inflammation of the liver was seen in all rats at 8000 mg copper sulfate per kg diet, all males and 6/10 females at 4000 mg copper sulfate/kg diet and one male at 2000 mg copper sulfate/kg diet. In the kidneys, cytoplasmic protein droplets were evident, particularly at the top two doses, and minimal nuclear enlargement of, and degeneration in, the tubule epithelium were seen at the top dose. From 2000 mg copper sulfate per kg diet, iron levels were reduced in the spleen (both sexes) and haematological changes indicative of microcytic anaemia were seen on day 21 and at the end of the study. Significant increases in red bloodcells and reticulocytes were seen in the high-dose males at the end of the study. A number of other clinical chemistry and urinalysis parameters were affected at the top two dose levels	NOEL: 17 mg Cu/kg body weight per day LOEL: 34 mg Cu/kg body weight per day	Hébert et al. (1993)

Table 10 (contd).

Rats (Wistar, groups of 16 males)	Rats fed diets containing 0 or 3000 mg Cu/kg as copper sulfate for 15 weeks (equivalent to 270 mg Cu/kg body weight/day). Four rats per group killed and livers removed for examination, remaining rats then fed diets containing 6000 mg Cu/kg as copper sulfate for a further 3 weeks	In group not supplemented with copper for first 15 weeks of experiment, clinical effects (lethargy, ruffled coats) seen on administration of 6000 mg Cu/kg diet. No such effect seen in 'copper-primed' group. Livers of rats given 3000 mg Cu/kg diet for 15 weeks showed only mild effects (believed to indicate ongoing recovery from damage that was assumed to have occurred in the earlier weeks) at 15 weeks, and feeding of 6000 mg Cu/kg diet for a further 3 weeks had no significant hepatotoxic effects. The unprimed group suffered hepatocellular necrosis and inflammation after the 3-week exposure to 6000 mg/kg	only one dose tested (effects at 270 mg Cu/kg body weight per day)	Haywood & Loughran (1985)
Rats (strain unspecified, groups of 24 treated and 12 control males)	Rats fed diet containing 2000 mg Cu/kg diet as copper sulfate (equivalent to about 100 mg Cu/kg body weight/day). Groups of 4 treated and 2 control rats killed after 1, 2, 3, 6, 9 and 15 weeks and their liver and kidneys examined histologically	Inflammation and extensive necrosis of the liver and bile duct hyperplasia were evident by week 6. By week 15 there was considerable recovery, although some fibrosis and less marked hyperplasia of the bile duct could still be seen Greenish discolouration of the kidneys was seen in some rats at week 6. Microscopic effects (eosinophilic droplets in the cytoplasm of cells in the proximal convoluted tubules and desquamation of these cells into the lumen) first appeared at week 3, and were more severe at 6 weeks. Regeneration was almost complete at 15 weeks The investigators concluded that repeated copper dosing elicits a similar response in the kidneys and the liver, both organs adapting to the excess copper, resulting in the development of tolerance in the treated rats	only one dose tested (effects at 100 mg Cu/kg body weight per day)	Haywood (1980)

Table 10 (contd).

Species	Protocol	Results	Effect level	Reference
	Blood was taken from the above rats prior to sacrifice and analysed for enzyme activity	Alanine aminotransferase activity was significantly increased ($P < 0.05$) at week 1 (indicative of liver damage), rose to a maximum around weeks 6–9, and remained at that level to the end of the study. Ceruloplasmin activity was elevated ($P < 0.05$) from week 6 until the end of the study. Alkaline phosphatase activity and bilirubin levels were unaffected by copper treatment		Haywood & Comerford (1980)
Rats (Wistar, groups of 28 males)	Rats fed diets containing 0, 3000, 4000, 5000 or 6000 mg Cu/kg diet as copper sulfate for up to 15 weeks (equivalent to 0, 270, 360, 450 and 540 mg Cu/kg body weight per day based on the mean final weight of the rats fed 3000 mg Cu/kg diet). Four rats at each dose level killed at 1, 2, 3, 4, 5, 6 and 15 weeks. Liver and kidneys removed for histological examination	Rats receiving 6000 mg Cu/kg diet did not grow and were in poor condition. Two died at 2 weeks. At 6 weeks the survivors developed diarrhoea, began to lose weight and were killed. At 3000–5000 mg/kg of copper sulfate, the animals showed clinical signs of toxicity (poor growth, ruffled fur) at around 3–5 weeks, but their condition subsequently improved; by week 15 they appeared sleek and active, but were only half the weight of controls Microscopic changes were evident in the liver (necrosis, inflammation, hepatocytic hypertrophy, nuclear enlargement) within 1–2 weeks, depending on the dose, but began to subside from week 6 onwards, with regeneration by week 15 (except at 6000 mg Cu/kg diet where the effects persisted).	LOEL: 270 mg Cu/kg body weight per day	Haywood (1985); Haywood & Loughran (1985)

Table 10 (contd).

		Microscopic effects on the kidneys (an increase in eosinophilic cytoplasmic droplets in cells of the proximal tubules followed by extrusion of the droplets and exfoliation of the cells, degenerative changes to proximal tubules) were seen at 2–5 weeks at all dose levels, with recovery from weeks 6–15		
Mice (B6C3F1, groups of 10 males and 10 females)	Copper sulfate pentahydrate given in the feed for 92 days at levels of 0, 1000, 2000, 4000, 8000 and 16 000 mg/kg diet. Estimated intakes were 0, 44, 97, 187, 398 and 815 mg Cu/kg body weight per day in males and 0, 52, 126, 267, 536 and 1058 mg Cu/kg body weight per day in females. Comprehensive microscopic examinations carried out at the top dose level, in the controls, and in the animals that died early. Liver, kidney and forestomach examined to establish a NOEL	Survival was unaffected. A dose-related depression in body weight gain was observed in both sexes (statistically significant from 2000 mg copper sulfate/kg diet in males and 4000 mg copper sulfate/kg diet in females, $P < 0.05$), although average feed consumption was similar in treated and control mice. No other clinical signs of toxicity were observed Gross and microscopic lesions of the forestomach (hyperplasia and hyperkeratosis of the limiting ridge) were seen at 4000 mg copper sulfate/kg diet and above There were no reported effects on the liver or kidneys, and iron levels in the spleen were normal	NOEL: 44 and 126 mg Cu/kg body weight per day in males and females respectively LOEL: 97 and 267 mg Cu/kg body weight per day in males and females respectively	Hébert et al. (1993)

Table 10 (contd).

Species	Protocol	Results	Effect level	Reference
Copper(I) chloride Rats (Sprague-Dawley, groups of 5 females)	Rats given drinking-water containing 0 or 100 mg CuCl/litre (equivalent to 0 or 10 mg Cu/kg body weight per day). Livers removed after 15, 30 or 90 days of treatment for determination of activity of glutathione *S*-epoxide transferase and glutathione *S*-aryl transferase	Activity of glutathione *S*-epoxide transferase was significantly inhibited ($P < 0.05$) after treatment for 15 days (–29% compared with controls) but not after 30 or 90 days. Glutathione *S*-aryl transferase activity was unaffected after 15 days, was significantly inhibited ($P < 0.05$) after 30 days (–7%), and was still slightly but not significantly reduced after 90 days (–6%). (These enzymes catalyse the metabolic inactivation of reactive substances)	only one dose tested (effects seen at 10 mg Cu/kg body weight per day)	Freundt & Ibrahim (1991)
Copper(II) carbonate Rats (Wistar or spontaneously hypertensive rats (SHR), groups of 10 males of each strain)	Rats given 18 or 100 mg Cu/kg diet as copper carbonate for 15 weeks (equivalent to about 1.7 and 9.6 mg Cu/kg body weight per day). Blood pressure measured 3 times/week	Body weight, urine output and feed and water intakes did not differ with copper intake. High-dose rats showed increased systolic blood pressure compared with low-dose rats, particularly in the Wistar strain (Wistar $P \leq 0.05$, SHR $P \leq 0.01$ at week 15). Haemoglobin levels were increased at high copper intake ($P \leq 0.05$), while total cholesterol, triglycerides and glucose levels in the blood were unaffected	only one dose tested (effects seen at 9.6 mg Cu/kg body weight per day)	Liu & Medeiros (1986)

7.3.1.2 Copper chloride

The task group was aware of an ongoing study in guinea-pigs which were orally dosed from their first day of life with milk formula containing copper(II) chloride (10, 15, 30 mg Cu/kg body weight per day) for 28 days in order to study the effect of exposure to copper in early life on copper homoeostasis and toxicity (Summer & Dieter, personal communication, 1996).

7.4 Long-term exposure chronic toxicity or carcinogenicity

The chronic toxicity/carcinogenicity of copper compounds has not been well characterized (see Table 11). Increased mortality and growth retardation or effects on the liver, kidneys or stomach have been observed in rats following long-term ingestion of 27–150 mg Cu/kg body weight per day as copper(II) sulfate, or 44–45 mg Cu/kg body weight per day as copper(II) acetate, in several limited studies. Long-term ingestion of copper(II) sulfate at 10 mg Cu/kg body weight per day induced marked hepatotoxicity in rabbits. An oral study in dogs did not show significant toxic effects at the highest dose of 8.4 mg Cu/kg per day, given as copper gluconate (Shanaman et al., 1972).

The available studies of the carcinogenicity of copper compounds in rats and mice have given no indication that copper salts are carcinogenic. However, the short duration or low level of exposure, the small group sizes employed, the limited extent of histopathological examination, or inadequate reporting limits the conclusions which can be drawn from such studies. The studies summarized in Table 11 are, therefore, inadequate to test the carcinogenic potential of copper compounds with any degree of certainty. In several studies, administration of copper compounds inhibited the development of tumours induced by known carcinogens (see Table 11).

7.5 Reproductive and developmental toxicity

As shown in Table 12, there is some limited evidence that exposure to copper compounds can affect reproduction in animals. In some studies of rats exposed by the oral route, the weights and/or histology of the testes, seminal vesicles, uterus or ovaries have been affected by chronic intakes of 27–120 mg Cu/kg body weight per day

Table 11 Chronic toxicity or carcinogenicity after long-term exposure

Species	Protocol	Results	Effect level	Reference
Copper(II) sulfate Oral Rats (Sprague-Dawley, groups of 25 males and 25 females)	Rats fed diets containing 0, 530 or 1600 mg Cu/kg diet as copper sulfate for 40–44 weeks (approx. 0, 27 or 80 mg Cu/kg body weight per day in males and 0, 40 or 120 mg Cu/kg body weight per day in females). (Reduced amounts fed for the first month of the experiment.) Microscopic examination of limited number of organs Study inadequately described	Body weight gain was retarded at 1600 mg Cu/kg diet in the males. Stomachs of the high-dose females were enlarged. Other findings at the high dose were 'bronzed' kidneys, 'bronzed' or yellowish livers, hypertrophied ridges between cardiac and peptic portions of stomach, and blood in the intestinal tract. Microscopic effects (not further described) were seen in the kidneys in the high-dose group (presumably in both males and females), and effects on the liver were seen in both males and females, presumably in both dose groups	LOEL (non-neoplastic effects): 27 mg Cu/kg body weight per day in males, 40 mg Cu/kg body weight per day in females	Harrisson et al. (1954)

Table 11 (contd).

Rats (Sprague-Dawley, groups of 50 or 58 males, additional groups of 55–102 males also given dimethyl nitrosamine (DMN) or acetylamino-fluorene (AAF)	Rats given diets containing deficient (1 mg Cu/kg diet) or excess (800 mg Cu/kg diet) levels of copper (as copper sulfate) for 9 months (equivalent to about 0.05 or 40 mgCu/kg body weight per day). Within each treatment group, separate groups given DMN in the drinking-water (50 mg Cu/kg diet) or AAF in the diet (0.06%), in both cases for 4 days in every 8 for 6 months, or no further treatment. Five rats per group killed after 90 days, and an additional 5/group killed every 30 days thereafter. Limited range of organs examined microscopically	Excess copper caused decreased body weight gain and increased mortality with or without DMN or AAF treatment. The only effects reported in the rats not exposed to these two carcinogens were liver necrosis and transitional nodules in the liver in 3/32 and 1/32 animals, respectively at 800 mg Cu/kg diet (none at 1 mg Cu/kg diet), and 1 kidney tumour the low-copper group (42 rats) Both DMN and AAF exposure markedly increased the incidence of liver necrosis and transitional nodules and each induced a similar incidence of liver tumours in rats fed excess copper or copper-deficient diets. There were no kidney neoplasms in the AAF-treated groups, but 57% of the rats in the DMN group on a copper-deficient diet (17/30) had kidney neoplasms compared with 0% (0/29) on the higher copper diet The incidence of AAF-induced extrahepatic neoplasms was apparently reduced by the excess copper diet (5/30 vs 11/27 in the low copper group)	Toxic effects at 40 mg Cu/kg body weight per day Exposure too short and group size inadequate to assess the carcinogenic potential of copper sulfate itself, but the data suggest it may have an inhibitory effect on DMN-induced kidney tumours and AAF-induced extra-hepatic tumours	Carlton & Price (1973)

Table 11 (contd).

Species	Protocol	Results	Effect level	Reference
Mice (C57BL/6J, groups of 10–12 females)	Copper sulfate pentahydrate supplied in the drinking-water at 198 mg/litre for 46 weeks (equivalent to about 10 mg Cu/kg body weight per day). One group received copper sulfate treatment alone, a second was given an intravenous injection of DMBA (a known carcinogen) 2 weeks after copper treatment began, and two further groups were untreated or received DMBA treatment only. Mice killed at 46 weeks and a limited range of organs studied microscopically	The numbers of mice with ovarian tumours were 0/10, 0/12, 11/11 and 6/11 in the untreated controls, copper-treated mice, DMBA-treated mice and DMBA + copper-treated mice respectively, suggesting that copper sulfate may inhibit tumour development to some extent. The corresponding figures for lymphomas were 1/10, 2/12, 3/11 and 3/11	Exposure too short and group size inadequate to assess the carcinogenic potential of copper sulfate itself, but the data suggest it may inhibit the development of DMBA-induced ovarian tumours	Burki & Okita (1969)

Table 11 (contd).

Rabbits (strain and numbers unspecified)	10 ml of a 1% solution of copper sulfate (equivalent to about 10 mg Cu/kg body weight) given to rabbits daily or on alternate days "for up to 400 days and over". Rabbits evidently killed at various time intervals, some as early as 33 days. Liver examined macroscopically and histologically	Effects on the liver included degeneration and vacuolation of the hepatocytes, granule formation in the cytoplasm, morphological changes in the nuclei, and atrophy and compensatory hypertrophy "in the late stage". Marked infiltration of round cells (mainly lymphocytes) into "interhepatic tissues" was seen after 200 days (and to a lesser extent after shorter periods of administration). Proliferation of the interstitial connective tissues was also evident after 200 days, and became much more marked after 300 days, "with a resulting picture of liver cirrhosis". Haemorrhage and necrosis of the liver occurred in some animals A dysfunction in sugar metabolism was evident after 30–60 days of copper administration, with temporary recovery after 90 days but further impairment after 120–150 days. There were no effects on serum bilirubin or total serum proteins.	Only one dose tested (effects at 10 mg Cu/kg body weight per day)	Tachibana (1952)

Table 11 (contd).

Species	Protocol	Results	Effect level	Reference
Copper gluconate Oral Rats (Sprague-Dawley, groups of 25 males and 25 females)	Rats fed diets containing 0 or 1600 mg Cu/kg diet as copper gluconate for 40–44 weeks (equivalent to about 0 or 80 mg Cu/kg body weight per day in males, and 0 or 120 mg Cu/kg body weight per day in females). (reduced amounts fed for the first month of the experiment). Microscopic examination of a limited number of organs. Study inadequately described	Mortality was increased, and food intake and body weight gain were retarded by 1600 mg Cu/kg diet in both sexes. Stomachs enlarged in both sexes, while hypertrophy of the uteri, ovaries, or seminal vesicles was observed. Other findings were "bronzed" kidneys, "bronzed" or yellowish livers, hypertrophied ridges between cardiac and peptic portions of stomach, and blood in the intestinal tract. Microscopic effects (not further described) were seen in the kidneys of copper-exposed rats (presumably in both sexes), and effects on the liver were seen on both males and females. Levels of copper in liver were nearly twice as high as in rats receiving an equivalent dose of copper as copper(II) sulfate, corresponding to their relative toxicities	Only one dose tested (effects at 80 mg Cu/kg body weight per day in males, and 120 mg Cu/kg body weight per day in females)	Harrisson et al. (1954)

Table 11 (contd).

Dogs (Beagle, groups of 6–8 males and 6–8 females)	Dogs fed diet containing 0, 0.012%, 0.06% and 0.24% copper gluconate for 6–12 months (equivalent to 0, 0.42, 2.1 and 8.4 mg Cu/kg per day). Detailed study of haematological biochemical and urinalysis parameters, and tissue copper concentrations in kidney, liver and spleen. Detailed necropsy, histopathology and organ weight information provided	No effect on mortality or body weight gain. Physical examinations, haematology, urinalysis and most blood biochemical analysis revealed no effect of the compound except in two of the 12 dogs on the highest dose which showed elevated levels of serum GPT; this was reversible. No compound related gross on microscopic pathologic lesions or changes in organ weight were seen. At 6 and 12 months, there was a gross-dependent increase in copper level in kidney, liver and spleen. Liver biopsy from 4 animals at 0, 4 and 12 weeks after withdrawal of 12 months dosing (0.24% copper gluconate) showed some reversibility of liver copper level	Elevated SGPT in 2 of 12 dogs on 8.4 mg Cu/kg body weight per day evaluated by the Task Group as not toxicologically significant	Shanaman (1972)
Copper(II) acetate Oral Rats (various strains, groups of 5 males and 5 females)	Rats fed diets containing 0 or 0.5% copper acetate (approximately 87 mg Cu/kg body weight per day) throughout their lifetimes. Second set treated in the same way, except 0.09% *p*-dimethylaminobenzene (DMAB), a known liver carcinogen, included in the diet for the entire period. Liver, spleen and grossly abnormal tissues were examined microscopically	Rats in all groups were reported to consume the same amounts of food. In one experiment, of animals treated with DMAB alone, 17/20 developed tumours, compared with 4/16 in those exposed to both DMAB and copper acetate. Comparable incidences for a subsequent experiment were 7/8 and 0/8, respectively	Study inadequate for assessing the carcinogenic potential of copper acetate itself, but the data suggest it has an inhibitory effect on DMAB-induced tumours	Howell (1958)

Table 11 (contd).

Species	Protocol	Results	Effect level	Reference
Rats (Holtzman, groups of 10 males)	Control group fed meal containing 18 mg Cu/kg diet, treated group fed meal supplemented with 2600 mg Cu/kg diet copper acetate (approximately 45 mg Cu/kg body weight per day) for 21 weeks. Limited number of organs weighed. Long bones radiographed and measured	Growth was reduced by 23% in the treated rats. Weights of the heart, spleen, lung and kidney were unchanged, while testis weights were increased. Effects on liver weight are unclear from the information provided Examination of the bones revealed no qualitative (osteoporosis, osteomalacia, modelling defects) or quantitative effects, although femur length was decreased relative to controls ($P < 0.05$)	Only one dose tested (non-neoplastic effects at 45 mg Cu/kg body weight per day)	Llewellyn et al. (1985)
Intraperitoneal injection Mice (Strain A/strong, groups of 10 males and 10 females)	Injection of copper acetate 3 times per week for 8 weeks at total doses of 36, 90 or 180 mg/kg body weight (roughly 12, 31 or 63 mg Cu/kg body weight). Control mice received vehicle alone (0.85% NaCl). Mice sacrificed 22 weeks after the last injection. Microscopic examination limited to the lungs and any tissues that appeared abnormal on gross examination of a small number of organs	Only 5/20 mice survived at the top dose. The numbers of mice with lung tumours were 4/15 (27%), 9/18 (50%) and 3/5 (60%) for the 36, 90 and 180 mg/kg body weight groups respectively, compared with 7/19 (37%) in the control group. The average number of lung tumours per mouse (0.40, 0.56 and 2.00 tumours per mouse in the low-dose, mid-dose and high-dose groups, respectively) increased dose-dependently but was not statistically significantly different from the control incidence (0.42) at any dose level. No other tumours were identified in a limited range of tissues	Inadequate group size to determine whether copper acetate increases the spontaneous lung tumour incidence in this susceptible strain of mice	Stoner et al. (1976)

Table 11 (contd).

Copper(II) 8-hydroxy-quinoline Oral Mice (B6C3F1 and B6AKF1, groups of 18 males and 18 females per strain)	Mice given 0 or 1000 mg copper 8-hydroxyquinoline/kg body weight (roughly 0 or 180 mg Cu/kg body weight) by gavage (in 0.5% gelatine) on days 7–28 of age, and then fed diets containing 2800 mg compound/kg diet (providing about 60 mg Cu/kg body weight per day) for remainder of the 18-month study. Extent of microscopic examination unclear, but certainly very limited	Study results inadequately reported. Survival was apparently unaffected by the treatment No statistically significant increases in tumour incidences were observed in either strain of mice compared with controls	The group sizes were too small and an inadequate number of doses were tested to assess the-carcinogenic potential of copper 8-hydroxyquinoline	Bionetics Research Labs. (1968)
Unspecified copper salts Oral Rats (Sprague-Dawley, groups of 10 males)	Rats maintained on diets containing 0.6, 25 or 100 mg Cu/kg diet copper (equivalent to 0.03, 1.25 or 5 mg Cu/kg body weight per day) for 25 weeks and then killed. A second series also received 16 weekly doses of a carcinogen (1,2-dimethylhydrazine, DMH, 20 mg/kg body weight)	No colonic tumours occurred in rats treated only with copper, while all DMH-treated rats had tumours. There was a significant increase ($P < 0.001$) in colonic tumours (3.14 ± 0.39 tumours/cm colon) in rats fed the copper-deficient diet (0.6 mg Cu/kg diet) and treated with DMH, compared with rats fed diets containing normal or high copper levels and treated with DMH (0.74 ± 0.07 and 0.76 ± 0.08 tumours per cm colon, respectively). A greater proportion of these tumours were malignant ($P < 0.01$) in the copper-deficient group (92% compared with 70 and 76% in the normal and high copper groups)	Carcinogenic potential of copper cannot be assessed from this study	Greene et al. (1987)

Table 12. Reproductive and developmental toxicity of copper

Species	Protocol	Results	Effect level	Reference
Copper(II) sulfate Oral Rats (F344/N, groups of 10 males and 10 females)	Copper sulfate pentahydrate given in the diet for 92 days at concentrations of 0, 500, 2000 or 4000 mg/kg. Estimated intakes 0, 8, 34 or 67 mg Cu/kg body weight per day. Sperm morphology and vaginal cytology evaluated	No effects were seen on testis, epididymis or cauda epididymis weight, spermatid counts or sperm motility in males of either species, at any tested dose. The length of the oestrous cycle in females was unaffected. A slight dose-related decrease was seen in the percentage of the oestrous cycle spent in oestrus but this effect did not achieve statistical significance ($P > 0.05$)	No effects observed at 67 mg Cu/kg body weight per day	Hébert et al. (1993)
Mice (C57BL and DBA, groups of 7–22 females, unspecified number of males)	Males and females given 0, 0.5, 1, 1.5, 2, 3 or 4 g copper sulfate/kg feed (approximately 0, 27, 53, 80, 106, 159 or 213 mg Cu/kg body weight per day) for 1 month prior to mating. Treatment presumably continued in females until sacrifice on day 19 of pregnancy	Developmental malformations (including hydrocephalus, encephalocoeles, and abnormalities of the ribs and vertebrae) occurred in groups of both strains given ≥ 3 g/kg feed. C57BL stock had abnormalities in 1/55 and 3/35 live fetuses and DBA stock in 2/56 and 4/45, in the 3 and 4 g/kg feed groups respectively. No abnormalities were found in controls (65 live C57BL fetuses, 76 live DBA fetuses). Mean values for litter size, live fetuses and mean fetal weight were reduced in groups of both strains given ≥ 1.5 g/kg feed. Statistical significance not reported, but reductions appear to have been dose-related in some cases	NOEL: 53 mg Cu/kg body weight per day LOEL: 80 mg Cu/kg body weight per day	Lecyk (1980)

Table 12 (contd).

Mice (C3H/HeN and C3H/HeJ, females, numbers unspecified)	Mice given 0 or 6 mg Cu/kg per litre as copper sulfate in drinking-water from day 13 of pregnancy to delivery (approximately 1.6 mg Cu/kg body weight per day). Half of the copper-treated animals then received 5 mg Cu/kg per litre as copper sulfate in the drinking-water during lactation (approximately 1.3 mg Cu/kg body weight per day) while the remainder received tap water alone. Neonates sacrificed and examined at 13 days of age	No data were presented on litter size or the incidence of abnormalities. Copper administration during pregnancy alone did not affect body weight or organ weights (cerebrum, liver and kidney) of the offspring within 24 h after birth, but continued copper administration during lactation resulted in significant reductions in neonatal body weight at 7–13 days of age ($P < 0.05$) and in the weight and protein content of the cerebrum, liver and kidney of neonates at 13 days of age ($P < 0.05$). The offspring of the copper-treated animals showed various changes in enzyme activity in these organs	One dose group only (effects observed at 1.3–1.6 mg Cu/kg body weight per day)	Kasama & Tanaka (1988)
Mice (B6C3F1, groups of 10 males and 10 females)	Copper sulfate pentahydrate given in the diet for 92 days at concentrations of 0, 1000, 4000 or 8000 mg/kg diet. Estimated intakes 0, 44, 187 or 398 mg Cu/kg body weight per day in males and 0, 52, 267 or 537 mg Cu/kg body weight per day in females. Sperm morphology and vaginal cytology evaluated	No effects were seen on testis, epididymis or cauda epididymis weight, spermatid counts or sperm motility in males at any tested dose. The length of the oestrous cycle in females was unaffected	No effects observed at 398 mg Cu/kg body weight per day in males, 537 mg Cu/kg body weight per day in females	Hébert (1993); Hébert et al. (1993)

Table 12 (contd).

Species	Protocol	Results	Effect level	Reference
Mink (standard dark, groups of 4 males and 12 females)	Males and females given 0, 25, 50, 100, 200 mg Cu/kg diet as copper sulfate pentahydrate (approximately 3, 6, 12 or 24 mg Cu/kg body weight per day), for 9 months before mating and for 3 months after mating	There were no overt toxic effects in the copper-treated adults. No information was provided on developmental malformations. Kit weight at 4 weeks (but not at birth) was significantly reduced in the 100 mg/kg group ($P < 0.05$). No such effect was evident at 200 mg/kg. Kit mortality (birth to 4 weeks) in the 100 and 200 mg/kg groups appeared to be increased (38% and 32% compared to 12% in controls (statistical significance not reported), and in all treated groups litter mass (at weaning) was reduced (statistical significance not reported), with some evidence of a dose-related effect. An adverse effect of copper on lactation was suggested	NOEL: 6 mg Cu/kg body weight per day LOEL: 12 mg Cu/kg body weight per day	Aulerich et al. (1982)
Copper(II) acetate Oral Rats (Holtzman, groups of 10 males)	Rats given 0 or 2600 mg/kg copper acetate in the diet (approximately 45 mg Cu/kg body weight per day) for 21 weeks followed by sacrifice. The control diet contained 18 mg/kg copper (roughly 1 mg Cu/kg body weight per day). Testis weights examined at termination	An increase in relative testis weight was seen in treated rats. No data were presented to support this statement	One dose group only (effect observed at 45 mg Cu/kg body weight per day)	Llewellyn et al. (1985)

Table 12 (contd).

Rats (Wistar albino, groups of 14 treated and 6 or 7 control females for each of the three times of sacrifice)	An increasing concentration (up to 0.185%) of copper acetate administered in the drinking-water for 7 weeks immediately prior to mating (up to approximately 65 mg Cu/kg body weight per day). Groups sacrificed at 11.5 or 21.5 days of pregnancy, or after delivery. (It is not clear whether copper acetate exposure continued during pregnancy)	There were no overt signs of toxicity in the treated females. In the groups that continued to normal delivery or were sacrificed at 21.5 days of pregnancy, the number of offspring per litter and the mean fetal weight were similar to the values in the control groups. External examination and serial sectioning revealed no malformations. Examination of the 11.5 day old embryos revealed significant reductions ($P < 0.005$) in mean yolk sac diameter, crown to rump length and mean somite number. In the 21.5 day old fetuses there was a significant reduction in ossification in 6 of the 7 ossification centres examined, while in newborn rats only 3 centres (cervical vertebrae, caudal vertebrae and hindlimb phalanges) showed a similar reduction ($P < 0.025$)	Only one dose group (effects observed at 65 mg Cu/kg body weight per day)	Haddad et al. (1991)
Copper(II) gluconate Oral Rats (Sprague-Dawley, groups of 25 males and 25 females)	0, 1600 mg Cu/kg as copper gluconate in the diet (approximately 0 or 82 mg Cu/kg body weight per day in males and 0 or 120 mg Cu/kg body weight per day in females) for 40–44 weeks. (Reduced amount fed for the first month of the experiment.) Microscopic examination of a limited number of organs. Study inadequately described	The authors reported hypertrophy of the uteri, ovaries and seminal vesicles. However, in the tabled data, it appears that the weight of the uterus and ovaries is reduced in females, and that the weight of the testes is reduced, while that of the seminal vesicles is unaffected in males. The histopathology of these tissues was evidently unremarkable. Levels of copper in liver were nearly twice as high as in rats receiving an equivalent dose of copper as copper(II) sulfate	One dose group only (effects observed at 82 mg Cu/kg body weight per day in males, 120 mg Cu/kg body weight per day in females)	Harrisson et al. (1954)

Table 12 (contd).

Species	Protocol	Results	Effect level	Reference
Copper(II) chloride Inhalation Rats (white, groups of 11 or 12 exposed and 12 control males)	Exposure to aerosols containing 5.2 or 41.4 mg copper chloride/m^3 (approximately 2.5 or 19.6 mg Cu/m^3) for 4 months. Functional state and morphology of gonads assessed after 2.5 and 4 months of exposure	The rats exposed at 19.6 mg Cu/m^3 showed overt signs of toxicity (not further described). Both concentrations significantly increased the incidence of dead and abnormal sperm ($P < 0.05$) in comparison with untreated controls. Sperm motility, testis weight and testosterone and oestradiol levels were all reduced in a dose-related manner, although statistical significance ($P < 0.05$) was reached only at the higher concentration. Significant reductions in the levels of luteinizing hormone, follicle-stimulating hormone and prolactin were evident at the lower concentration ($P < 0.05$), but no dose–response relationship was apparent	LOEL: 2.5 mg Cu/m^3	Gabuchyan (1987)

as copper(II) sulfate, acetate, or gluconate, although the results are inconsistent between studies and the reporting of some studies is deficient. In mice, there were no effects on male or female reproductive organs at 398–537 mg Cu/kg body weight per day as copper(II) sulfate in the diet. In a single study of rats inhaling copper(II) chloride aerosol, there were effects on sperm, testis weight and circulating levels of reproductive hormones.

In a limited number of studies, oral exposure of rodents to copper compounds during gestation induced embryo/fetotoxic effects and (at higher doses) developmental effects. Exposure to copper(II) sulfate induced effects on neonatal body weight, and on organ weights and biochemistry in mice at 1.3–1.6 mg Cu/kg body weight per day, while higher doses were embryolethal to mice (at 80 mg Cu/kg body weight per day) and to mink (at 12 mg/kg body weight per day). Developmental effects, including delayed ossification, were induced in rats exposed to 65 mg Cu/kg body weight per day as copper(II) acetate, and terata were induced in mice at 159 mg Cu/kg body weight per day as copper(II) sulfate.

7.6 Mutagenicity and related end-points

7.6.1 Copper sulfate

7.6.1.1 In vitro

The genotoxicity of most copper compounds has not been extensively studied.

Copper (II) sulfate, when studied in strains T98, T100 and TA102 of *Salmonella typhimurium* with and without metabolic activity, even at cytotoxic concentrations or the limit of solubility, did not exhibit mutagenic activity (Moriya et al., 1983; Marzin & Phi, 1985). A similar lack of activity was reported, at up to cytotoxic concentrations, in the absence of a metabolic activation system in the SOS Chromotest with *Escherichia coli* PQ37 (Olivier & Marzin, 1987), in a test for reversion to streptomycin independence in *E. coli* Sd4-73 (Iyer & Szybalski, 1958), in the rec-assay with *Bacillus subtilis* H17 and M45 (Matsui, 1980) and in tests for penicillin and/or streptomycin resistance in *Micrococcus aureus* FDA209 (Clark, 1953).

When rat hepatocytes were incubated for 20 h with 7.9, 15.7, 31.4 or 78.5 µmol/litre copper(II) sulfate solution (the highest concentration being moderately cytotoxic), there was a significant increase in unscheduled DNA synthesis at each concentration in a roughly dose-related manner. Copper was shown to have accumulated in the nucleus at these dose levels (Denizeau & Marion, 1989).

7.6.1.2 In vivo

A single intraperitoneal injection of copper(II) sulfate pentahydrate in mice induced a dose-related increase in the incidence of chromatid type chromosome aberrations in the bone marrow 6 h after dosing between 0.28 and 1.7 mg Cu/kg body weight (Agarwal et al., 1990). Only at the highest dose tested (1.7 mg Cu/kg body weight) were chromosomal breaks enhanced significantly. In the micronucleus test no evidence of genotoxic activity was found in mice given a single injection of copper(II) sulfate pentahydrate at 1.7, 3.4 and 5.1 mg Cu/kg body weight (Tinwell & Ashby, 1990). Bhunya & Pati (1987) reported a significant dose-related increase in the incidence of micronuclei after two injections at doses between 1.3 and 5 mg Cu/kg body weight per injection; however, this study did not utilize a positive control and is thus difficult to interpret.

7.6.2 Other copper compounds

7.6.2.1 In vitro

Copper(II) chloride also showed no evidence of mutagenic activity in *Salmonella typhimurium* strains TA98, TA102, TA1535 and TA1537 in the presence or absence of a metabolic activation system when studied at concentrations up to those causing cytotoxicity (Wong, 1988). It was similarly inactive in the rec-assay with *Bacillus subtilis* H17 and M45, as was copper(I) chloride (Nishioka, 1975; Kanematsu et al., 1980).

Copper(II) 8-hydroxyquinoline showed evidence of weak mutagenic activity in one strain (TA100) of *S. typhimurium* in the presence, but not in the absence, of a metabolic activation system. No activity was evident in four other *Salmonella* strains, nor in *Escherichia coli* WP2 her, in either the presence or the absence of a metabolizing system (Moriya et al., 1983). An earlier study reported

negative results in strains TA98, TA100, TA1535 and TA1537, with or without metabolic activation, but the maximum concentration tested was very low (Räsänen et al., 1977).

In Chinese hamster V79 cells, copper(II) nitrate produced dose-related increases in the mutation frequency (resistance to 8-azaguanine) at 0.01 and 0.1 mmol/litre and in the frequency of sister chromatid exchanges at 0.01–0.5 mmol/litre (Sideris et al., 1988). The investigators reported an increase in the molecular weight of DNA isolated from the cells, which was attributed to binding of the copper ions to the DNA.

7.7 Other studies

7.7.1 Neurotoxicity

There are few studies of the neurological effects of copper compounds. In rats, oral exposure to copper(II) sulfate in two studies did not affect the results of behavioural tests, but did alter brain neurochemistry. Injection of copper(II) chloride altered levels of neurotransmitters in the brain of rats.

7.7.1.1 Copper sulfate

Dietary administration of 250 mg/kg Cu (as copper(II) sulfate pentahydrate) to groups of six male rats for 30 days, providing 5 mg Cu/rat per day (equivalent to about 20 mg Cu/kg body weight per day) did not affect their locomotor activity, learning ability or relearning capacity and memory (Murthy et al., 1981). Analysis of biogenic amines in the brain revealed a significant increase in dopamine and norepinephrine (noradrenaline) levels ($P < 0.02$).

In another study using rats loaded with copper through administration of 0.125% copper(II) sulfate in the drinking-water for 11 months (equivalent to about 46 mg Cu/kg body weight per day), there were no overt effects on the behaviour of the eight treated females (de Vries et al., 1986). Neurological effects in the brain included a disturbance in striatal dopamine metabolism (reduced levels of the dopamine metabolite, 3,4-dihydroxyphenylacetic acid), a three-fold increase in the affinity of D_2-dopamine receptors and a 50%

reduction in the number of these receptors. Brain levels of dopamine and noradrenaline, and that of the noradrenaline metabolite, 3,4-dihydroxyphenylethylene glycol, were unaffected in copper-loaded animals (de Vries et al., 1986).

7.7.1.2 Copper chloride

Daily intraperitoneal injections of copper(II) chloride to 12 male rats at a dose of 2 mg Cu/kg body weight per day for 21 days resulted in significant increases in dopamine and norepinephrine (noradrenaline) levels in the brain ($P < 0.05$), while the level of 5-hydroxytryptamine in the brain was similar to that in saline-treated controls (Malhotra et al., 1982).

7.7.2 Immunotoxicity

Only copper(II) sulfate has been tested for its immunomodulatory effect. In studies summarized in this section, oral exposure of mice to this compound affected measures of both humoral and cell-mediated immune function, while inhalation adversely affected host resistance and pulmonary macrophage activity.

7.7.2.1 Copper(II) sulfate

The administration of copper(II) sulfate in the drinking-water of mice at 50, 100 and 200 mg Cu/litre for up to 10 weeks resulted in the dose-related inhibition of a number of immune system parameters in two studies. (These levels would normally be equivalent to 10, 20 or 40 mg Cu/kg body weight per day, but water consumption decreased with increasing copper concentrations. It was reported that total copper intake increased with increasing level, though no further detail was provided.) At 50 mg Cu/litre, the lymphoproliferative response to lipopolysaccharide from *E. coli* was depressed, while the production of autoantibodies against bromelain-treated mouse red blood cells was increased (Pocino et al., 1991). These parameters were also affected at 100 and 200 mg Cu/litre, along with decreased lymphoproliferative response to concanavalin A, and decreased antibody response and delayed-type hypersensitivity response to sheep erythrocytes (Pocino et al., 1990, 1991). A NOEL could not be established in these two studies.

In an inhalation study in mice, single or repeated 3 h exposures to copper(II) sulfate aerosol resulted in significant immunosuppressive effects, including reduced bactericidal activity of the alveolar macrophages to *Klebsiella pneumoniae* and reduced resistance to infection by *Streptococcus zooepidemicus*. These effects were evident after a single exposure at 0.28 mg Cu/m^3 and above and after 5 or 10 daily exposures at 0.06–0.07 mg Cu/m^3. A NOEL was not established in these studies (Drummond et al., 1986).

In hamsters, a single 4 h exposure to copper(II) sulfate pentahydrate aerosol at 0.3–7.1 mg Cu/m^3 resulted in reduced pulmonary macrophage activity and volume from 3.2 mg Cu/m^3 within 1 h after exposure; no effect was observed at 0.3 mg Cu/m^3 (Skornik & Brain, 1983).

7.8 Biochemical mechanisms of toxicity

The mechanism(s) by which copper may lead to cell injury are discussed in section 6.

8. EFFECTS ON HUMANS

8.1 General population: copper deficiency and toxicity

Copper is an essential element. Most tissues therefore have measurable amounts of copper associated with them and, in general, cells, tissues and organisms have mechanisms to maintain its availability while limiting its toxicity (homoeostasis).

In most situations, if we explore the indices of function affected by copper excess or deficit we will find altered indicators prior to the onset of clinical signs or symptoms. In some situations we can use the functional indicators instead of clinical signs, since they are closely associated. The least significant manifestations in terms of human health are the physiological changes that occur in response to high or low copper intakes. Most of the changes observed in these situations represent adaptive or homoeostatic mechanisms to prevent deficit in response to low intake or prevent toxicity in response to high intake.

8.2 Copper deficiency

Characteristic clinical features of copper deficiencies in infants are anaemia refractory to iron, and low copper plasma levels (Sturgeon & Brubaker, 1956). Copper deficiency has been considered the likely cause of the anaemia, but it was not until the completion of a series of controlled case studies of copper deficit in infants recovering from malnutrition (Cordano et al., 1964) that the full spectrum of copper deficiency was demonstrated. Subsequent reports during the 1970s of acquired copper deficiency in low-birth-weight neonates and in infants and children receiving copper-free total parenteral nutrition, clarified the indispensable nature of copper as an essential nutrient for humans (Widdowson et al., 1974; Shaw, 1992).

8.2.1 Clinical manifestations of copper deficiency

Clinically evident copper deficiency occurs relatively infrequently in humans. The most consistent clinical manifestations of copper deficiency are anaemia, neutropenia and bone abnormalities including fractures. The haematological changes are characterized by the existence of a hypochromic, normocytic or macrocytic anaemia,

accompanied by a reduced reticulocyte count, hypoferraemia, neutropenia and thrombocytopenia. In a small proportion of cases there is microcytic anaemia (Williams, 1983). Bone marrow cytological examination reveals megaloblastic changes and vacuolization of the erythroid and myeloid progenitors. There is also an arrest of the maturation of myeloid precursors and the appearance of ringed fibroblasts. These alterations are unresponsive to iron therapy but are readily corrected by copper supplementation (Schubert & Lahey, 1959; Prohaska et al., 1985). The current prevailing view is that anaemia in copper deficiency is due to defective iron mobilization resulting from reduced ceruloplasmin (ferroxidase I) activity.

A summary of some reports of clinical manifestations of copper deficiency in humans is given in Table 13. As seen clearly from the table, many of the reports of deficiency originate in infants and young children, particularly those with low birth weight or malnourished after birth. Healthy infants receiving less than 0.1 mg Cu/kg body weight per day are at risk of deficit. For those with low birth weight or affected by protein energy malnutrition the figure is close to 0.2 mg/kg per day. These latter conditions affect a sizeable proportion of children at a global level. It has been estimated that about 16% of live births or some 20 million infants per year are of low birth weight (< 2500 g) (WHO, 1990). The presence of bone abnormalities is very common in copper deficiency in low-birth-weight infants and in young children (Heller et al., 1978; Danks, 1988; Shaw, 1992). These abnormalities, which mimic the changes observed in scurvy, include osteoporosis, fractures of the long bones and ribs, epiphyseal separation, fraying and cupping of the metaphyses with spur formation, and subperiosteal new bone formation (Danks, 1988; Shaw, 1992). Less frequent manifestations of copper deficiency are hypopigmentation of the hair and hypotonia (Danks, 1988; Shaw, 1992), impaired growth (Castillo-Duran & Uauy, 1988), increased incidence of infections (Castillo-Duran et al., 1983), and alterations of phagocytic capacity of the neutrophils (Heresi et al., 1985). In addition, abnormalities of cholesterol and glucose metabolism have been reported, but are not so well established (Klevay et al., 1984, 1986; Reiser et al., 1987). Prevalence of cardiovascular disease has been linked to high zinc and low copper in the diet but this hypothesis has not been validated (Lukaski et al., 1988).

Table 13. Clinical copper deficiency

Subjects	Study and results	Reference
11 copper-deficient infants (plasma copper < 70 µg/litre and ceruloplasmin < 200 mg/litre) and 10 control infants	In a prospective case control, growth was evaluated 1 month before and 1 month after copper supplementation with 80 mg/kg body weight. Weight/age and weight/length indices increased significantly after supplementation in the copper-deficient group. Daily energy intake was significantly higher in the copper-deficient group after supplementation than it was in the control group. Daily weight gain after supplementation increased significantly in the copper-deficient group and the value for daily weight gain after supplementation was significantly higher than that of the control group for the equivalent amount of time	Castillo-Duran et al. (1988)
24 males aged 21–57 years	The subjects received diets low in copper (1.03 mg/day per 2850 kcal [12 MJ]) and containing either 20% of the calories as fructose or cornstarch. During the course of feeding the diets for 11 weeks, four of the subjects exhibited heart-related abnormalities and were removed from the study (1 myocardial infarction, 2 severe tachycardia and 1 a type II second-degree heart block). There were no changes in serum copper and ceruloplasmin. However, fructose ingestion significantly reduced erythrocytic SOD. Repletion of the subjects with 3 mg Cu/day for 3 weeks significantly increased SOD levels in subjects previously fed fructose but not starch. These results suggest that the type of dietary carbohydrate fed can differentially affect indices of copper status in humans. Copper deficiency could play a role in human heart disease	Reiser et al. (1985)
24 males aged 21–57 years	The subjects were fed an experimental diet inadequate in copper (0.36 mg/day per 1000 kcal [4.18 MJ]) for 11 weeks showed significant increase in LDL cholesterol and significant decrease in HDL cholesterol when compared to either their pretest self-selected diets (0.57 mg Cu/day per 1000 kcal) or a repletion diet (1.41 mg Cu/day per 1000 kcal [4.18 MJ])	Reiser et al. (1987)

Table 13 (contd).

8 men aged 18–36 years	The subjects were fed diets low in copper (0.89 ± 0.10 mg/day), for periods ranging from 105 to 120 days. One man who was in a negative balance showed a significantly reduction in plasma copper, immunoreactive ceruloplasmin and erythrocyte SOD. Serum cholesterol was significantly elevated by the end of the 15 week depletion. Another two men presented a slightly negative balance and a trend to lower plasma copper and SOD. Two of four subjects tested had impaired glucose clearance during depletion. Conclusion: intakes of below 0.9 mg/day apparently result in signs of copper depletion in healthy adults	Milne et al. (1990)
11 men aged 21-32 years	The effects of low-copper diets on indexes of immune response were examined in 11 subjects during a 90 day metabolic study. Daily copper intake for the first 24 days, the next 42 days and the last 24 days of the study was 0.66, 0.38 and 2.49 mg, respectively. Feeding the diet with 0.38 mg/day was associated with a significant decrease in the proliferation of peripheral blood mononuclear cells cultured with phytohemagglutinin, concavalin A, or pokeweed, and an increase in the percentage of circulating B cells (CD 19+)	Kelley et al. (1995)
3 month old infant	An infant with a birth weight of 1140 g fed an infant formula low in copper developed low plasma copper and ceruloplasmin, anaemia, neutropenia, apnea, metaphyseal flaring and cupping. These changes were reversed after copper supplementation	Al-Rashid & Spangler (1971)
6 month old infant	An infant with a birthweight of 1140 g fed exclusively with cow's milk presented hypocupraemia, low ceruloplasmin, sideroblastic anaemia, neutropenia, osteoporosis with blurring and cupping of the metaphyses, depigmentation of skin, enlarged and distended blood vessels of the scalp, and hypotonia. Treatment with 3 mg Cu/day reversed these abnormalities	Ashkenazi et al. (1973)

Table 13 (contd).

Subjects	Study and results	Reference
7 month old infant	An infant receiving total parenteral nutrition (TPN) from birth to 7 months showed osteoporosis and soft tissue calcifications. Plasma copper and ceruloplasmin levels were markedly reduced. The infant died and postmortem examination showed a reduced liver copper content. A 10 month preterm infant required TPN during the first 4 months of life because of bowel resection at age 10 days presented hypocupraemia, anaemia, neutropenia, osteoporosis, irregularity of the metaphyses and subperiosteal new bone formation. These changes were reversed by the feeding of a formula containing 1 mg Cu/litre	Heller et al. (1978)
7 month old infant	A preterm infant (birth weight 2050 g) fed only powdered milk who presented a persistent diarrhoea, developed hypocupraemia, neutropenia, and severe anaemia. Bone radiography showed generalized osteoporosis, flaring and cupping of the metaphyses of the long bones and a fracture of the right fibula. All these abnormalities were alleviated after treatment with copper sulfate	Tanaka et al. (1980)
Two 6 month old infants	One infant fed only cow's milk since birth presented decreased serum copper and ceruloplasmin, microcytic anaemia and neutropenia. Another infant fed a diet predominantly mainly of cow's milk, presented reduced concentration of serum copper and ceruloplasmin, and microcytic anaemia. A radiological study showed increased density of the preparatory calcification areas with spur formation at the proximal parts of the femurs. In both cases the abnormalities were recovered after the addition of chicken, meat and vegetables	Levy et al. (1985)
30 year old woman	Following extensive bowel resection, a woman received parenteral nutrition not supplemented with copper. The patient developed hypocupraemia, subnormal ceruloplasmin levels, anaemia and severe neutropenia. Following supplementation of the parenteral solution with 4 mg Cu/day an increase in reticulocyte count, haemoglobin and neutrophils was observed	Zidar et al. (1977)

It has been shown that copper deficiency is associated with increased incidence of infection and impaired weight gain in infants recovering from malnutrition (Castillo-Duran et al., 1983; Castillo-Duran & Uauy, 1988). The initial randomized controlled trial included 27 infants recovering from protein energy malnutrition: 13 received 80 µg/kg per day of copper supplement for 3 months while 14 matched infants received a placebo. Plasma copper and ceruloplasmin dropped in the placebo group, 30% of whom had low copper plasma levels, while values rose in the supplemented group during the rapid growth phase of recovery. The mean number of upper respiratory infections, febrile days, and number of febrile episodes per child per month were similar in both groups. However, seven infants presented clinical evidence of severe lower respiratory infection (mainly pneumonia) in the placebo group versus only one subject in the copper supplemented group ($P < 0.025$) (Castillo-Duran et al., 1983). In a separate case control study, 11 infants identified as copper-deficient, based on low plasma copper and low ceruloplasmin, and 10 matched copper-sufficient infants at a similar stage of their nutritional recovery, were supplemented with 80 µg Cu/kg, as copper sulfate, daily for 30 days. The daily weight gain and daily energy intake were significantly higher relative to controls in the copper-deficient group shortly after supplementation (Castillo-Duran & Uauy, 1988).

Copper deficiency is associated with altered immunity in humans (Prohaska & Failla, 1993). Heresi et al. (1985) studied 19 hypocupraemic infants before and after 1 month of copper supplementation. The phagocytic activity of polymorphonuclear leukocytes increased by 30% after copper supplementation while immunoglobulins remained unchanged. Kelley et al. (1995) described a decrease in the proliferation of peripheral blood mononuclear cells cultured with different mitogens in 11 men receiving a low-copper diet.

An increased concentration of total cholesterol and low density lipoprotein (LDL) cholesterol and a reduction of high density lipoprotein (HDL) cholesterol concentration have been observed in subjects fed an experimental diet low in copper (Klevay et al., 1984). Low copper intake has also been demonstrated to diminish glucose tolerance (Klevay et al., 1986), alter cardiac rhythm and electrocardiogram, and modify the hypertensive response to a hand-grip test (Lukaski et al., 1988). However, other studies have not validated the results of changes in cholesterol and glucose metabolism.

The role of copper deficit in altered neurodevelopment has been postulated on the basis of the high copper content of the brain, especially of the basal ganglia. The existence of a prenatal critical phase in central nervous system (CNS) development during which copper deficiency can cause CNS damage has been suggested (Danks, 1988). This could explain the severe mental deficiency associated to prenatal tissue deficit found in Menkes disease while postnatally acquired nutritional copper deficiency is not accompanied by neurological abnormalities.

8.2.2 Biological indicators of copper deficiency: balance studies

The determination of the levels of copper intake which will prevent deficiency without resulting in toxicity (homoeostasis) has been discussed fully in section 6.3. Several of the most promising biological indicators for copper deficiency as well as toxicity, for example, cytochrome c oxidase, levels of LDL, ceruloplasmin and serum copper are also discussed in section 6.3.

In view of the importance of this subject for the determination of human health risks (deficit and excess) from exposure to copper, it is repeated here for emphasis.

8.3 Toxicity of copper in humans

8.3.1 Single exposure

Acute toxicity due to ingestion of copper is infrequent in humans and is usually a consequence of the contamination of beverages (including drinking-water) or from accidental or deliberate ingestion of high quantities of copper salts.

Numerous case reports of single oral exposures to high levels of copper have been reported. Such exposures, including suicide attempts with copper sulfate, have occurred in youths and adults at doses ranging from 0.4 to 100 g Cu (Chuttani et al., 1965; Mittal, 1972; Stein et al., 1976; Walsh et al., 1977; Chugh et al., 1977; Williams, 1982; Jantsch et al., 1985). Symptoms including vomiting, lethargy, acute haemolytic anaemia, renal and liver damage, neurotoxicity, increased blood pressure and respiratory rates. In some cases, coma and death followed. There are also a number of reports of high dose copper

ingestion in beverages (35–200 mg/litre; Hopper & Adams 1958; Semple et al., 1960).

8.3.2 Repeated oral exposures

8.3.2.1 Gastrointestinal and hepatic effects

In case reports and cross-section studies, consumption of drinking-water contaminated with copper has been associated with nausea, abdominal pain, vomiting and diarrhoea (Table 14). In none of these studies have the doses of copper ingested been well characterized. In addition, microbiological quality of the water supplies or other contributing factors were not assessed. Also, symptoms may have been over-reported owing to lack of blinding of subjects.

An often cited report is that of Wyllie (1957) in which acute gastrointestinal symptoms were reported in 10 people consuming a cocktail contaminated with copper from the cocktail shaker. Owing to limitations in reporting and confounding, this study is considered inadequate to serve as a basis for characterization of concentrations of copper which results in adverse health effects.

In a family in Vermont, USA, living at the end of a copper main, there were recurrent episodes of gastrointestinal illness. There were no symptoms in two other families of similar age and sex distribution on the same street exposed to lower levels (Spitalny et al., 1984). Symptoms ceased with a change of water source.

Knobeloch et al. (1994) reported on five investigations of gastrointestinal upset associated with ingestion of copper-contaminated water. Data were obtained from questionnaires on age, weight, water use habits, duration of exposure and symptoms. There was generally a higher incidence of intermittent or constant symptoms of diarrhoea, abdominal cramps or nausea in those who consumed first-draw water, in infants and young children and among residents of newly constructed or renovated houses. In one study, gastrointestinal symptoms occurred in 8 of 14 people ingesting 0.6–3.8 mg Cu/day from drinking-fountains (1.6–7.7 mg Cu/litre) compared with 3/26 people ingesting ≤ 0.55 mg Cu/day from drinking-water.

Table 14. Gastrointestinal effects associated with copper in potable water or beverages

Observations	Comments	Reference
10 of 13 nurses experienced nausea, vomiting, diarrhoea, weakness, abdominal cramps and headache following ingestion of an alcohol lemon cocktail from cocktail shakers containing copper; reconstruction of the episode suggested that copper ingestion varied between 5.3 and 32 mg	owing to limitations in reporting and confounding (alcohol, fasted state); unknown whether 5.3 mg is a LOAEL or NOAEL; study considered inadequate to establish effect levels	Wyllie (1957)
In three of four family members residing in Vermont at the end of a copper main, there were recurrent episodes over 1.5 years of gastrointestinal illness 5–20 min after drinking tap water in the morning (median level of copper in incoming water, 3.1 mg/litre; single maximum level 7.8 mg/litre); no symptoms in two other families of similar age and sex distribution on the same street exposed to lower levels (medians, 1.58 and 0.02 mg/litre); copper levels in hair significantly higher in symptomatic family; symptoms ceased with change of water source	well-conducted study that provides useful information on levels of copper in water which induce acute effects	Spitalny et al. (1984)

Table 14 (contd).

Observations	Comments	Reference
Three children (1–2.5 years old) with prolonged diarrhoea and weight loss exposed to tap water containing 0.22–1 mg/litre. Symptoms disappeared when water replaced with that of lower copper content	limited usefulness for risk assessment	Stenhammar (1979)
Association between the copper content in drinking water (0.35–6.5 mg/litre in first-draw water) at 7 new Swedish kindergartens and diarrhoea in attending children < 3 years old. The symptoms disappeared when the children went home for a few days but reappeared when they returned to the kindergarten	viral or other microbiological causes of diarrhoea were not studied. Limited usefulness for risk assessment	Berg & Lundh (1981)
Five different case reports of gastrointestinal illness in individuals, families or residents completing questionnaires. Higher incidence of gastrointestinal effects with first-draw water compared with flushed water	data inadequate to establish effect levels	Knobeloch et al. (1994)

Micronodular cirrhosis and acute liver failure was described in a case report (O'Donohue et al., 1993). A 26-year-old male consumed copper tablets at 30 mg/day (tablet formulation unspecified) for 2 years, followed by 60 mg/day for an unspecified period, before presenting with symptoms of liver failure. The patient had Kayser–Fleisher rings; laboratory investigations revealed normal serum copper (22.6 mmol/litre) and serum ceruloplasmin (0.27 mmol/litre) but very high urinary excretion of copper (207 mmol/24 h) compared to the normal (< 1.2 μmol/24 h). An emergency liver transplant was performed and the patient made a good recovery. The mean copper content of the removed liver was 3230 μg/g (normal 20–50 μg/g). Histology resembled that of Indian childhood cirrhosis and Wilson disease (see section 8.4).

8.3.2.2 *Reproduction and development*

After adjusting for confounding variables, there was no association between the risk of spontaneous abortion in a population of Massachusetts women exposed to copper in drinking-water (> 1 mg/litre) during 1976–1978 (Aschengrau et al., 1989). In a small study of trace element status, there was a significant positive relationship between placental copper and birth weight, and a negative correlation between the copper/zinc ratio and birth weight (Mbofung & Subbarau, 1990). These data are inadequate to assess the reproductive/developmental effects of copper in humans.

8.3.2.3 *Cancer*

Epidemiological studies in which the association between copper intake and/or levels of copper in serum and cancer has been investigated are presented in Table 15.

In geographical/ecological studies in China (Chen et al., 1992) and the USA (Schrauzer et al., 1977), associations between serum copper or copper intake and some cancers were reported. However, owing to the lack of consideration of individual exposure and confounding factors in such studies, they contribute little to assessment of the weight of evidence for carcinogenicity.

Interpretation of the available analytical epidemiological (case-control or cohort) studies is complicated by the fact that increased

Table 15. Epidemiological studies on cancer in the general population

Study protocol	Results	Comments	Reference
A nested, matched case-control study was conducted to compare the serum copper levels of 133 cancer cases identified between 1974 and 1984 among 5000 members of a North West Washington State employee cohort, with 241 controls selected at random from the same initial cohort. Cases and controls were matched for age (in 5-year groupings), sex, race (white/nonwhite) and year and season of blood sampling. 48% of the study population was male and 97% was white. Blood had been collected in the initial study in 1972–1974 (before diagnosis)	The mean serum copper level in the control group was 115 ± 36 µg/dl, whereas the case group mean was 123 ± 37 µg/dl. The groups were split into quartiles with copper serum levels corresponding to 43–92, 93–107, 108–125 and 126 276 µg/dl. The relative risk estimates of cancer, all sites combined, by quartile levels of serum copper, increased steadily, with that in the upper quartile reaching statistical significance (RR=1.0, 1.1, 1.3 and 2.4 for the quartiles and 95% CI=0.6–2.2, 0.7–2.7 and 1.1–5.1 for the 2nd–4th quartiles, respectively)	When adjusted for other factors which might influence both the serum copper levels and the risk of all cancer sites combined (i.e. occupational status, family history of cancer, cigarette smoking, alcohol consumption and use of exogenous oestrogens), the relative risk estimates did not differ appreciably from the unadjusted risk estimates	Coates et al. (1989)

Table 15 (contd).

Study protocol	Results	Comments	Reference
A case–control study of 35 early-diagnosed oesophageal cancer patients who had not received treatment and were attending, for the first time, a cancer hospital in India. Dietary habits over the preceding 6 months and blood biochemical parameters were assessed and compared with 35 control subjects matched for age, sex, socioeconomic status, rural/urban residence, and chewing, smoking and drinking habits (minimal control for confounders)	There was no difference in the serum copper levels of the cases compared with the controls (1.29 ± 0.03 and 1.24 ± 0.04 mg/litre, respectively). When the cohorts were analysed according to blood copper levels corresponding to 0.75–0.99, 1.00–1.25 and > 1.25 mg/litre, more cases occurred in the highest group compared with the controls (20 and 13, respectively; $P < 0.025$). There was no difference between the daily copper intake values for cases and controls (3.6 ± 0.64 and 3.4 ± 0.43 mg)	Numbers in the individual tertiles were small; limited control for confounders; serum analyses for copper after diagnosis; though more cases in highest tertile based on serum copper, no difference between daily copper intake for cases and controls	Prasad et al. (1992)

Table 15 (contd).

A 6–9 year prospective follow-up study of an initial cohort of a Dutch population of 10 532, aged 5 years or more, was conducted to the end of December 1983. The serum copper concentrations (sampled on initial entry into the study) of 64 cancer death patients and 62 cardiovascular death patients were compared with those from randomly selected, sex- and age- (in 5 year intervals) matched members of the original cohort, still alive on 31 December 1983. Each case was matched with two controls. Cancer cases and their controls were matched for smoking status	The mean levels of serum copper were not significantly increased in the cancer death patients over those in the controls (1.33 mg/litre compared with 1.25 mg/litre; P=0.08). For subjects in the highest serum quintile (> 1.43 mg/litre), the relative risk, adjusted for various factors, of death from cancer, was 3.7 (95% CI=1.5–9.1) compared with the adjusted relative risk pooled from quintiles 2–4 (serum copper range 1.05–1.43 mg/litre). For the lowest serum quintile (< 1.05 mg/litre), the adjusted relative risk of death from cancer was 1 8 (95% CI=0.7–4.7)	Kok et al. (1988)

Table 15 (contd).

Study protocol	Results	Comments	Reference
A case–control study was conducted on 214 patients, first diagnosed for primary carcinoma of the breast and not previously undergoing therapy, randomly selected among consecutive admissions to a cancer institute in Milan, Italy, from May 1982 to June 1985. Controls (*N*=215) were patients with a variety of diagnoses other than breast cancer. Dietary copper intakes were estimated from dietary questionnaires. Blood samples were taken the day after admission and the serum copper levels determined	The mean dietary intakes of copper in the control and case cohorts were estimated to be 2.8 ± 1.1 and 2.7 ± 1.1 mg/day, respectively. The correlation between copper intake and copper blood level was examined and was found not to be significant. Both groups were split into quartiles of dietary copper intake for comparison. No significant trend in the OR estimates for breast cancer were found	Results essentially negative but serum copper concentrations determined after admission	Cavallo et al. (1991)
A second set of 47 cases and 46 age-matched controls from Montpellier, France, which represented a subsample of a larger study concerning diet and breast cancer, was investigated. Controls consisted of patients admitted, for the first time, to neurology or neurosurgery wards. Blood samples were taken the day after admission and the serum copper levels determined	Mean serum copper levels were significantly decreased in the cases when compared with the controls. The mean serum copper level was found to be significantly higher in the cases than the controls		

Table 15 (contd).

	When the results of the mean blood copper levels in the two areas were pooled, the difference between the cases and controls was found to be substantially less, but the mean level was still statistically higher in controls. When the groups were split into quartiles of serum copper level, the pooled ORs were not significantly different from each other nor was there any significant trend in values. Adjustment for dietary zinc, which competes in the absorption of copper, and other elements, in particular iron, vitamin C and raw fibre, did not allow the correlation between copper intake and blood level to reach significance		
Serum copper and zinc levels were measured in 20 healthy women and 100 women with gynaecological tumours. 70 patients had benign and 30 had malignant genital tumours	Mean serum copper levels increased from control to benign to malignant groups	Serum levels measured after diagnosis. No control for potential confounders	Çetinkaya et al. (1988)

Table 15 (contd).

Study protocol	Results	Comments	Reference
The plasma copper concentrations of a group of 46 women who developed breast cancer between 1968 and 1985 were compared with an age-stratified random sample of 138 women. Both groups were taken from an initial cohort of 5100 ostensibly healthy women studied between 1968 and 1975, aged 28-75 years and living on the island of Guernsey, United Kingdom. Plasma samples were collected at the start of the study and on development of breast cancer, and the levels of copper analysed	The breast cancer cases were diagnosed an average of 11 years (range 1-17 years) after entry into the study cohort. The mean initial copper levels were 1.26 mg/litre in the control group and 1.31 mg/litre in the cases (95% CI for overall difference=-0.07–0.17). The groups were split into quartiles corresponding to initial copper concentrations of ≤ 1.03, 1.04–1.19, 1.20–1.33 and > 1.34 mg/litre, and the adjusted odds ratio for the 1.04–1.19 mg/litre quartile set at 1.0. The adjusted odds ratios were: 1.8 (95% CI=0.6–5.4), 1.6 (95% CI=0.5–5.4) and 3.2 (95% CI=1.1–9.4) for the ≤ 1.03, 1.20–1.33 and > 1.34 mg/litre quartiles, respectively, with only the last group reaching statistical significance	Adjustments were made for possible confounding by known indicators of breast cancer, i.e. family history of breast cancer, age, age at first live birth, parity, weight and oral contraceptive use The authors suggest a U-shaped risk response although this is not supported by the reported results	Overvad et al. (1993)

Table 15 (contd).

Total serum copper and ceruloplasmin levels were determined in 13 pre- and 10 postmenopausal breast cancer patients aged 39 ± 7 and 66 ± 6 years, respectively. The levels were compared with those in a group of 14 pre- and 11 postmenopausal omnivorous women aged 33 ± 6 and 57 ± 5 years, respectively and with those in a group of 12 pre- and 11 postmenopausal vegetarian women aged 34 ± 7 and 59 ± 5 years, respectively who were all free of breast cancer. Fasting serum samples were collected on three consecutive days, typically four times in a year	The serum copper concentrations did not alter significantly with time during the study year. A significantly higher serum copper level was noted in the premenopausal breast cancer patients (mean = 18.7 ± 0.62 µmol/litre) when compared with the two premenopausal control groups (means = 16.5 ± 0.30 and 16.7 ± 0.43 µmol/litre, respectively; $P < 0.03$). No such difference was noted in the postmenopausal patients. Postmenopausal patients showed significantly lower ceruloplasmin levels (mean = 0.309 ± 0.011 g/litre) than the corresponding control groups (means = 0.387 ± 0.013 and 0.355 ± 0.11 g/litre, respectively; $P < 0.01$), this being more pronounced when the control groups were pooled ($P < 0.001$). Again, there was no overall significant change with time during the study year. The copper/ceruloplasmin ratios were higher in both groups of patients, these increases being significant in the premenopausal group when compared with the corresponding omnivorous controls ($P < 0.05$) and in the postmenopausal patients when compared with both the omnivorous ($P < 0.001$) and vegetarian ($P < 0.01$) control groups. The ratio in the postmenopausal patients (mean = 3.94 ± 0.096 µg/g) was significantly higher than in the premenopausal patients (mean = 3.44 ± 0.061 µg/g; $P < 0.001$)	The average estimated daily dietary copper intakes were apparently lower in the patients (1.46 mg/day) than in the normal control subjects (1.63 mg/day; difference $P = 0.05$) and this could not, therefore, directly explain the results No control for smoking The investigators concluded that the high serum copper/ceruloplasmin ratio in the breast cancer patients may reflect disordered copper metabolism in this disease (serum levels determined after diagnosis)	Dabek et al. (1992)

serum concentrations of copper could be related to alterations in copper handling resulting from the disease state. Available analytical epidemiological studies in which concentrations of copper in serum were determined only following diagnosis of cancer (Çetinkaya et al., 1988; Cavallo et al., 1991; Prasad et al., 1992; Dabek et al., 1992) are uninformative, therefore, with respect to the possible aetiological role of cancer in the disease. In prospective studies where concentrations of copper in serum have been determined prior to disease development, associations between serum copper levels generally greater than 1.25 mg/litre and either total or breast cancer have been observed, though there is no convincing evidence of a dose–response trend in this regard (Kok et al., 1988; Coates et al., 1989; Overvad et al., 1993). Moreover, there has been no association between intake of copper and cancer, in those few analytical epidemiological studies in which it has been investigated (Cavallo et al., 1991; Dabek et al., 1992; Prasad et al., 1992).

There is therefore little convincing evidence that copper plays an aetiological role in the development of cancer in humans.

8.3.3 Dermal exposure

Sources of topical exposure to copper have come from its use in pigments, ornaments, jewellery, dental amalgams, and IUDs, and as an antifungal agent and an algicide. Though copper algicides are used in the treatment of water in swimming pools and reservoirs, there are no reports of toxicity from these applications.

Copper or copper salts may induce allergic contact dermatitis in susceptible individuals. Signs and symptoms include itching, redness, swelling, vesicle formation and pustulation. Patch-testing to identify the sensitized state generally involved using covered 24–48 h contact with 0.5–5.0% copper sulfate in water or petrolatum. Numerous reports have been published on the allergic response to unintentional and defined dermal exposure to copper or preparations containing copper (Hackel et al., 1991; Nordlend & Linden, 1991; Klapheck et al., 1994; Krolczyk et al., 1995), however, the exposure concentrations leading to any effect are poorly characterized in most cases.

Routine patch testing of 1190 eczema patients found that only 13 (1.1%) cross-reacted to 2% copper sulfate in petrolatum. The investigators warned of the possibility that contamination of copper with nickel (a well-established contact allergen) might have been the cause of the apparent reaction to copper (Karlberg et al., 1983). In an investigation of copper and zinc status in 22 asthmatic, 21 eczematous and 19 healthy Italian children (age-matched), the asthmatic group had higher mean values for serum and hair copper concentrations, and the eczematous group had higher mean hair copper concentrations, than did healthy controls. Estimated dietary copper intakes were said to be similar for the three groups and ranged from 90 to 111% of the "safe and adequate" intakes (Di Toro et al., 1987).

8.4 Disorders of copper homoeostasis: populations at risk

Because copper is an essential metal, there are homoeostatic mechanisms to maintain copper levels within defined limits. However, there are a number of disorders in homoeostatic mechanisms which can result in deficiency or toxicity from exposure to copper at levels which are tolerated by the general population. In addition to this, gross overexposure to copper can overwhelm the homoeostasis mechanisms in the normal individual. The hereditary copper metabolic disorders are Menkes disease and Wilson disease.

8.4.1 Menkes disease

Menkes disease is an X-linked recessive disorder of copper metabolism that occurs in approximately 1 in 200 000 live births. Clinically the condition resembles a copper deficiency state and is characterized by skeletal abnormalities, severe mental retardation, neurological degeneration and death in early childhood. The symptoms of Menkes disease result from a deficiency of copper and its effects on the function of copper-dependent enzymes.

The gene for the condition has been isolated (Chelly et al., 1993; Mercer et al., 1993; Vulpe et al., 1993) and designated *MNK*. The gene codes for a 1500-amino-acid P-type cation transporting ATPase, with strong homology to the bacterial and yeast cation transporting ATPases. The *MNK* gene also has strong homology to the gene that is

defective in Wilson disease (see section 8.4.2) (Bull et al., 1993; Thomas et al., 1995).

Although the gene involved is widely expressed (except in liver), and copper actually accumulates in some cells (such as fibroblasts, kidney and placenta), the primary defect is a marked reduction in the first phase of copper transport. Most of the copper entering mucosal cells from the diet does not enter the portal circulation and travel to the liver and elsewhere. As a result, in most tissues, enzymes that depend upon copper for their functions will be inactive or have reduced activity. This may be the reason for the diverse clinical symptoms observed in Menkes patients. The MNK protein has structural similarities to Mg(II), Na(I), K(I), and Ca(II) transporters from various organisms. P-type ATPases have a conserved aspartate residue which is phosphorylated in the course of cation transport and have specific metal-binding sequences. The metal binding sequences are similar to those of P-type ATPases of bacteria, characterized by a G-M-T-C-XX-C motif. The Menkes disease and Wilson disease genes both encode proteins with six of these metal-binding sequences in the N-terminal half of the molecules, and multiple hydrophobic (probably membrane spanning) sequences nearer the C-terminal. They share a 59% amino-acid sequence identity with each other, and, respectively, share 43% and 33% identities with the bacterial transporter CopA (Solioz et al., 1994). In Menkes disease the liver is not overtly affected, whereas in Wilson disease the liver is the primary site of damage. The gene for Menkes disease (also called *Mc1*) has been mapped to band q13 on chromosome X (Mercer et al., 1993), and cloned, again by three independent research groups (Mercer et al., 1993; Vulpe et al., 1993; Chelly et al., 1993).

The primary defect appears to involve defective expression of a transporter that transfers copper across the basolateral membrane of intestinal mucosal cells. The transporter also may play a role in other cells, because it is widely expressed. It seems possible that its function might be to aid in copper efflux from cells, since Menkes fibroblasts accumulate the metal and fail to express the *MNK* gene. This may not be the case in other tissues where accumulation has not been observed. In Menkes disease, intestinal absorption of copper, or its transfer across the placenta to the fetus, does not totally exclude copper from the body, since this is incompatible with life. Some cell types in tissues

such as the intestine accumulate copper (Waldrop & Ettinger, 1990) which is subsequently lost as the intestinal cells are sloughed. The bulk of the metal is believed to accumulate in the Menkes-affected cell in metallothionein complexes. The lack of copper transport across the gut is one factor in production of a copper deficiency in most tissues. Most likely, transporters for other metal ions can be used, at least to some extent, for copper transfer. Nevertheless, there is a still a serious copper deprivation in most tissues of the body, with the consequence that copper-dependent enzymes in all areas are affected and have a diminished function.

Lysyl oxidase has been shown to be important in cross-linking collagen and elastin and its lack of activity may explain the connective tissue lesions. Low levels of cytochrome c oxidase may contribute to poor thermal regulation. Tyrosinase deficiency would be expected to lead to hypopigmentation of the skin and hair. The pili torti (twisted or "kinky" hair) observed in Menkes patients is related to the cross-linking failure of keratin which is dependent on copper. Deficiency of cytochrome c oxidase, SOD and dopamine beta-hydroxylase may result in neurological degeneration, mainly by oxygen free radicals (Bankier, 1995).

The clinical features observed in Menkes patients are a direct result of the failure of copper to be incorporated into specific copper-dependent enzymes (Kodama, 1993). Hence, Menkes disease mimics a deficiency in copper. Babies with Menkes disease are often born prematurely; and although they appear to have fine, normal-looking hair they often have problems associated with temperature instability, jaundice and feeding (Bankier, 1995). Many pass the developmental milestones of head control and responsive smile, but by the age of 3 months they develop loss of head control and begin to have seizures. They have truncal hypotonia (a condition of diminished tone of the skeletal muscles and diminished resistance of muscles to passive stretching) and progressive spasticity of the limbs. The hair becomes fragile, lustreless and hypopigmented. The hair feels to the touch like steel wool, owing to pili torti. The skin becomes hypopigmented and hyperextensible (cutis laxa) and the joints become hypermobile (Martin et al., 1994).

The bones are osteoporotic with flared metaphyses of the long bones, rib fractures and possible wormian bones (small irregular bones in the sutures between the bones of the skull) visible by cranial radiography. In the case of severe occipital horn syndrome the main effect is bone spurs, perhaps because of disordered connective tissue function and neurological problems (Kaler et al., 1994). The vasculature is tangled and elongated owing to numerous splits and fragmentations in arterial elastic fibres and thickened intima. Alterations in the central nervous system include severe mental retardation, seizures and ataxia which are due to intense degenerative changes of the brain and the cerebellum with a pronounced alterations of the Purkinje cells (Iwata et al., 1979). Subdural and cerebral haematoma may occur. There is progressive deterioration until death occurs, usually by the age of 5. Urinary tract diverticulum (a pouch or sac produced by herniation of the mucous membrane through a defect of the lining of the urinary tract) is common.

The majority of patients with Menkes disease present with severe, classical symptoms although individuals with milder symptoms and/or longer survival have been observed (Haas et al., 1981; Gerdes et al., 1988). A spectrum of mutations adversely affecting protein expression has been observed in severely affected Menkes patients. The diseases X-linked cutis laxa (Levinson et al., 1993; Yeowell et al., 1994), occipital horn syndrome (Kaler et al., 1994) and milder Menkes phenotypes result from mutations that only diminish or alter *MNK* expression.

8.4.2 Wilson disease

Samuel A.K.Wilson described a disorder of the nervous system associated with liver cirrhosis. Wilson wrote that the disease, "...is familial, invariably fatal (and caused by) a toxin generated in connection (with) the hepatic cirrhosis that is always found after death" (Wilson, 1912). Following this lead in 1920, Hall concluded that Wilson disease occurred only in individuals who inherited a defective gene (Hall, 1921) which Bearn later showed to be recessive (Bearn, 1960). It was not until 1948 that Cumings identified that copper was indeed the toxin in Wilson disease, finding that the liver and brain of patients had an extremely high content of the metal (Cumings, 1948).

Wilson disease is the most extensively described inherited disorder of copper metabolism. The gene is distributed worldwide, having been demonstrated in virtually all races. Current global estimates indicate that the incidence rate of the disease is approximately 1 in 30 000 live births, with prevalency ranging from 15 to 30 per million. The gene frequency varies between 0.3 and 0.7%, corresponding to a heterozygote carrier rate of slightly greater than 1 in 100.

Genetic studies from a large Israeli-Arab kindred identified a linkage between the Wilson disease locus and the erythrocyte enzyme esterase D, thereby establishing that the gene mutation responsible for Wilson disease was located on chromosome 13 (Frydman et al., 1985). Using multipoint linkage techniques, the abnormal gene for Wilson disease was localized more specifically to 13q14-q21. In 1993, a candidate gene for Wilson disease (*WND*) was reported independently by several different groups of investigators, using slightly different strategies for positional cloning (Bull et al., 1993; Petrukhin et al., 1993; Tanzi et al., 1993). The *WND* gene consists of a transcript of approximately 7.5 kilobases, which is expressed primarily in liver, kidney and placenta; it has also been detected in heart, brain, lung, muscle and pancreas, albeit at much lower levels. The full-length cDNA sequence of the *WND* gene (Bull et al., 1993; Tanzi et al., 1993) predicts a protein of 1411 amino acids which is a member of the cation-transporting P-type ATPase subfamily, highly homologous to the Menkes disease gene product and the copper-transporting ATPase (CopA) found in copper-resistant strains of *Enterococcus hirae*.

From sequence analysis of the cDNA, the WND protein is predicted to possess a metal-binding domain (containing five specific binding sites), an ATP-binding domain, a cation channel and phosphorylation region, and a transduction domain responsible for the conversion of the energy of ATP hydrolysis to cation transport. To date, more than 30 disease-specific mutations in the Wilson disease gene have been identified, and it has been postulated that different mutations at that locus may explain the clinical variability. Moreover, the variety of mutations identified in the Wilson disease gene potentially may affect copper transport to varying degrees, and at different cellular sites (Schilsky, 1994). However, detailed genetic and epidemiological studies suggest that the variability in clinical expression observed in Wilson disease patients may not be solely a

consequence of allelic heterogeneity, since marked differences in presentation, age of onset and disease course have been observed in family members who have inherited two identical mutant alleles (Walshe, 1995).

Developments in the molecular genetics of Wilson disease have provided a means for carrier detection and early diagnosis (Sternlieb, 1993). In fact, several studies using haplotype analysis of relatives with closely linked markers have permitted precise carrier detection with less than 1–2% error. There also is a report of prenatal exclusion of Wilson disease by analysis of DNA polymorphism in a chorionic villus biopsy performed at 9 weeks gestation (Cossu et al., 1992). Unfortunately, the use of genetic techniques in the diagnosis of Wilson disease has significant limitations. Currently, DNA marker studies can be performed only within families, and under circumstances where the diagnosis has already been established definitely in at least one family member by standard biochemical methods. The index patient's DNA is then used as a reference to recognize the disease-carrying chromosomes in other members of the family. However, spontaneous chromosomal rearrangements can cause such markers to be uninformative, thereby limiting the diagnostic reliability. These findings indicate considerable potential difficulties for DNA-based genetic screening, since most patients will possess alleles with two different mutations of the Wilson disease gene (Schilsky, 1994). Given the rapidity and accuracy of biochemical analyses in establishing the diagnosis of Wilson disease, as well as the aforementioned limitations of genetic testing, standard biochemical methods should continue to be utilized in the evaluation of most suspected cases. In addition, genetic screening of young family members of patients afflicted with the disorder would facilitate early diagnosis and permit initiation of therapy in the presymptomatic state.

It is postulated that the harmful effects of excess copper are mediated by the generation of free radicals, which deplete cellular stores of glutathione and oxidize lipids, enzymes and cytoskeletal proteins. Indeed, it has been shown that a number of intracellular systems are disrupted by elevated copper concentrations, including organellar membranes, DNA, microtubules, and various enzymes and proteins, although the principal cellular target of copper toxicity is unknown. In the earliest stages of hepatocellular injury, ultrastructural

abnormalities involving the endoplasmic reticulum, mitochondria, peroxisomes and nuclei have all been identified (Sternlieb, 1990). These changes, in conjunction with diminished mitochondrial enzyme activities, may be important steps in the pathophysiological events leading to lipid peroxidation and triglyceride accumulation in the hepatocyte.

Wilson disease patients exhibit impaired biliary excretion of copper, which is believed to be the fundamental cause of copper overload. The prompt reversal of abnormal copper metabolism in Wilson disease patients following orthoptic liver transplantation confirms that the primary defect resides in the liver. It has been proposed that the Wilson disease gene product is responsible for copper secretion from the liver cell, either across the canalicular (apical) membrane of the hepatocyte or into a subcellular compartment that communicates with the bile canaliculus (Tanzi et al., 1993). The latter is consistent with a putative lysosomal defect underlying the diminished biliary excretion and systemic accumulation of copper observed in patients with Wilson disease. In addition, in an animal model of Wilson disease, the Long-Evans Cinnamon (LEC) rat, excessive hepatic copper accumulation occurs in the setting of diminished biliary excretion. These rodents exhibit impaired entry of copper into the lysosomes, with normal delivery of lysosomal copper to the bile (Schilsky et al., 1994). The LEC rat is a mutant strain of the Long-Evans rat which spontaneously develops fulminant hepatitis at 3–4 months of age, resulting in a 40% mortality rate. Surviving animals manifest chronic hepatic disease, low serum ceruloplasmin levels and increased copper concentrations in the liver. Thus, the LEC rat shares many important clinical, biochemical and histological features with Wilson disease, and the recent availability of this animal model will probably provide new insight into the pathogenesis of the human disorder.

The biochemical defect which leads to the accumulation of copper in Wilson disease is present at birth; however, clinical symptoms rarely are observed before the age of 5 years. The initial signs of Wilson disease are generally detected in older children, adolescents and young adults, although case reports have documented the clinical onset as early as 4 years. Wilson disease patients typically present with hepatic and/or neurologic dysfunction. Less commonly, patients present with

skeletal, cardiac, ophthalmologic, endocrinologic or dermatologic symptoms. Approximately 25% of patients have involvement of two or more organ systems at initial evaluation, although, with the advent of aggressive screening, there has been a significant increase in the number of asymptomatic patients diagnosed. The clinical manifestations of Wilson disease are summarized in Table 16.

Table 16. Clinical manifestations of Wilson disease (hepatolenticular degeneration)

Organ system	Symptoms
Hepatic	cirrhosis, chronic active hepatitis, fulminant failure
Neurologic	bradykinesia, rigidity, tremor, ataxia, dyskinesia, dysarthria, seizures
Psychiatric	behavioural disturbances, cognitive impairment, affective disorders, psychosis
Ophthalmologic	Kayser–Fleischer rings, sunflower cataracts
Haematologic	haemolysis, coagulopathy
Renal	renal tubular defects, diminished glomerular filtration, nephrolithiasis
Cardiovascular	cardiomyopathy, arrhythmias, conduction disturbances, autonomic dysfunction
Musculoskeletal	osteomalacia, osteoporosis, degenerative joint disease
Gastrointestinal	cholelithiasis, pancreatitis, spontaneous bacterial peritonitis
Endocrine	amenorrhoea, spontaneous abortion, delayed puberty, gynaecomastia
Dermatologic	azure lunulae, hyperpigmentation, acanthosis nigricans

Hepatic involvement in Wilson disease tends to manifest at a younger age (mean 8–12 years) than does neurological dysfunction, and is nonspecific, mimicking the features of a variety of acute and chronic liver diseases. Three major clinical patterns of liver disease are observed: cirrhosis, chronic active hepatitis and fulminant hepatic failure. In the early asymptomatic phase of Wilson disease, or in the

presence of inactive cirrhosis, liver tests may be normal or only minimally elevated. In the majority of cases, hepatic injury develops insidiously and, if untreated, pursues a chronic and relentless course to cirrhosis. Hepatocellular carcinoma is uncommonly associated with Wilson disease, in contrast to haemochromatosis.

An estimated 5–30% of patients with Wilson disease exhibit clinical, biochemical and histological features similar to those observed in chronic active hepatitis (Scott et al., 1978; Schilsky et al., 1991). The diagnosis may be overlooked in these patients, since a significant number, almost 50% in one series (Scott et al., 1978), have no evidence of neurologic dysfunction or Kayser–Fleischer rings on ophthalmologic examination. Serum ceruloplasmin levels also may be normal in the setting of severe hepatic inflammation. It has been estimated that Wilson disease represents the underlying aetiology in 5% of patients with idiopathic chronic active hepatitis who are under 35 years of age (Schilsky et al., 1991). A distinctive feature of wilsonian chronic active hepatitis is the relatively modest elevations of serum aminotransferase levels in the presence of severe hepatocellular necrosis and inflammation.

More dramatically, Wilson disease occasionally manifests as fulminant hepatic failure. These patients may be indistinguishable from individuals with viral-induced hepatic necrosis, and many of the biochemical tests used to establish the diagnosis of Wilson disease are abnormal in patients with other forms of fulminant hepatic failure (McCullough et al., 1983). The clinical features most suggestive of fulminant wilsonian hepatitis include the presence of intravascular haemolysis, splenomegaly, and Kayser–Fleischer rings. Biochemical markers indicative of Wilson disease include relatively mild elevations in serum transaminases despite massive hepatic necrosis, hyperbilirubinaemia with normal or low alkaline phosphatase levels, and a markedly elevated serum copper concentration. The serum level of aspartate aminotransferase (ASAT) typically is higher than that of alanine aminotransferase (ALAT), as a result of the associated haemolysis. Although uncommonly observed in wilsonian fulminant hepatic failure, Kayser–Fleischer rings are not pathognomonic, since they are occasionally seen in patients with other cholestatic hepatic disease. Liver biopsy with measurement of quantitative copper may be helpful, although deranged clotting function may preclude this

procedure, or necessitate the transjugular approach. If a biopsy specimen is obtained, histological evidence of cirrhosis (predominantly micronodular) in a young patient with fulminant hepatitis is suggestive of Wilson disease, as is an elevated hepatic copper content. Wilson disease patients with acute hepatic failure tend to be young and to have a fulminant clinical course, with survival generally no longer than days to weeks unless liver transplantation is performed. Even when transplantation is unavailable for patients, it remains imperative to make the diagnosis of Wilson disease for the purpose of aggressive medical therapy and family screening.

The simplest screening procedure includes a slit-lamp examination of the eyes, and measurement of serum ceruloplasmin and transaminase (ALAT, ASAT) levels. If Kayser–Fleischer rings are present on ophthalmologic examination and ceruloplasmin levels are below 200 mg/litre in a patient with neurologic signs or symptoms, the diagnosis of Wilson disease is established. If a patient is asymptomatic, exhibits isolated liver disease, or lacks corneal rings, the coexistence of a hepatic copper concentration above 250 µg/g (dry weight) and a low serum ceruloplasmin level also is sufficient to make the diagnosis.

The normal serum concentration of ceruloplasmin is 200–400 mg/litre. Although a decreased ceruloplasmin level *per se* is not diagnostic of Wilson disease, approximately 90% of all patients, and 85% of individuals presenting with hepatic manifestations of the disease, have levels that are below the normal range.

The 10% of heterozygous carriers of the gene for Wilson disease who manifest diminished serum levels of ceruloplasmin, yet never develop clinical symptoms or signs of the disease, may cause diagnostic confusion. These individuals, who represent approximately 1 in 2000 of the general population, may present a difficult diagnostic dilemma if they fortuitously develop chronic active hepatitis or cirrhosis (of another aetiology), thereby mimicking the clinical, biochemical and histological features of Wilson disease. Normal ceruloplasmin concentrations are found in up to 15% of patients with Wilson disease and active liver involvement (Scott et al., 1978).

The urinary excretion of copper is greater than 100 µg/24 h (normal < 40 µg/24 h) in most patients with symptomatic Wilson disease, reflecting increased serum levels of the readily filterable fraction of nonceruloplasmin copper.

If Kayser–Fleischer rings or neurological abnormalities are absent, a liver biopsy for quantitative copper determination is essential to establish the diagnosis of Wilson disease. Care must be taken to ensure that the biopsy needle and specimen container are free from copper contamination. The normal hepatic copper concentration varies from 15 to 55 µg/g (0.24–0.87 µmol/g) dry liver. Virtually all untreated patients with Wilson disease have elevated hepatic copper levels, ranging from 250 to as high as 3000 µg/g dry liver. Values below 250 µg/g are usually attributable to the irregular distribution of copper in the liver, particularly in the presence of cirrhosis, when small fragmented biopsy samples are obtained. The finding of a normal hepatic copper concentration effectively excludes the diagnosis of untreated Wilson disease. However, an elevated liver copper level alone is insufficient to establish the diagnosis of Wilson disease, since concentrations above 250 µg/g may be found in other chronic hepatic disorders (most cholestatic conditions). In the great majority of individuals with prolonged cholestasis, serum ceruloplasmin concentrations are either normal or increased. The histochemical staining of liver biopsy specimens for copper is of little diagnostic value in patients with Wilson disease.

8.4.3 Hereditary aceruloplasminaemia

Although no defect in copper metabolism has been identified in cases of aceruloplasminaemia, this condition is included here because ceruloplasmin is a genetically regulated, copper-binding protein with a role in iron metabolism (Harris & Gitlin, 1996) (see chapter 6).

Recent evidence indicates that genetic abnormalities of ceruloplasmin synthesis occur as an autosomal recessive condition (Logan et al., 1994). Clinical signs and symptoms in these patients include mental confusion, memory loss, dementia, cerebellar ataxia, altered motor function, retinal degeneration and diabetes (Miyajima et al., 1987; Logan et al., 1994; Harris, 1995; Morita et al., 1995). Biochemical signs are decreased serum copper levels and absent or

nonfunctional ceruloplasmin in plasma and impaired copper absorption (Harris, 1995). Isotopic tracer studies demonstrate enhanced copper incorporation into liver with limited release into plasma since ceruloplasmin synthesis is absent, yet copper delivery to tissues is preserved (Miyajima et al., 1987; Harris, 1995). In fact, copper homoeostasis appears to be minimally affected while striking abnormalities in iron metabolism are found.

There is a significant decrease in serum iron, normal iron-binding capacity, markedly elevated serum ferritin and low urinary iron excretion. Iron deposition in liver, brain, pancreas and other tissues is markedly increased. The alterations in iron homoeostasis are correctable by the intravenous administration of ceruloplasmin (Ragan et al., 1969). On the basis of this evidence the clinical symptoms are most like the result of iron overload in brain, pancreas and other critical organs, rather than induced by a copper deficit.

8.4.4 Indian childhood cirrhosis

Indian childhood cirrhosis (ICC) was once a major cause of infant mortality on the Indian subcontinent (Kumar, 1984). The peculiar epidemiological, clinical and histopathological features, the enigmatic aetiology and the uniformly fatal outcome have baffled many for over a century now (Achar et al., 1960; Chawla et al., 1973; Bhagwat & Walia, 1980; Sethi et al., 1993).

Epidemiologically, the illness normally strikes between the ages of 6 months and 3 years (Bhave et al., 1992) although it can occur up to 5 years of age (Nayak & Ramalingaswamy, 1975). There is a male predominance and high rates of parental consanguinity, and up to 22% of siblings are affected.

Clinically, the onset is generally insidious (86%). In the early stage of the disease the complaints are nonspecific such as abdominal distention, irregular fever, excessive crying and altered appetite. In a few children, the disease begins with jaundice, but commonly jaundice is a late feature. In the second clinical stage of the disease, the liver is characteristically firm with a "leafy" edge. The progress is relentless and within a few months, the patient progresses on to the terminal stages with jaundice, hepatosplenomegaly, oedema and ascites. Death

is usually due to intercurrent infections or terminal hepatocellular failure leading to haemorrhagic complications or hepatic coma.

The standard liver function tests are usually deranged but not specific for the differentiation of early ICC from other childhood liver disorders. Serum copper is raised significantly in ICC. The mean serum copper values increase with the clinical progression of the disease (Tanner et al., 1979; Sharda & Bhandari, 1984; Sethi et al., 1993). Serum ceruloplasmin levels, however, are normal or elevated, in contrast to Wilson disease. Hepatic copper is increased. A hepatic copper level > 800 μg/g dry weight helps distinguish ICC from other liver disorders occurring at this age.

Histopathology remains the cornerstone of definitive diagnosis. (Parekh & Patel, 1972; Bhave et al., 1982, 1983). The two most discriminatory features of ICC now recognized are typical widespread coarse dark brown orcein staining and intralobular pericellular fibrosis (Pradhan et al., 1983). Hepatocytic necrosis (seen in 97%) and hyaline (66%) are also diagnostic though late features. Portal fibrosis, inflammation and disruption of the limiting plate are seen in most cases, but also are seen in other liver disorders and hence are not of discriminatory value. Parenchymal fat is usually absent and cholestasis is a late feature (Pandit & Bhave, 1983). Raised hepatic copper, indicated by orcein staining, is seen consistently in ICC. Intensity of orcein staining correlates significantly with the histopathological grade of the disease (Sethi et al., 1993).

Various aetiological agents have been implicated in ICC, but none has so far been confirmed. Tanner et al. (1983) stated that "early introduction of copper-contaminated animal milk is of aetiological importance", based on the observation that ICC was predominantly seen in children who were bottle-fed rather than breast-fed, and that milk stored in brass vessels prior to feeding became contaminated with high levels of copper. Experimentally, boiling and storing of milk in untinned brass vessels raises its copper concentration more than 60 times, and copper and brass vessels have been used traditionally in some parts of India to boil and store milk and water. Although ingestion of large amounts of copper in early infancy may be a factor in the aetiology, it cannot fully explain the disease. Approximately half

of the patients presenting with ICC had received milk which had been previously stored in brass vessels (Sharda & Bhandari, 1984).

In a study in India, a group of 32 children who developed cirrhosis had a significantly higher mean value of serum copper measured after diagnosis than a control group of 10 healthy age-matched children. The use of brass utensils to carry, boil and store milk occurred in only 14 (44%) of the cases, and increased serum copper levels were not limited to these. In another 82 children suffering from cirrhosis, liver biopsies revealed raised liver concentrations of copper in all cases, and levels increased with the severity of the disease (Sethi et al., 1993).

In some cases, other family members and siblings had received milk from the same source as the ICC cases but were found to have normal serum and urinary copper levels (Sharda & Bhandari, 1984). Furthermore, that ICC has been seen in children who have been breast-fed suggests that copper is unlikely to be the sole cause of the illness (Sethi et al., 1993).

Because of the familial occurrence and high consanguinity, a genetic aetiology of ICC has heen suspected (Agrawal et al., 1979; Sethi et al., 1993). Chandra (1976) reported a pedigree analysis compatible with autosomal recessive inheritance. Although both serum and hepatic concentrations increased with the severity of the disease, the copper content is variable at the same stage of the disease. Thus, genetic heterogeneity in ICC has been postulated (Sethi et al., 1993).

The copper chelator *d*-penicillamine has been given to early ICC patients, and histological improvement and remission in up to 65% of patients has been claimed (Tanner et al., 1987). This is a single study on only 29 patients; therefore, more work needs to be done to definitely determine the role of *d*-penicillamine in the treatment of ICC.

There has been a reduction of ICC in India (Bhave et al., 1992). Whether this reduction is due to the reduction of the use of brass vessels, or due to increasing intercaste marriages leading to genetic dilution, or both, is yet unclear.

A similar reduction in fatal infantile liver cirrhosis in a region of Austria has been reported (Müller et al., 1996). An ecogenetic

aetiology proposed in these conditions requiring a convergence of a genetic predisposition with a high copper intake could also be a prerequisite for the development of ICC. However, whether ICC represents a specific form of infantile copper toxicosis (ICT) or is an unrelated infantile cirrhosis is yet to be determined. The relative importance of the role of environmental exposure to copper and the genetic predisposition to copper accumulation have not yet been determined.

8.4.5 Idiopathic copper toxicosis, or non-Indian childhood cirrhosis

Scattered reports of early childhood cirrhosis similar to ICC, referred to as copper-associated idiopathic copper toxicosis (ICT) have appeared from some Western countries (Walker-Smith & Blomfield, 1973; Müller-Höcker et al., 1987; Adamson et al., 1992; Gormally et al., 1994). It is unclear whether the aetiology of this disease is the same as that of ICC as seen in India (section 8.4.4). Müller-Höcker et al. (1987, 1988) described the first three cases in Germany with histological and clinical features of ICC, including very high liver copper levels. Eife et al. (1991) reported a total of 22 such cases (13 fatal) in Germany up to 1990 and attributed them to ICT. All the families involved from Germany and elsewhere, lived in rural areas and were supplied with soft and acidic water from private wells using copper pipes. The exposed children were breast-fed only briefly or not at all and their formula had been made up with well water, presumably contaminated with copper. Details on three of the aforementioned German cases were given by Müller-Höcker et al. (1987, 1988), Schramel et al. (1988) and Weiss et al. (1989). The water copper levels (non-representative siugle values) varied from 0.4 to 15.5 mg Cu/litre. These values were not measured during the time of exposure, but several months later. The authors attributed the illness to copper toxicosis, possibly in connection with an unproven genetic predisposition and/or unusual high copper exposure of the babies via the formulas.

Müller et al. (1996) reported on the largest non-Indian series of cases of a disease they regarded as identical to ICC or ICT. Unfortunately they were unable to obtain liver samples to confirm high copper values, and relied on photographs for histology to demonstrate the similarity with ICC. In the Tyrol region of Austria between 1900

and 1974, 138 fatal cases of this cirrhosis were found. Detailed family pedigree analysis suggested that susceptibility to the disease was inherited in an autosomal recessive fashion and that the copper-rich diet of the region induced the symptoms (experiments duplicating methods of milk preparation using copper vessels suggested copper levels of up to 60 µg/litre). Many similarly fed infants did not develop cirrhosis. There have been no cases since 1974. The authors speculated that this could be due to the replacement of copper aud brass vessels, although increased mobility of the population and fewer consanguineous marriages may have diluted the gene pool reducing the number of homozygous children. This report provides a likely explanation for the causation and natural history of copper-associated ICT in Austria and possibly elsewhere.

A number of case reports on childhood cirrhosis associated in most cases with only intermediate hepatic copper levels (≤ 400 µg/g dry weight) have been described worldwide, but no environmental copper exposure was evident (Lim & Choo, 1979; Maggiore et al., 1987; Aljajeh et al., 1994; Baker et al., 1995).

In order to test the hypothesis that ICT is an entirely environmental coudition, Scheinberg & Sternlieb (1994) reported on three Massachusetts, USA, towns where drinking-water was known to contain high levels of copper (8.5–8.8 mg Cu/litre on first-draw samples after 6 h of stagnation). Between 1969 and 1991, mortality of 3000 children under the age of 6 years with liver and other diseases were studied. During that period there were 135 deaths among the study population but none from cirrhosis or any form of liver disease. The sample size of this study was insufficient to fully test the proposed hypothesis.

Fewtrell et al. (1996) reported 220 patients aged up to 7 years with liver disease in the United Kingdom in 1991–1993. Copper exposure in tap water was mostly below 3 mg/litre, but in 15 cases higher levels may have occurred. In this series of patients too no cases of ICT were detected.

A retrospective, multicentre study (Schimmelpfennig et al., 1996) detected a total of 103 cases of early childhood cirrhosis of different causes for the years 1982–1994 in Germany. The three cases described

in detail by Müller-Höcker et al. (1987, 1988) were not included in this study. In only two cases were the exact conditions of increased copper exposure reliably reconstructed and other aetiologies of cirrhosis excluded. The concentrations of copper in the tap water in these two cases were 9–26 mg/litre owing to specific conditions of the individual water supplies. These concentrations may have been the cause of one fatal case and may have led to severe liver disease in the other. Recently a case of adult liver cirrhosis associated with a daily copper intake of 0.5–1.0 mg Cu/kg body weight was described (see section 8.3.2) (O'Donohue et al., 1993). Based on these collective data, a purely environmental basis for ICT cannot be confirmed or excluded; thus, the cause of liver injury remains uncertain.

8.4.6 Chronic liver diseases

Copper retention occurs as a result of impaired biliary excretion. As reviewed recently by Zucker & Gollan (1996), conditions such as primary biliary cirrhosis, primary sclerosing cholangitis, extrahepatic biliary obstruction or atresia, intrahepatic cholestasis of childhood and chronic active hepatitis can lead to liver copper levels above 250 µg Cu/g dry weight. These patients can be distinguished from those with Wilson disease on the basis of history, physical findings and elevated or normal serum ceruloplasmin levels. The presence of hepatic disease requires caution in the provision of dietary copper. Correction of biliary output in the cholestatic condition may lead to decrease in liver copper levels (Ohi & Lilly, 1980).

8.4.7 Copper in infancy

Fetal copper metabolism is different from that in children or adults. Neonates have high levels of copper in the liver and low levels of serum copper and ceruloplasmin (Epstein, 1983) and elevated levels of metallothionein that decrease after birth. After the age of about 6 months both liver copper and serum copper levels come within the adult range. The ratio of hepatic concentration of copper in newborns to that of an adult human is 15 : 4 (Goyer, 1991).

Acquired copper deficiency is a clinical syndrome that occurs mainly in infants (Shaw, 1992), although it has also been described in children and in adults. Copper deficiency is usually the consequence of decreased copper stores at birth (see chapter 6), inadequate dietary

copper intake, poor absorption, elevated requirements induced by rapid growth or increased copper losses. Excretion of copper is usually via the bile, but if renal tubular reabsorption is impaired urinary losses may be quite high. The multiple factors that may lead to deficiency commonly coexist in copper-deficient subjects. Copper deficiency is more frequent in preterm infants, especially of very low birth weight, owing to their reduced copper stores at birth given the smaller relative size of the liver and higher requirements determined by their high growth rate (Widdowson & Dikerson, 1964; Widdowson et al., 1974; Dauncey et al., 1977; Sutton et al., 1985; Hurley & Keen, 1988).

Infants fed exclusively diets based on cow's milk are more prone to develop copper deficiency because of the low copper content of milk and limited absorption of this mineral in cow's milk. In contrast, breast-fed infants absorb more copper; this may be due to the lower casein content of human milk or to factors present in human milk which enhance copper absorption (Naveh et al., 1981; Lönnerdal et al., 1985). In developing countries, where infant feeding is often based on cow's milk enriched with a high concentration of refined carbohydrates, copper deficit may be more prevalent because fructose and other refined sugars lower copper absorption.

On the basis of published information, the most common cause of copper deficiency is insufficient copper supply during the nutritional recovery of malnourished children (Shaw, 1992). These infants present several factors which are frequently associated to copper deficiency: history of low birth weight, short duration of breast-feeding, a diet based on cow's milk and a highly refined carbohydrate, or increased losses of nutrients due to diarrhoeal disease and frequent infections. During nutritional recovery they grow 5–10 times as fast as normal for their age group, thus increasing the nutrient requirement.

8.4.8 Malabsorption syndromes

Copper deficiency has been reported in subjects with malabsorption syndromes such as coeliac disease, tropical sprue, cystic fibrosis, partial gastrectomy or short bowel syndrome due to intestinal resection (Williams, 1983; Rodriguez et al., 1985; Hayton et al., 1995). Copper deficit should be suspected in infants with prolonged or recurrent diarrhoeal episodes, abnormal bile loss, intestinal resections,

or loss of intestinal contents from intestinal fistula (Williams, 1983; Castillo-Duran et al., 1988). Castillo-Duran et al. (1988) evaluated the magnitude of copper loss in 14 infants during acute diarrhoeal episodes requiring hospitalization. The results were compared with those obtained in 15 matched control infants. Faecal losses of copper were twice as high in the diarrhoea group as in the control subjects. This group presented a negative copper balance up to 7 days after hospital admission. Copper losses were directly related to faecal weight. Furthermore, Rodriguez et al. (1985) compared the copper status of 19 children exhibiting chronic diarrhoea with two control groups (19 healthy and 11 malnourished children). Plasma copper levels were 30% lower and hair copper content decreased 3–4-fold in the group with chronic diarrhoea relative to the control groups.

High oral intakes of zinc and iron decrease copper absorption and may lead to copper deficiency (Prasad et al., 1978; Williams, 1983). This phenomenon is used as a therapeutic strategy in Wilson disease where high zinc intake (40–50 mg/day) has been demonstrated to lower copper absorption. Copper deficiency has been also documented in subjects receiving penicillamine or other cation chelating agents, or high doses of oral alkali therapy which enhance copper losses (Williams, 1983).

8.4.9 Parenteral nutrition

Patients fed with intravenous nutrient mixtures lacking sufficient copper will develop symptomatic deficiencies after 3–12 months (Shike et al., 1981). In adults, this presents as an iron-resistant anaemia, with a mark fall in neutrophils. In children, as well as the haematological abnormality, there are marked effects in bone: characteristic radiological changes, greater ease of fracture and reduced bone age (Shaw, 1992).

It has been shown that infusion of 0.3 mg Cu/day will maintain a 70 kg adult in copper balance (Shike et al., 1981). However, in patients with high volume fistula or diarrhoeal losses additional copper may be needed. The adult normative requirements of 1.3 mg Cu/day will maintain plasma copper within the reference interval and prevent the development of deficiency disease (Shenkin et al., 1987). An

increased amount of copper may be required in patients who have high volume fistula fluid or diarrhoeal losses.

The neonatal requirements for copper will vary according to such factors as premature delivery and low birth weight. It has been suggested that approximately twice as much copper is required by the pre-term infant compared to the term infant (Shaw, 1992; WHO, 1996).

Where there is evidence of cholestasis, copper supplements in both adults and children should be reduced or withheld and the patient monitored for any signs of developing copper toxicity.

8.4.10 Haemodialysis patients

Copper homoeostasis mechanisms available for regulating gastrointestinal absorption of copper are bypassed by parenteral administration. Copper toxicity in patients on haemodialysis is not common. In two studies of four patients exposed to poorly defined concentrations of copper in the dialysis fluid (0.056 to > 0.11 mg/litre) headache, sweating, nausea, hypotension, stupor and coma were reported (Klein et al., 1972; Lyle et al., 1976). Similar signs and symptoms were reported in three patients exposed to copper concentrations between 5.1 and 8.8 mg/litre of dialysate (Manzler & Schreiner, 1970).

8.4.11 Cardiovascular diseases

Changes in copper concentrations have been associated with ischaemia (Kinsman et al., 1990), as well as various cardiovascular and cerebrovascular related problems (Peterson et al., 1990). Reviewing the relationship between ischaemic heart disease and copper deficiency, Sorenson (1989) found evidence that copper deficiency can elevate blood pressure. Impaired tissue formation has been associated with copper deficiency, particularly with the cardiovascular system (Farquharson et al., 1989; McCormick et al., 1989; Saari & Johnson, 1990; Tinker et al., 1990). Variation in copper intake may cause significant changes in the SOD level in certain cardiac tissue (Askari et al., 1990).

There are some reports concluding that elevated serum copper levels (nondietary copper exposure) are implicated in the onset of cardiovascular disease. In two double-blind studies, groups of 7 or 8 males took a supplement of copper gluconate providing 2 or 3 mg Cu/day, respectively, for 6 weeks. Groups of 6 males formed control groups in each case. The data suggested that 2 and 3 mg Cu/day could increase LDL cholesterol and total serum cholesterol, respectively. However, the control groups showed a variability in levels that made these findings questionable. At 3 mg Cu/day, there was an increase in the haemoglobin level after 6 weeks (Medeiros et al., 1991). An earlier study found no significant changes in the serum levels of copper, zinc, magnesium, triglyceride, serum glutamic-oxaloacetic transaminase (SGOT), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH) or alkaline phosphatase, in a group of 7 subjects ingesting 10 mg Cu/day for 12 weeks as copper gluconate. Both treated and placebo groups reported nausea, diarrhoea, heartburn and back pain. The small group sizes should be noted (Pratt et al., 1985).

In England a correlation study, with measurements made after diagnosis of coronary heart disease, has shown higher serum copper levels in cardiovascular disease patients (Punsar et al., 1975). A follow-up study in the Netherlands compared the copper and zinc intake in cardiovascular mortality; the adjusted risk of death from cardiovascular disease showed a U-shaped pattern which was four times higher in subjects in the highest quartile for serum copper (> 1.43 mg/litre), but a twofold excess mortality was also observed in subjects with low serum copper (< 1.05 mg/litre) (Kok et al., 1988). It is noteworthy that causal interpretation of these data is difficult because the disease might have affected serum copper levels. Furthermore, the possibility that elevated serum copper levels are the result of preclinical disease could not be ruled out. Also, information on vitamin C, iron status and other nutrients that are associated with copper is not available. In another prospective study, baseline serum copper levels were measured in 1666 randomly selected Finnish males aged 42–60 years in 1984–1988, and the cohort followed until December 1989. When divided into tertiles of initial serum copper, the highest tertile experienced acute myocardial infarction in 4.6% of the subjects, compared with 3.6% in the medium tertile and only 0.9% in the lowest tertile. After adjustments, the relative risks for the three groups were 4.0, 3.5 and 1.0, respectively (Salonen et al., 1991). It

should be stressed that elevated serum copper could be a consequence rather than a causal factor for acute myocardial infarction.

The same group of authors reported that the mean increase in the maximal common carotid intima media thickness after 2 years was greater in men with high serum copper concentrations, those with low serum selenium concentrations and those with raised serum LDL cholesterol concentrations. They concluded that there was a synergistic effect of copper, a low serum concentration of selenium, and LDL cholesterol concentration in atherogenesis (Salonen et al., 1991).

The association between serum ceruloplasmin level and the subsequent incidence of myocardial infarction and stroke were studied in a nested case–control study in Finland. High serum ceruloplasmin levels were significantly associated with higher future odds of myocardial infarction but not of stroke, which support the hypothesis that a high serum ceruloplasmin level is a risk factor for myocardial infarction (Reunanen et al., 1992). This was consistent with the described positive relationship between high serum copper and the aggregation of classical risk factors (McMaster et al., 1992). Several investigators (Taggart et al., 1986; Fraser et al., 1989) reported that ceruloplasmin is a positive acute-phase reactant and increases in response to injury and infection in parallel with other plasma protein markers such as C-reactive protein.

All these observations may seem incongruous when juxtaposed with the copper-deficiency theory (Klevay, 1975), but they are not in conflict with the theory because high serum copper does not prove high copper absorption. Experiments with animals reveal that the opposite may be true (Klevay, 1988, 1992). Thus, the role of elevated serum copper (unrelated to dietary copper exposure) in the aetiology of cardiovascular disease remains a matter of controversy and conjuncture.

8.5 Occupational exposure

It has been reported that occupational exposure to copper fume results in metal fume fever (Armstrong et al., 1983) and a similar condition has been reported from inhalation of finely ground copper-

oxide dust (Schiatz, 1949). Air concentrations capable of producing these effects are not well defined. Schiatz (1949) reported on conditions in a postwar factory in which ventilation systems were inoperative. In this case, exposures were likely to be unusually high compared to plants with adequate industrial hygiene.

Most industrial exposures are to a mixture of copper and other contaminants, and assessing the effects of copper alone from such studies is extremely difficult. This restricts the usefulness of much of the data on Bordeaux mixture sprayers (Pimentel & Menezes, 1977; Plamenac et al., 1985), from the mining and smelting of copper (Ruoling & Mengxuan, 1990; Chen et al., 1993) and from the maintenance of moulds in a paper mill (Srivastava et al., 1992). Copper refinery studies are less likely to be confounded by mixed exposures. Studies where effects could reasonably be attributed to copper are discussed below.

A large historical prospective study of 3550 men working for at least 1 year in the tank house of nine copper refineries in the USA (Logue et al., 1982) provided no statistically significant evidence of an increased risk of cancer.

Suciu et al. (1981) reported on a clinical study of workers exposed to copper dust during the sieving and electrolysis processes. Exposures at the time of the clinical examinations were very high, ranging from 464 mg Cu/m^3 in 1971 to 111 mg Cu/m^3 in 1973 [present widely recognized exposure limits are typically 1 mg Cu/m^3 (ILO, 1991)]. Signs and symptoms studied and their occurrence included hepatomegaly in 55.6%, digestive disorders in 10–15%, and a range of respiratory signs and symptoms. Normal serum copper values in unexposed workers were reported as 0.76–1.17 mg/litre. In 1970–1973, the proportion of workers with serum copper above the normal range increased from 40% to 92%. Using a number of assumptions, absorption of copper can be estimated as being in the range of 200 mg/day. The absence of control data and information on methods used for measuring exposure severely limit the usefulness of this study (Suciu et al., 1981).

In another study, Gleason (1968) reported symptoms similar to the common cold with sensations of warmth and stuffiness of the head in

workers polishing copper plates using an aluminium oxide abrasive on buffing wheels. Air samples in front of the buffing wheel were reported at 0.12 mg Cu/m^3 but at times estimated to be a factor of 2–3 times higher. Microscopic examination indicated the particulates to be metallic copper rather than copper-oxide dust.

No adequate studies were found on the effects of occupational exposures to copper on fertility or fetal development.

9. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

9.1 Bioavailability

Copper usually has limited bioavailability in environmental media, and this needs to be carefully considered in all assessments of its environmental impacts. Bioavailability refers to the degree to which total chemical in the environment (e.g. water, sediment, food items) can actually be taken up by organisms (Rand & Petrocelli, 1985). The more bioavailable a chemical is, the greater the potential for toxicity or bioaccumulation. Bioavailability can be affected by the speciation of a chemical (i.e. certain species will be more or less able to interact with and pass through the absorptive surfaces of organisms), but can also be affected by other physicochemical properties of the media which regulate uptake of chemicals.

9.1.1 Bioavailability in water

A large body of environmental literature demonstrates that bioavailability is generally poorly related to the concentration of total metal in water. Major factors reported to limit copper bioavailability are adsorption to suspended particles, complexation by dissolved organic matter and complexation by some inorganic ligands such as carbonate (Sunda & Guillard, 1976; Brungs et al., 1976; Allen & Brisben, 1980; Giesy et al., 1983; Borgmann & Ralph, 1983, 1984; Borgmann & Charlton, 1984; Meador, 1991; Verweij, 1992; Erickson et al., 1996). Copper toxicity is usually found to decrease with increasing water hardness, possibly because calcium and copper compete for adsorption sites on biological surfaces, so that greater calcium concentrations will limit copper adsorption (Zitko & Carson, 1976; Howarth & Sprague, 1978; Chakoumakos et al., 1979; Miller & Mackay, 1980; Pagenkopf, 1983). Copper toxicity has also been reported to be affected by pH, which may be due either to hydrogen ion affecting copper speciation or to the interactions of copper with biological surfaces (Howarth & Sprague, 1978; Miller & Mackay, 1980; Borgmann, 1983; Meador, 1991; Erickson et al., 1996).

Particular attention has been paid to the possibility that the principal bioavailable species is the free copper (cupric) ion. Several studies have shown a close correlation of copper toxicity to cupric ion activity as the concentrations of organic ligands vary (Sunda & Guillard, 1976; Allen & Brisbin, 1980; Meador, 1991; Verweij et al., 1992). However, other studies have shown that this correlation is not always good for some organic ligands and organisms (Giesy et al., 1983; Borgmann & Charlton, 1984; Borgmann & Ralph, 1983, 1984; Erickson et al., 1996). In fact, certain hydrophobic copper complexes appear to have high bioavailability (Ahsanullah & Florence, 1984). Studies which evaluated the effect of pH on copper toxicity also do not show a close correlation of toxicity with cupric ion activity (Borgmann 1983; Meador, 1991). Toxicity on the basis of cupric ion will also vary with varying water hardness, although if this is due to competitive interactions it does not contradict the notion that cupric ion is the principal bioavailable species. More information and analysis regarding the "free ion activity model" for metal toxicity and metal bioavailability is provided in a review by Campbell (1995).

The bioavailability of Cu(I) has been largely ignored since soluble or complexed forms of Cu(I) have not been thought to occur in significant amounts in aerobic environments. However, studies by Moffett & Zika (1987) speculate that Cu(II) can be directly or indirectly reduced to Cu(I) by photochemical processes. If this should occur in seawater, chloride ions might stabilize the Cu(I) through complex formation.

Whatever the mechanisms, bioavailability can vary widely and must be considered in any interpretation and application of toxicity data such as those presented later in this chapter. Additional consideration must be given to the condition of organisms and any physicochemical exposure conditions which affect organism susceptibility without affecting bioavailability, such as temperature and sodium concentrations (Erickson et al., 1987, 1996). Some empirical strategies exist for doing this. The US EPA water quality criteria for copper (US EPA, 1984) are adjusted for hardness, based on regression analysis of studies in which toxicity was evaluated at various hardness levels. This addresses only some aspects of bioavailability, and EPA procedures allow for criteria to be modified based on toxicity tests in site water which evaluate bioavailability. Welsh et al. (1993) provide empirical

equations for the effects of pH and organic carbon on the acute toxicity of copper to fathead minnows. Erickson et al. (1987) proposed similar equations for several physicochemical factors affecting acute copper toxicity. Such empirical approaches have considerable utility, but can be expensive to develop. Some recent research has introduced predictive models which are more mechanistically based and have a potential for providing better extrapolations.

9.1.1.1 Predicting effects of copper on fish gill function

Gills of freshwater fish have two important physiological functions; transport of gas (oxygen, carbon dioxide, ammonia) and uptake of active ions (sodium, calcium) (Wood, 1992; Playle, 1997). At environmentally realistic levels for anthropogenically contaminated waters, metals exert their toxic effects by binding to these sodium and calcium pump-associated ligands in a highly specific fashion, thereby inhibiting the inward transport of the essential nutritive ions. These ligands are, therefore, the proximate receptors for the metals; the free cationic forms of the metals are the most potent in binding to these receptors. For cupric and cadmium ions, strong relationships between the gill metal burden and mortality have been determined experimentally (MacRae et al., in press). Thus, it may be possible to predict toxicity from gill metal burden for these two metals and potentially other cationic metals.

Viewed in the above context, the specific receptor ligands on the gill are entirely analogous to other anionic ligands in the water column which may also bind the cationic metal – for example chloride, hydrogen carbonate and dissolved organic carbon (DOC) – and indeed the gill ligands will compete with these natural ligands for the metal (Playle et al., 1993a,b). The final metal partitioning will depend in part on the affinities and numbers of natural ligands relative to gill ligands. Naturally occurring cations in the water column (e.g. sodium, calcium, hydrogen) will compete with the metal for both the natural anionic ligands and gill receptor ligands. Aquatic geochemical speciation programs such as MINEQL' and MINTEQA2 (Allison et al., 1991; Schecher & McAvoy, 1992) are specifically designed to deal with these competitive interactions and can be used to produce accurate equilibrium models of the metal partitioning among the various ligands in the water, provided the water chemistry is known. At present, these

programs do not contain binding constants for the gill receptor ligands and therefore deal only with partitioning within the water column. However, they allow the user to add constants for other ligands at will. A problem with these modelling approaches is that the biomembrane–water interaction is treated as an equilibrium situation, whereas it is, in fact, a dynamic reaction and kinetic factors (rate constants) should also be taken into account.

Recently, methods have been developed to determine conditional equilibrium binding constants of copper and other metals to the gill receptor ligands (Janes & Playle, 1995). In brief, these involve experimental determination of equilibrium gill metal burden after exposure of the fish (3 h) to environmentally relevant levels of the metal in the presence of various concentrations of natural and/or synthetic ligands with known metal-binding constants. Analogous competition experiments can be run in the presence of various concentrations of natural cations to determine the conditional binding constants of the gill receptors for such cations. These constants can then be added into chemical speciation calculation programs to make a prediction of gill receptor loading with metal, and therefore toxicity, in any water with known chemistry.

The advantages of this predictive modelling approach include the following:

- it is mechanistically based
- for the first time in aquatic toxicology it allows estimation of metal dose at the receptor surface directly associated with toxicity
- it takes all important water chemistry factors into account (not just hardness, for example)
- it can deal with multiple metals simultaneously.

This approach to modelling toxicity allows for flexible, site-specific criteria based on the known chemistry of the receiving water and the known chemistry of the gill surface. This approach is also currently being investigated for freshwater invertebrates.

9.1.2 Bioavailability of metals in sediments

Determining the bioavailability of metals sorbed to sediments is a key to understanding their potential to accumulate in aquatic organisms

and to induce toxic effects. Considerable published data indicate that total metal concentrations on sediments are not a good estimator of the bioavailable fraction of the total chemical present (Ruiz et al., 1991; DeVevey et al., 1993; Allen & Hansen, 1996). Total metal concentrations in sediments which produce toxic effects can differ by a factor of 10–100 for different sediments. In order to assess the potential for toxicity based on chemical measurements, the bioavailable fraction of the total metal present needs to be estimated. A number of approaches to determining metal bioavailability associated with sediments have been evaluated, including carbon normalization and sorption of metals in oxic freshwater sediments to particulate carbon and the oxides of iron and manganese (Jenne, 1987).

Recently, the dominant role of the sediment sulfides in controlling metal bioavailability has been demonstrated (DiToro et al., 1990, 1991; Ankley et al., 1991). Sulfides are common in many freshwater and marine sediments and are the predominant form of sulfur in anaerobic sediments (usually found as iron sulfide). The ability of sulfide and metal ions to form insoluble precipitates with water solubilities well below the toxic threshold of dissolved metal is well known (DiToro et al., 1990). This accounts for the lack of toxicity from sediments and sediment pore waters even when high metal concentrations are present (Ankley et al., 1991). The same authors have shown that the solid-phase sediment sulfides that are soluble in weak cold acid, termed acid volatile sulfides (AVS), are a key factor in controlling the toxicity of heavy metals (copper, cadmium, nickel, lead, zinc). Toxicity due to these metals is not observed when they are bound to sediment and when, on a molar basis, the concentration of AVS is greater than the sum of the molar concentrations of metals. When the ratio of the sum of the simultaneously extracted metals to AVS concentration exceeds 1.0 on a molar basis, toxic effects due to metals may be expressed, if the metal(s) are not complexed by other ligands. The key concept here is that the metal : AVS ratio can be used to predict the fraction of the total copper concentration present in sediment that is bioavailable.

Limitations to the AVS : metal ratio approach occur when the AVS concentration is low. This could occur in fully oxidized sediments. Most sediments have at least a small zone where the sediments are oxic near the sediment–water interface. The importance of this zone has

been demonstrated for copper relative to AVS and accumulation of copper in midge (*Chironomus tentans*) (Besser et al., 1996). In these situations, other phases (i.e. iron and manganese oxides, dissolved organic carbon and particulate organic carbon) can play an important and more dominant role in determining the bioavailability of copper. The available data suggest that AVS concentrations may be sufficient in both freshwater and marine ecosystems to be the dominant sorbing phase for copper and other metals, except in fully aerobic sediments.

9.2 Essentiality

Copper is an essential element for all biota. Copper was identified in plant (Bucholtz, 1816; Meissner, 1817) and animal (Sarzeau, 1830; Harless, 1847) systems in the nineteenth century and postulated to be a biological catalyst in the early twentieth century (Fleurent & Levi, 1920; Guerihault, 1920). Subsequent nutritional studies demonstrated that copper and other metals were necessary for optimal growth of plants and animals (McHargue, 1925, 1926, 1927a,b; Arnon & Stout, 1939; Woolhouse, 1983). Copper was shown to be an essential element for animals by Hart et al. (1928) who demonstrated that copper, as well as iron, is necessary to prevent anaemia in rats. Copper is also essential for the utilization of iron in the formation of haemoglobin (Friberg et al., 1979); hence its involvement in anaemia.

9.2.1 Animals

To satisfy their internal metabolic demands, all species in a given habitat are adapted to the natural concentration range of essential elements. Therefore, laboratory-generated no-observed-effect concentrations (NOECs) substantially below the natural background concentration of copper require further attention as they appear to violate evolutionary principles. This may be explained by the concept of the optimal concentration band of essential elements (OCEE). This concept is well known in the field of ecotoxicology of essential elements, but has not so far been accommodated in the regulatory context. Thus although ecotoxic at high concentrations, copper may also be limiting or cause symptoms of deficiency at low ambient bioavailable concentrations.

Most crustaceans and molluscs possess the copper-containing haemocyanin as their main oxygen-carrying blood protein. Haemocyanin doubles their requirement for copper compared to other invertebrates (Hopkin, 1993).

White & Rainbow (1985) calculated theoretical estimates for the minimum metabolic requirements of copper in molluscs and crustaceans. Enzymatic requirements for both groups were estimated to be 26.3 mg Cu/kg (dry weight). The possession of haemocyanin as a respiratory pigment adds a further nonenzymatic metabolic requirement of 125 mg Cu/kg for certain gastropod molluscs and 57.4 mg Cu/kg for some crustaceans such as decapods. However, Depledge (1989) recalculated the amount of copper required by decapod crustaceans to be 82.8 mg/kg (dry weight). Hopkin (1993) estimated that terrestrial isopods require a minimum whole-body concentration of 50 mg Cu/kg. Evidence on copper concentrations of certain decapod crustaceans in the deep sea suggests that circumstances exist where there is insufficient bioavailable copper for the decapods to meet all their metabolic copper requirements (Rainbow, 1988). Small specimens of the mesopelagic caridean *Systellaspis debilis*, for example, have low copper concentrations (30 mg/kg dry weight), body concentrations reaching only 100 mg/kg in large adults. According to the theoretical calculations of Depledge (1989) the smaller *S. debilis* would only have sufficient absorbed copper to match enzymatic needs, whereas larger adults have sufficient copper for haemocyanin requirements as well. This is indeed the case; Rainbow & Abdennour (1989) found that small *S. debilis* contained little, if any haemocyanin, large animals containing a more typical haemocyanin complement. Moreover, juvenile *S. debilis* undertake limited vertical migrations. This may be related to the shortage of haemocyanin in juveniles, indicating that insufficient bioavailable copper in the mesopelagic environment may limit activity levels until sufficient copper has been accumulated to allow the synthesis of increased haemocyanin concentrations. Ambient copper availability in the deep ocean is so low that levels of copper in juvenile crustaceans are a reflection of copper deficiency. Any such deficiency is only overcome in adults which have had sufficient time to accumulate body copper concentrations meeting all metabolic requirements.

Analysis of concentrations of copper in invertebrates from uncontaminated sites suggests that some terrestrial invertebrate species may be copper deficient (Hopkin, 1993). In mammals, molybdenum has been shown to influence the tissue and blood levels of copper. Copper deficiency may occur in mammals when the intake of molybdenum is excessive (Friberg et al., 1979). This is thought to be due to the formation of copper molybdate.

Problems related to copper and molybdenum metabolism have been widely reported in grazing domestic livestock, and there are some reports of concern for wildlife (Ward & Nagy, 1976; Flynn et al., 1977; Robbins, 1983). The metabolism of copper, molybdenum and inorganic sulfate is extremely complex and interrelated (Underwood, 1977). The interactions of copper and molybdenum can result in two toxic scenarios; excess copper–deficient molybdenum, or deficient copper–excess molybdenum. In the presence of inorganic sulfur it is impossible to delineate between the toxicity of one and deficiency of the other (Buck et al., 1976). Deficiency or excess of copper and molybdenum are most prominent among ruminants and directly related to copper–molybdenum balance in soil and forage.

King et al. (1984) examined copper and molybdenum levels in white-tailed deer from a uranium-mining district of Texas, USA, where molybdenosis was reported in cattle. Liver copper levels ranged from 0.47 to 0.94 µg/g in all samples, and there was no difference between mined and unmined areas. Only 1 deer of 36 examined contained detectable levels of molybdenum. The authors suggest that 6 deer with liver copper levels < 1.0 µg/g were probably suffering from copper deficiency that was not molybdenum-induced. Keinholz (1977) reported that mean copper and molybdenum levels in liver of deer from a molybdenum mining area were 40 and 1 µg/g, respectively, above control levels.

Ward & Nagy (1977) demonstrated that mule deer were able to withstand much higher dietary levels of molybdenum (1000 µg/g) than domestic livestock. The authors point out, however, that the diet used was a pelleted concentrate which may have affected availability of molybdenum to the deer. They did observe that mule deer rejected feed with excess molybdenum. The ability of wildlife to select feeds low in molybdenum would reduce the chances of toxicity.

A copper deficiency in moose on the Alaskan Kenai peninsula impaired hair and hoof keratinization, and reduced reproduction (Flynn et al., 1977). Adult females in the Kenai moose population had a 53.5% pregnancy rate compared with 91.6% for moose in another area of Alaska. Copper levels in the moose browse (5.7 μg/g) are considered marginal for domestic livestock. Examination of tissue molybdenum and sulfur levels led the authors to believe that the copper deficiency was not molybdenum induced (Flynn et al., 1976).

Aulerich & Ringer (1976) showed that addition of 25 or 50 μg Cu/g to the diet stimulated growth of young mink (dark ranch phase). Up to 200 μg Cu/g in the diet had no effect on adult mink reproduction but there was increased kit mortality at this level (Aulerich et al., 1982). Liver copper levels increased in proportion to dietary levels, but supplemental copper had no effect on the concentration of zinc or iron in mink liver. The acute (21-day) LC_{50} (intraperitoneal injection) of copper sulfate and copper acetate in adult mink was 7.5 and 5.0 mg/kg, respectively (Aulerich et al., 1982).

There is a marked difference between species in their ability to tolerate high levels of copper. Levels that are toxic to ruminants (30–50 μg Cu/g) are well tolerated by nonruminants. A difference in the rate of copper absorption from the diet between ruminants and nonruminants may partially explain the difference in sensitivity (Buck et al., 1976). Rats, swine and mink can tolerate up to 200–250 μg Cu/g in the diet (Aulerich et al., 1982).

There is also some indication that the source or quality of dietary protein may be a factor in copper toxicity. Suttle & Mills (1966) observed severe copper toxicosis in swine receiving whitefish meal but not in those receiving soybean-oil meal, with both diets containing up to 425 μg Cu/g. It is also possible that the effects of dietary protein source on copper toxicity are related to their concentrations of elements such as zinc and iron, both of which have been shown to protect swine from the adverse effects of high (250–750 μg/g) levels of dietary copper (Ritchie et al., 1963).

9.2.2 Plants

9.2.2.1 Aquatic plants

Copper must be provided as a micronutrient (as copper chloride or copper sulfate) in the culture media for growing algae (McLachlan, 1973). Copper participates, as part of the plastocyanin molecules, in the electron transport during photosynthesis, and as co-factor in a number of enzymatic reactions and metabolic pathways (Bidwell, 1979; De Boer, 1981; Lobban et al., 1985).

9.2.2.2 Terrestrial plants

Copper is an essential micronutrient for normal plant nutrition (Woolhouse, 1983; Marschner, 1986; Fernandes & Henriques, 1991; Larcher, 1995), because this element is constituent of a number of plant enzymes (Adriano, 1986; Fernandes & Henriques, 1991), some of which are listed in Table 7. Copper is required in small amounts: 5–20 mg/kg in plant tissue is adequate for normal growth (Nriagu, 1979; Clarkson & Hanson, 1980; Howeler, 1983; Stevenson, 1986), less than 4 mg/kg is considered deficient (Robson & Reuter, 1981; Howeler, 1983; Marschner, 1986) and more than 20 mg/kg is considered toxic (Stevenson, 1986). However, depending on the plant species, plant organ, developmental stage, and nitrogen supply, these ranges can be larger (Thiel & Finck, 1973; Robson & Reuter, 1981). Adriano (1986) reports a variety of soil types which are deficient in copper for normal plant growth including peat and muck soils, alkaline and calcareous soils, highly leached sandy and acid soils, and soils heavily fertilized with nitrogen, phosphorus or zinc. Zinc is expected to serve as an uptake competitor. Typical visible symptoms of copper deficiency are stunted growth, distortion of young leaves, necrosis of the apical meristem, and wilting and bleaching of young leaves (Rahimi & Bussler, 1973). Copper deficiency results in insufficient lignification of the cell walls of the xylem vessels (Rahimi & Bussler, 1974; Pissarek, 1974) indicating that the degree of lignification is a good indicator of nutritional copper status in plants.

9.3 Toxic effects: laboratory experiments

Since copper is an essential metal for aquatic and terrestrial organisms, care must be taken when interpreting toxicity test results.

For all organisms there will be an optimum concentration range, with copper being toxic or deficient above or below this optimum range. A wide variety of factors will influence this optimum range including previous exposure, test conditions and species sensitivity.

9.3.1 Microorganisms

9.3.1.1 Water

Dutka & Kwan (1981) reported a 15-min Microtox EC_{50} at 3800 µg Cu/litre. Microtox EC_{50} (15 min) values were reported at 1200 µg/litre for a copper chloride solution and at 600 µg/litre in sewage (Codina et al., 1993). Blaise et al. (1994) calculated 5-, 15-, 30- and 60-min EC_{50}s in Microtox tests to be 1100, 150, 70 and 60 µg Cu/litre, respectively. Carlson-Ekvall & Morrison (1995) report that the 30-min EC_{50} for *Photobacterium phosphoreum* was 136 µg Cu/litre. The toxicity of copper in the presence of various organic substrates identified in sewage sludge was found to vary from < 20 µg/litre for ethyl xanthogenate to > 500 µg/litre for tannic acid.

Codina et al. (1993) calculated copper EC_{50} values for two *Pseudomonas fluorescens* growth inhibition tests, a baker's yeast (*Saccharomyces cerevisiae*) test, a respiratory inhibition test with baker's yeast and a respiratory inhibition test with *P. fluorescens*. The EC_{50} values were 51.7, 48.7, 73.2, 78.8 and 150.9 mg Cu/litre, respectively.

Berk et al. (1985) calculated a 15-min EC_{50}, based on inhibition of ciliate chemotactic response, to be 150–160 µg Cu/litre for the freshwater ciliate *Tetrahymena* sp. Copper concentrations of 5 and 50 µg/litre were found to be significantly inhibitory to chemotactic responses of the marine ciliates *Miamiensis avidus* and *Paranophrys* sp., respectively.

In a static test system Schafer et al. (1994) exposed the freshwater ciliate *Tetrahymena pyriformis* to copper. They calculated 48-h and 96-h EC_{50}s, based on growth inhibition to be 8.017 and 10.18 mg Cu/litre, respectively; NOECs were 3.563 and 3.818 mg Cu/litre, respectively.

Madoni et al. (1992) isolated seven ciliate species from the activated sludge of a sewage treatment works. The 24-h LC_{50}s ranged from 1.45 µg Cu/litre for *Blepharisma americanum* (free-swimming form) to 64 µg Cu/litre for *Euplotes affinis* (a crawling form). Madoni et al. (1994) isolated a further two ciliates (*Spirostomum teres* and *Drepanomonas revoluta*) and found 24-h LC_{50}s to be 3.51 and 1.75 µg Cu/litre, respectively.

Tijero et al. (1991) studied the effect of copper on an anaerobic digester system. A concentration threshold of 20 mg Cu/litre was reported, and a 50% reduction in digester yields was found at a copper concentration of 40 mg/litre.

Isolda & Hayasaka (1991) studied the effect of copper (20 and 1000 mg/litre) on the microbial processes in pond sediment for 4 weeks. Copper had no significant effect on glucose mineralization, nitrogen fixation or dehydrogenase activity. Methanogenesis was significantly reduced at both copper concentrations and the highest exposure significantly reduced phosphatase activity.

Flemming & Trevors (1988) studied the effect of copper on nitrous oxide (N_2O) reduction in anaerobically incubated freshwater sediment at 15 °C. A concentration-dependent decrease in sediment pH and a significant decrease in nitrous oxide reduction were observed at copper concentrations ranging from 500 to 5000 mg/kg. However, when copper-amended microcosms were pre-incubated to allow the sediment pH to return naturally to pH 7.1, an inhibitory effect on nitrous oxide reduction was only observed at 5000 mg Cu/kg.

Martínez et al. (1991) calculated the 60-min EC_{50}, based on ^{3}H-thymidine incorporation (a measure of bacterial heterotrophic activity), to be 32 µg Cu^{2+}/litre for naturally occurring bacteria from the river Rhone (Mediterranean Sea) plume. Tubbing et al. (1995) found EC_{50}s, based on ^{3}H-thymidine and ^{3}H-leucine incorporation and proteolytic activity, to be 28–100, 28–90 and 585–1997 µg Cu/litre, respectively.

Schreiber et al. (1985) exposed the marine bacterium *Vibrio alginolyticus* to copper under aerobic and anaerobic conditions. The copper concentration at which there was a 50% reduction in heat

production (TC_{50}) was used to compare the toxicity under aerobic and anaerobic conditions. Copper was more toxic to the bacterium in anaerobic culture (TC_{50} = 133 µg/litre (2.1 µmol/litre)) than in aerobic culture (TC_{50} = 406 µg/litre (6.4 µmol/litre)). The addition of organic chelators (EDTA and nitrilotriacetic acid) protected the anaerobic cultures from the toxic effects of copper, indicating that copper-organic complexes are not toxic to the bacterium.

9.3.1.2 Soil

Toxicity of copper to soil microorganisms is summarized in Table 17.

Chang & Broadbent (1981) calculated the threshold (EC_{10}) and EC_{50} concentrations, based on the inhibition of carbon dioxide production in a silt loam soil amended with alfalfa and sewage sludge, to be 4.2 and 22 mg/kg (65.6 and 347 nmol/g) for DTPA-extractable copper (bioavailable copper).

Rogers & Li (1985) incubated soil for 6 days in the presence of copper. EC_{50}s, based on inhibition of soil dehydrogenase activity, were 29 mg Cu/kg for soil enriched with 1% alfalfa and 53 mg Cu/kg for soil that was not enriched.

Lighthart et al. (1983) measured soil microbial respiration in five soil types after treatment with copper. After a 45-day incubation at 20 °C the lower level treatments (3.2 and 32 mg Cu/kg, 0.05 and 0.5 mmol/kg) had little effect, with mean inhibitions of less than 20%. Higher levels of 320 and 3200 mg Cu/kg (5 and 50 mmol/kg) inhibited respiration by up to 35% and 60%, respectively. Bremner & Douglas (1971) report that copper concentrations of 50 mg/kg inhibited soil urease activity by 13–16% following a 5-h incubation period.

Doelman & Haanstra (1984) found that short-term (2 weeks) exposures to copper (150–8000 mg/kg) caused decreases in the rate of soil respiration. Long-term (up to 18 months) exposure was less clear cut. In sand there was a significant decrease at copper concentrations of 400 mg/kg and in sandy peat there was a significant decrease at 1000 mg/kg. The effect of copper in silty loam and clay was less apparent with a significant decrease and increase at 8000 mg/kg for the

Table 17. Toxicity of copper to soil microorganisms

Organisms	Parameter	End-point	Concentration	Reference
Soil microorganisms	EC_{10} and EC_{50}	inhibition of CO_2 production	4.2 and 22 mg/kg for silt loam soil amended with alfalfa and sludge	Chang & Broadbent (1981)
	EC_{50}	inhibition of soil dehydrogenase activity	29 mg/kg for soil enriched with 1% alfalfa; 53 mg/kg for soil not enriched	Rogers & Li (1985)
	45-day EC_{50}	soil respiration	320 and 3200 mg/kg resulted in 35 and 60% inhibition	Lighthart et al. (1983)
	5-h EC_{50}	inhibition of soil urease activity	inhibition between 13% and 16%	Bremner & Douglas (1971)
	6-week EC_{50}	inhibition of urease activity	260 mg/kg in sand to 4200 mg/kg in sandy peat	Doelman & Haanstra (1986)

Table 17 (contd).

	18-month EC_{50} (significant reduction)	glutamic acid reduction time	55 mg/kg in sand to 1000 mg/kg in sandy peat	Haanstra & Doelman (1984)
	18-month ED_{50}	reduction of arylsulfatase activity	287 mg/kg in sand to 6991 mg/kg in sandy peat	Haanstra & Doelman (1991)
	6-month EC_{50}	microbial biomass	890 mg/kg in sandy loam; 4321 mg/kg in humus	Frostegard et al. (1993)
	15-week EC_{50}	population growth	up to 5000 mg/kg when exposed in soil; 10 mg/kg when exposed in agar	El-Sharouny et al. (1988)
Soil ciliate (*Colpoda cucculus*)	7-day EC_{10} and EC_{50}	population growth	331.5 and 971.6 µg/litre	Janssen et al. (1995)

two soil types, respectively. Doelman & Haanstra (1986) calculated EC_{50}s, based on inhibition of soil urease activity. After 6 weeks EC_{50}s were 260, 570, 1370 and 4200 mg Cu/kg for sand, saudy loam, clay and sandy peat, respectively, and after 18 months they were 680, 1990, 1080 and 1970 mg Cu/kg, respectively.

Haanstra & Doelman (1984) report that copper significantly reduced glutamic acid decomposition time, in an 18-month incubation, at 55 mg/kg in sand, at 400 mg/kg in silty loam and clay and at 1000 mg/kg in sandy peat. Haanstra & Doelman (1991) calculated 18-month ED_{50}s, based on reduction of arylsulfatase activity, raugiug from 287 mg Cu/kg (4.51 mmol/kg) iu sand to 6991 mg Cu/kg (110 mmol/kg) in sandy peat.

Frostegård et al. (1993) incubated forest humus and arable soil (sandy loam) with copper for 6 months at 22 °C. EC_{50}s, based on a decrease in the ATP content, were 4321 aud 890 mg Cu/kg (68 and 14 mmol/kg) for the two soils, respectively. An EC_{50}, based on a reduction in respiration, was > 8134 mg Cu/kg (> 128 mmol/kg) for forest humus. In both soil types, copper exposure caused gradual changes in thc phospholipid fatty acid composition.

El-Sharouny et al. (1988) studied thc effects of copper (500, 2000 or 5000 mg/kg) on soil mycoflora. Thc application of copper sulfate to the soil resulted in a significant increase in the count of total fungi after 1 week. There was little further increase after 5 weeks but at the end of the 15-week exposure there were significant increases. The increases were mainly due to *Aspergillus niger*, *A. sydowii*, *A. versicolor*, *Penicillium chrysogenum* and *Rhizopus stolonifer*. When similar species werc exposed via agar medium there were siguificant decreases at all copper exposures (10, 50 and 100 mg/kg), the highest exposure eliminating all but *Aspergillus niger* which survived at very low levels.

Janssen et al. (1995) fouud the 7-day EC_{10} and EC_{50} for thc soil ciliate *Colpoda cucculus*, based on population growth, to be 331.5 and 971.6 µg Cu/litre (5.22 and 15.3 µmol/litre), respectively.

9.3.2 Aquatic organisms

9.3.2.1 Plants

Care should be taken in interpreting published algal assay results for copper. Most of the algal assay EC_{50} results reported in the literature refer to studies of cell division rate carried out in full culture media. Culture media contain chemicals such as iron, manganese, citrate, silicate and EDTA which bind copper and reduce its toxicity. When the algal cells are removed from the culture medium, washed, and the assay carried out in a natural water (seawater or river water) the cell division rate is usually much more sensitive to copper (Stauber & Florence, 1987; Stauber, 1995). Acute toxicity of copper to freshwater and marine algae is summarized in Table 18.

Wurtsbaugh & Horne (1982) exposed a natural phytoplankton association from Clear Lake, California, USA, to copper for a period of 6 days. Chlorophyll *a* and nitrogen fixation were significantly reduced at copper concentrations of > 20 µg/litre and carbon fixation was significantly reduced at > 10 µg/litre. Biomass estimates indicated that the blue-green alga *Aphanizomenon flos-aquae* was more sensitive to copper than were diatoms.

Wong & Chang (1991) reported that copper concentrations of 250 µg/litre significantly reduced the growth rate of *Chlorella pyrenoidosa*: the alga did not grow at copper concentrations of 500 and 750 µg/litre. Photosynthetic rate and chlorophyll *a* during the log phase were significantly reduced at 100 µg Cu/litre.

Metaxas & Lewis (1991) found that the marine diatoms *Skeletonema costatum* and *Nitzschia thermalis* did not grow at total copper concentrations above 32 µg/litre (0.5 µmol/litre) and 38 µg/litre (0.6 µmol/litre), respectively. At lower concentrations *Skeletonema* showed increasing growth rate and lag phase with increasing copper concentrations whereas *Nitzschia* showed decreasing growth with increasing copper exposure.

Visviki & Rachlin (1994b) studied the effects of copper on the algae *Dunaliella salina* and *Chlamydomonas bullosa* in acute (96 h) and chronic (8 month) exposures. Acute exposures of 378 and 49.6 µg Cu/litre (5.94 and 0.78 µmol/litre) for the two species, respectively,

Table 18. Toxicity of copper to algae

Organism	Conditions[a]	Temperature (°C)	Copper salt	Parameter	End-point	Concentration (µg/litre)	NOEC (µg/litre)	Reference
Green alga (*Chlamydomonas reinhardii*)	stat	20	sulfate	72-h EC_{50}	growth inhibition	79	5	Schäfer et al. (1994)
	flow	24	sulfate	96-h EC_{50}	growth inhibition	47	ND	Schäfer et al. (1993)
Green alga (*Selenastrum capricornutum*)	stat	24–26	sulfate	72-h EC_{50}	growth inhibition	47	ND	Nyholm (1990)
	stat	24–26	sulfate	72-h EC_{50}	biomass	35	ND	Nyholm (1990)
Marine alga (*Chlamydomonas bullosa*)		15	chloride	96-h EC_{50}	growth inhibition	50	ND	Visviki & Rachlin (1994a)
Green alga (*Scenedesmus subspicata*)	stat	20	sulfate	72-h EC_{50}	growth inhibition	120	5.6	Schäfer et al. (1994)
Marine alga (*Dunaliella minuta*)	ND	15	chloride	96-h EC_{50}	growth inhibition	481	ND	Visviki & Rachlin (1991)
Marine alga (*Dunaliella salina*)	ND	15	chloride	96-h EC_{50}	growth inhibition	377	ND	Visviki & Rachlin (1994a)

[a] Stat = static conditions (water unchanged for duration of test); flow = flow-through conditions (copper concentration in water continuously maintained); ND = no data available.

had no significant effect on the ultrastructure of cells. However, chronic exposure (0.03 µg Cu/litre (4.9×10^{-4} µmol/litre)) caused significaut increases in lipid number and relative volume of *Dunaliella* and significant increases in cell volume, and decreases in periplasmalemmal space and cell wall relative volumes in *Chlamydomonas*.

A 50% reduction in the total algal cell volume of *Selenastrum capricornutum* in standard algal assay medium (SAAM) occurred at 85 µg Cu/litre after 14 days. For *Chlorella stigmatophora* growu in 28‰ artificial seawater plus SAAM for 21 days a value of 70 µg Cu/litre was found for the same parameter (Christensen et al., 1979).

Winner & Owen (1991a) found that copper (20 and 40 µg/litre) caused significant reductions in community richness of phytoplankton exposed for 5 week periods during different seasons of the year. Copper significantly changed the algal divisions (percentage composition of total phytoplankton) during the spring and autumn but not during the summer.

Winner & Owen (1991b) exposed the green alga *Chlamydomonas reinhardii* to copper in 72-h tests. The NOECs based on deflagellation and changes in cell density varied from 12.2 to 49.1 µg Cu/litre and from 12.2 to 43.0 µg Cu/litre for the two parameters, respectively.

Schäfer et al. (1993) found 7-day and 10-day EC_{50}s, based on growth inhibition, to be 31.5 µg Cu/litre for the green alga *Chlamydomonas reinhardii* in flow-through tests with copper sulfate.

Shanmukhappa & Neelakantan (1990) exposed the unicellular algae *Synechosystis aquatilis* to copper. They found 6-h EC_{50}s, based on chlorophyll reduction, were 650 µg Cu/litre. A slightly reduced EC_{50} (720 µg Cu/litre) was found when algae were exposed to copper in the presence of humic acid (10 µg/litre).

There are several studies which have assessed the effects of copper ou varions marine algae. Hall et al. (1979) found that the growth rate (as measured by an increase in wet weight) of *Ectocarpus siliculosus* (a tolerant strain) decreased from a mean value of 756% in controls to 86% in algae exposed to 500 µg Cu/litre. The nontolerant strain was

unable to grow under the two experimental copper exposures (250 and 500 µg/litre).

Reed & Moffat (1983) studied the responses of tolerant and nontolerant isolates of the green alga *Enteromorpha compressa* to copper concentrations of up to 610 µg/litre (9.6 µmol/litre). They found that none of the physiological processes that were tested (cell viability, net photosynthesis, intracellular potassium and dimethylsulfoniopropionate content) were affected by the highest exposure concentration with the tolerant isolate. However, the nontolerant isolate showed symptoms of copper toxicity at all copper exposures ranging from 114 to 610 µg/litre (1.8 to 9.6 µmol/litre). The authors concluded that this copper tolerance was genetically determined as the progeny retained the same pattern of response to copper enrichments. On the other hand, Correa et al. (1996) found that the progeny of copper-tolerant isolates of *Enteromorpha compressa* from northern Chile responded in the same manner as the progeny of non-tolerant isolates of the same species. Chilean *Enteromorpha compressa* grew well at 100 µg Cu/litre (from a copper-polluted site) and at 10 µg Cu/litre (from a nonpolluted site) and it was concluded that physiological plasticity rather than genotype was involved in tolerance to copper.

Stauber & Florence (1987) found that copper ions depressed both cell division and photosynthesis in the marine diatom *Asterionella glacialis* (101.6 µg Cu/litre; 1.6×10^{-6} mol/litre) and the freshwater green alga *Chlorella pyrenoidosa* (63.5 µg Cu/litre; 10×10^{-7} mol per litre). Ionic copper concentrations (176.5 µg Cu/litre; 27.8×10^{-7} mol per litre) which were inhibitory to cell division in the marine diatom *Nitzchia closterium* had no effect on photosynthesis, respiration, ATP production, electron transport or membrane ultrastructure. The authors suggest that the main toxic effect of copper on *N. closterium* is to act within the cytosol; a different toxic mechanism was apparently operating with *A. glacialis* and *C. pyrenoidosa* because both cell division and photosynthesis were affected by copper. Lipid-soluble organocopper complexes were found to be much more toxic than ionic copper. Stauber & Florence (1985a,b) showed that the toxicity of ionic copper to *Nitzchia closterium* was reduced by the addition of manganese(III) and iron(III) hydroxides to the culture medium. Stauber & Florence (1987) demonstrated that the addition to the algal growth medium of trivalent metal ions such as aluminium, iron, chromium, or divalent metals such as manganese and cobalt (which can

be oxidized by algae to trivalent species) reduced the toxicity of copper ions. The trivalent species form a layer of hydrated metal oxide around the cell which adsorbs copper ions.

Chung & Brinkhuis (1986) assessed the effects of copper at 5, 10, 50, 100 and 500 µg/litre on the early stages of development in the kelp *Laminaria saccharina*. It was found that the release of meiospores from copper-tested sorus materials was reduced by concentrations of 50 µg Cu/litre. Settlement and germination of meiospores were not affected by concentrations of up to 500 µg Cu/litre. Development of gametophytes and gametogenesis were delayed at concentrations of > 50 µg Cu/litre. Growth of the sporophytes was inhibited at concentrations > 10 µg Cu/litre. Hopkin & Kain (1978) found that sporophyte growth and gametophyte germination of *Laminaria hyperborea* were inhibited at 10 and 100 µg Cu/litre, respectively.

Brown & Rattigan (1979) exposed the pondweeds *Elodea canadensis* and *Lemna minor* to copper. A 24-h IC_{50} (photosynthetic oxygen evolution) was calculated to be 150 µg Cu/litre for *Elodea*. In 28-day tests copper concentrations of 3100 and 130 µg/litre caused 50% plant damage in *Elodea* and *L. minor*, respectively. Dirilgen & Inel (1994) calculated a 7-day IC_{50}, based on frond growth, to be 1540 µg Cu/litre for the duckweed *Lemna minor*.

9.3.2.2 *Invertebrates*

Acute and short-term toxicity

The acute toxicity of copper to freshwater and marine invertebrates are summarized in Tables 19 and 20, respectively. For freshwater invertebrates 48-h $L(E)C_{50}$s range from 5 µg Cu/litre for a daphnid species to 5300 µg Cu/litre for an ostracod; 96-h LC_{50}s for marine invertebrates range from 29 µg Cu/litre for the bay scallop to 9400 µg Cu/litre for the fiddler crab.

Kaitala (1988) reported an 8-day LC_{50} for mussels (*Mytilus edulis*) at 127 µg Cu/litre and a 10-day LC_{50} for clams (*Macoma baltica*) at 54 µg Cu/litre. Beaumont et al. (1987) exposed veliger larvae of common mussel (*M. edulis*) and scallop (*Pecten maximus*) to copper for 15 days. LC_{50}s were calculated to be 400 and 85 µg Cu/litre for the two species, respectively.

Table 19. Acute toxicity of copper to freshwater invertebrates (24-h to 96-h L(E)C_{50}s)[a]

Organism	Size/ age	Conditions[b]	Temperature (°C)	Hardness (mg $CaCO_3$/litre)	pH	Copper salt	Parameter	Concentration (µg/litre)	Reference
Snails									
Amnicola sp.	eggs	stat	17	50	7.6	ND	24-h LC_{50}	4500	Rehwoldt et al.
	eggs	stat	17	50	7.6	ND	96-h LC_{50}	9300	(1973)
	adult	stat	17	50	7.6	ND	24-h LC_{50}	1500	Rehwoldt et al.
	adult	stat	17	50	7.6	ND	96-h LC_{50}	900	(1973)
Goniobasis livescens	ND	stat	15	154	8.5	sulfate	96-h LC_{50}	390	Paulson et al. (1983)
Lithoglyphus virens	ND	flow	15	22	7.2	chloride	96-h LC_{50}	8	Nebeker et al. (1986)
Juga plecifera	ND	flow	15	22	7.2	chloride	96-h LC_{50}	15	Nebeker et al. (1986)
Physa integra	ND	flow	15	45	7.7	sulfate	96-h LC_{50}	39	Arthur & Leonard (1970)
Campeloma decisum	ND	flow	15	45	7.7	sulfate	96-h LC_{50}	1700	Arthur & Leonard (1970)

Table 19 (contd).

Gastropod									
Potamopyrgus jenkinsi	juvenile	flow	15	298	8.0	sulfate	48-h LC_{50}	58	Watton & Hawkes (1984)
	juvenile	flow	15	298	8.0	sulfate	96-h LC_{50}	54	
	prime adult	flow	15	298	8.0	sulfate	48-h LC_{50}	112	
	prime adult	flow	15	298	8.0	sulfate	96-h LC_{50}	77	
	senescent adult	flow	15	298	8.0	sulfate	48-h LC_{50}	87	
	senescent adult	flow	15	298	8.0	sulfate	96-h LC_{50}	79	
Bristle worm									
Nais sp.	ND	stat	17	50	7.6	ND	24-h LC_{50}	2300	Rehwoldt et al. (1973)
	ND	stat	17	50	7.6	ND	96-h LC_{50}	90	
Oligochaete									
Lumbriculus variegatus	ND	stat	25	290	6.6	nitrate	96-h LC_{50}	130	Shubauer-Berigan et al. (1993)
	ND	stat	25	290	7.3	nitrate	96-h LC_{50}	270	
	ND	stat	25	290	8.3	nitrate	96-h LC_{50}	500	
Water fleas									
Daphnia magna	6–24 h	stat	25	80–100	7.4–7.8	sulfate	24-h LC_{50}	380	Ferrando et al. (1992)
	ND	stat	17–19	44–53	7.4–8.2	chloride	48-h EC_{50}	9.8	Biesinger & Christensen (1972)
	ND	stat	17–19	44–53	7.4–8.2	chloride	48-h EC_{50}	60 with food	
	< 24 h	stat	21.6	143	7.1–7.9	oxide	48-h EC_{50}	26	Lewis (1983)
	ND	stat	ND	175	8.1	sulfate	48-h EC_{50}	23–27[c]	LeBlanc (1982)
	< 24 h	stat	20	ND	6.5	sulfate	48-h LC_{50}	7	Oikari et al. (1992)
	< 24 h	stat	20	ND	6.5	sulfate	48-h LC_{50}	45 humic water	

Table 19 (contd).

Organism	Size/ age	Conditions[b]	Temperature (°C)	Hardness (mg $CaCO_3$/litre)	pH	Copper salt	Parameter	Concentration (µg/litre)	Reference
D. magna (contd).	< 24 h	stat	ND	45	7.2–7.4		48-h LC_{50}	54	Mount &
	< 24 h	stat	ND	45	7.2–7.4		48-h LC_{50}	53	Norberg (1984)
	< 24 h	stat	20	130–160	8.2–9.5	sulfate	72-h LC_{50}	86.5	Winner &
Daphnia pulex	< 24 h	stat	20	130–160	8.2–9.5	sulfate	72-h LC_{50}	86	Farrell (1976)
Daphnia parvula	< 24 h	stat	20	130–160	8.2–9.5	sulfate	72-h LC_{50}	72	Winner &
Daphnia ambigua	< 24 h	stat	20	130–160	8.2–9.5	sulfate	72-h LC_{50}	67.7	Farrell (1976)
	ND	stat$	28.5	200	7.9		48-h LC_{50}	54.6	Vardia et al.
Daphnia lumholtzi	ND	ND	ND	ND	ND	ND	96-h LC_{50}	9.4	(1988)
Daphnia hyalina	1.27 mm	stat	10	ND	7.2	chloride	48-h LC_{50}	5	Baudouin & Scoppa (1974)
Moina irrasa	< 24 h	stat	20	< 5	8.0	chloride	48-h LC_{50}	5.9	Zou & Bu (1994)
Moina macrocopa	ND	stat$	24–27	ND	6.5	sulfate	48-h LC_{50}	80	Wong (1992)
Ceriodaphnia dubia	< 12 h	stat	25	57	8.2	sulfate	48-h LC_{50}	13.4	Oris et al. (1991)
	< 12 h	stat	25	45	7.7	ND	48-h LC_{50}	17–32[d]	Belanger et al.
	< 12 h	stat	25	179	8.3	ND	48-h LC_{50}	67[e]	(1989)
	< 12 h	stat	25	94	8.15	ND	48-h LC_{50}	34–37[d]	Belanger et al.
	< 12 h	stat	25	179	8.3	ND	48-h LC_{50}	78–81[e]	(1989)

Table 19 (contd).

C. dubia (contd).	ND	stat	25	290	6.2	nitrate	48-h LC_{50}	9.5	Schubauer-Berigan et al. (1993)
	ND	stat	25	290	7.1	nitrate	48-h LC_{50}	28	
	ND	stat	25	290	8.6	nitrate	48-h LC_{50}	200	
Ceriodaphnia reticulata	< 4 h	stat	ND	45	7.2–7.4	ND	48-h LC_{50}	17	Mount & Norberg (1984)
Simocephalus vetulus	< 24 h	stat	ND	45	7.2–7.4	ND	48-h LC_{50}	57	Mount & Norberg (1984)
Copepods									
Cyclops abyssorum	1.27 mm	stat	10	ND	7.2	chloride	48-h LC_{50}	2500	Baudouin & Scoppa (1974)
Eudiaptomus padanus	1.27 mm	stat	10	ND	7.2	chloride	48-h LC_{50}	500	Baudouin & Scoppa (1974)
Amphipods	ND	stat	17	50	7.6	ND	24-h LC_{50}	1200	Rehwoldt et al. (1973)
Gammarus sp.	ND	stat	17	50	7.6	ND	96-h LC_{50}	910	
Gammarus pulex	3–5 mm	stat$	12	151	6.8–7.2	ND	48-h LC_{50}	47	Taylor et al. (1991)
	3–5 mm	stat$	12	151	6.8–7.2	ND	96-h LC_{50}	37	
	ND	stat$	ND	104	8.33	ND	48-h LC_{50}	41	Stephenson (1983)
	ND	stat$	ND	104	8.33	ND	96-h LC_{50}	21	

Table 19 (contd).

Organism	Size/ age	Conditions[b]	Temperature (°C)	Hardness (mg $CaCO_3$/litre)	pH	Copper salt	Parameter	Concentration (µg/litre)	Reference
G. pulex (contd).	ND	stat$	ND	249	8.33	ND	48-h LC_{50}	183	Stephenson (1983)
	ND	stat$	ND	249	8.33	ND	96-h LC_{50}	109	
Gammarus italicus	ND	stat	8	240	8.1	chloride	24-h LC_{50}	179	Pantani et al. (1990)
Gammarus pseudolimnaeus	ND	flow	15	45	7.7	sulfate	96-h LC_{50}	20	Arthur & Leonard (1970)
Hyallela azteca	ND	stat	25	290	6.2	nitrate	96-h LC_{50}	17	Scubauer-Berigan et al. (1993)
	ND	stat	25	290	7.1	nitrate	96-h LC_{50}	24	
	ND	stat	25	290	8.4	nitrate	96-h LC_{50}	87	
Echinogammarus tibaldii	ND	stat	7.5 8.5	240	7.9	chloride	96-h LC_{50}	720	Pantani et al. (1995)
Crangonyx pseudogracilis	4 mm	stat	13	50	6.75	sulfate	48-h LC_{50}	2440	Martin & Holdich (1986)
	4 mm	stat	13	50	6.75	sulfate	96-h LC_{50}	1290	
Isopod *Asellus aquaticus*	7 mm	stat	13	50	6.75	sulfate	96-h LC_{50}	9210	Martin & Holdich (1986)

Table 19 (contd).

Ostracod *Cypris subglobosa*	ND	statS	28.5	200	7.9	ND	48-h LC_{50} 96-h LC_{50}	5363 277.3	Vardia et al. (1988)
Rotifers *Brachionus calyciflorus*	juvenile	stat	20	36.2	7.3	sulfate	24-h LC_{50}	200	Couillard et al. (1989)
	ND	stat	25				24-h LC_{50}	26	Snell et al. (1991)
	ND	stat	25	80–100	7.4–7.8	sulfate	24-h LC_{50}	76	Ferrando et al. (1992)
Brachionus rubens	neonate	stat	25	80–100	7.4–7.8	sulfate	24-h LC_{50}	19	Snell & Persoone (1989b)
Keratella cochlearis	ND	stat	20	ND	8.3	chloride	24-h LC_{50}	101	Borgmann & Ralph (1984)
Anostracan crustacean *Streptocephalus proboscideus*	2nd/3rd instar	ND	20	8–10	6.4–6.6	sulfate	24-h LC_{50}	120	Centeno et al. (1993)
	2nd/3rd instar	ND	20	71–110	7.6–7.8	sulfate	24-h LC_{50}	210	Centeno et al. (1993)

Table 19 (contd).

Organism	Size/ age	Conditions[b]	Temperature (°C)	Hardness (mg $CaCO_3$/litre)	pH	Copper salt	Parameter	Concentration (µg/litre)	Reference
S. proboscideus (contd).	2nd/3rd instar	ND	20	250–327	7.9–8.2	sulfate	24-h LC_{50}	520	Centeno et al. (1993)
Freshwater shrimp	adult	flow	15	17	6.96	sulfate	96-h LC_{50}	34 (total Cu)	Daly et al. (1990a)
Paratya australiensis	adult	flow	15	17	6.96	sulfate	96-h LC_{50}	16 (Cu ion)	Daly et al. (1990a)
Midges									
Chironomus sp.	ND	stat	17	50	7.6	ND	24-h LC_{50}	650	Rehwoldt et al. (1973)
	ND	stat	17	50	7.6	ND	96-h LC_{50}	30	
Chironomus tentans	3rd instar	stat	13	25	6.3	sulfate	48-h EC_{50}	327	Khangarot & Ray (1989)
	1st instar	stat	19–22	42.7	7.6	sulfate	96-h EC_{50}	16.7	Gauss et al. (1985)
	1st instar	stat	19–22	109.6	7.8	sulfate	96-h EC_{50}	36.5	
	1st instar	stat	19–22	172.3	8.1	sulfate	96-h EC_{50}	98.2	
	4th instar	stat	19–22	42.7	7.6	sulfate	96-h EC_{50}	211	Gauss et al. (1985)
	4th instar	stat	19–22	109.6	7.8	sulfate	96-h EC_{50}	977	
	4th instar	stat	19–22	172.3	8.1	sulfate	96-h EC_{50}	1184	

Table 19 (contd).

C. tentans (contd).	1st instar	stat$	20	71	ND	chloride	96-h LC_{50}	298	Nebeker et al. (1984a)
	2nd instar	flow	20	84	ND	chloride	96-h LC_{50}	773	
	3rd instar	flow	20	84	ND	chloride	96-h LC_{50}	1446	Nebeker et al. (1984a)
	4th instar	flow	20	84	ND	chloride	96-h LC_{50}	1690	
Chironomus decorus	4th instar	stat	20	40–48	7.2–7.6	sulfate	48-h LC_{50}	739	Kosalwat & Knight (1987a)
Chironomus riparius	2nd instar	stat$	12	151	6.8 7.2		48-h LC_{50}	1200	Taylor et al. (1991)
	2nd instar	stat$	12	151	6.8–7.2		96-h LC_{50}	700	

[e] EC_{50}s based on immobilization; ND = no data available.
[f] Stat = static conditions (water unchanged for duration of test); stat$ = static renewal conditions (water changed at regular intervals); flow = flow-through conditions (copper concentration in water continuously maintained).
[g] Range of means for three duplicate tests.
[h] Range of tests from different culture sources; parental diet was synthetic.
[i] Range of tests from different culture sources; parental diet was algal.

Table 20. Acute toxicity of copper to marine invertebrates (24-h to 96-h L(E)C_{50}s)[a]

Organism	Size/age	Conditions[b]	Temperature (°C)	Salinity (‰)	pH	Salt	Parameter	Concentration (µg/litre)	Reference
Bay scallop *Argopecten irradians*	juvenile	statS	20	25	ND	chloride	96-h LC_{50}	29	Nelson et al. (1988)
Surf clam *Spisula solidissima*	juvenile	statS	20	25	ND	chloride	96-h LC_{50}	51	Nelson et al. (1988)
Squid *Loligo opalescens*	larvae	stat	8.6	30	8.1	chloride	96-h LC_{50}	309	Dinnell et al. (1989)
Cabezon *Scorpaenichthys marmoratus*	larvae	stat	8.3	27	7.9	chloride	96-h LC_{50}	95	Dinnell et al. (1989)
Amphipod *Allorchestes compressa*	24 h adult	stat stat	20 20	32 32	ND ND	sulfate sulfate	96-h LC_{50} 96-h LC_{50}	110 500	Ahsanullah & Florence (1984)
American lobster *Homarus americanus*	larvae	stat	20	30.5	ND	nitrate	96-h LC_{50}	48	Johnson & Gentile (1979)

Table 20 (contd).

Dungeness crab *Cancer magister*	larvae	stat	8.5	30	8.1	chloride	96-h LC_{50}	96	Dinnell et al. (1989)
Fiddler crab	24–29 mm	stat	29	25	ND	sulfate	96-h LC_{50}	9420	Devi (1987)
Uca annulipes	24–29 mm	stat	29	25	ND	sulfate	96-h LC_{50}	12 820[c]	Devi (1987)
Fiddler crab	24–29 mm	stat	29	25	ND	sulfate	96-h LC_{50}	8380	Devi (1987)
Uca triangularis	24–29 mm	stat	29	25	ND	sulfate	96-h LC_{50}	14 810[c]	Devi (1987)
Ragworm	20 mm	stat	12	7.3	ND	nitrate	96-h LC_{50}	357	Ozoh (1992a)
Hediste	20 mm	stat	12	29.2	ND	nitrate	96-h LC_{50}	512	Ozoh (1992a)
diversicolor	20 mm	stat	22	7.3	ND	nitrate	96-h LC_{50}	247	Ozoh (1992a)
	20 mm	stat	22	29.2	ND	nitrate	96-h LC_{50}	500	Ozoh (1992a)
Copepods									
Tisbe battagliai	nauplius	statS	20	34–35	6.2–8.2	nitrate	96-h LC_{50}	64	Hutchinson et
	adult	statS	20	34–35	6.2–8.2	nitrate	96-h LC_{50}	88	al. (1994)
Tisbe holothuriae	ND	stat	22	38	ND	sulfate	48-h LC_{50}	370	Verriopoulos & Dimas (1988)
Rotifer									
Brachionus	neonate	stat	25	15	7.7	ND	24-h LC_{50}	120	Snell &
plicatilis	neonate	stat	25	30	7.7	ND	24-h LC_{50}	130	Persoone (1989a)

Table 20 (contd).

Organism	Size/age	Conditions[b]	Temperature (°C)	Salinity (‰)	pH	Salt	Parameter	Concentration (µg/litre)	Reference
Sand shrimp *Crangon* sp.	adult	flow	13.7	30.1	7.9	chloride	96-h LC_{50}	898	Dinnell et al. (1989)
Penaeid shrimp *Metapenaeus ensis*	protozoeal	stat	27	30–34	8.7	sulfate	48-h LC_{50}	160	Wong et al. (1993)
	mysid	stat	27	30–34	8.7	sulfate	48-h LC_{50}	1580	
	postlarva	stat	27	30–34	8.7	sulfate	48-h LC_{50}	4760	
Mysid shrimp *Holmesimysis costata*	3 days	stat		ND	ND	chloride	96-h LC_{50}	17	Martin et al. (1989)
Grass shrimp *Palaemonetes pugio*	juvenile	stat$	20	10	ND	ND	48-h LC_{50}	2100	Burton & Fisher (1990)
	ND	stat	22	25	8.3–8.7	acetate	96-h LC_{50}	3700	Curtis et al. (1979)

[a] EC_{50}s based on immobilization; ND = no data available.
[b] Stat = static conditions (water unchanged for duration of test); stat$ = static renewal conditions (water changed at regular intervals); flow = flow-through conditions (copper concentration in water continuously maintained).
[c] Animals collected from a polluted site (6.8–30.6 µg Cu/litre).

Centeno et al. (1993) studied the effect of temperature (10–30 °C) on the 24-h LC_{50} of copper on the third instar of the crustacean *Streptocephalus proboscideus*. A significant increase in toxicity was observed at the highest temperature tested. Zou & Bu (1994) observed an increase in the acute toxicity of copper to the water flea *Moina irrasa* with increasing temperature (20–30 °C). Snell et al. (1991) found that the acute toxicity of copper to the rotifer *Brachionus calyciflorus* was significantly increased at 10, 25 and 30 °C when compared with tests at both 15 and 20 °C.

Ozoh (1992c) found that sediment affected the acute response of both juvenile and adult ragworms (*Hediste diversicolor*) to copper. Without sediment, increasing salinity (7.3 to 30.5‰) and increasing temperature (12 to 22 °C) reduced the acute toxicity of copper, whereas in the presence of sediment increasing temperature and increasing salinity increased the acute toxicity of copper.

Snell & Persoone (1989a,b) exposed neonate rotifers to copper for 24 h; NOEC was 20 µg Cu/litre at a salinity of 15‰, 50 µg Cu/litre at 30‰ for the marine rotifer *Brachionus plicatilis* and 9.4 µg/litre for the freshwater rotifer *Brachionus rubens*. Ozoh & Jones (1990) studied the effects of salinity and temperature on the toxicity of copper to ragworm (*Hediste diversicolor*) in 96-h tests. Larvae at 1 day old were more susceptible to copper than at 7 days old. Increasing salinity from 7.6 to 30.5‰ reduced copper toxicity.

Stephenson (1983) reported that copper was 4–6 times more toxic to *Gammarus pulex* in soft water (100 mg $CaCO_3$/litre) than in hard water (250 mg $CaCO_3$/litre). Similar findings were reported by Gauss et al. (1985) for first and fourth instar midge *Chironomus tentans* exposed to copper in acute toxicity tests. First instar larvae were the most sensitive with 96-h EC_{50}s, based on immobilization, at 16.7 µg Cu/litre in soft water (40 mg $CaCO_3$/litre) and 98.2 µg Cu/litre in hard water (170 mg $CaCO_3$/litre). The 48-h LC_{50}s of copper to *Ceriodaphnia dubia* increased from 35 to 79 µg/litre when the water hardness was increased from 94 to 170 mg $CaCO_3$/litre (Belanger et al., 1989). Increasing the hardness from 8–10 to 250–327 mg $CaCO_3$/litre decreased the 24-h LC_{50} of copper to the third instar of the crustacean *Streptocephalus proboscideus* irrespective of the temperature (Centeno et al., 1993). In similar tests a significant increase in toxicity was

noted at pH 6.0 when compared with tests carried out at pHs ranging from 7.6 to 10.0.

Shaner & Knight (1985) found that alkalinity had a significant effect on the acute toxicity of copper-bearing sediments to *Daphnia magna*. The 24-h LC_{50}s were 1332 and 1578 mg/kg at alkalinities of 600 and 1000 mg bicarbonate/litre, respectively. LC_{50}s predicted by multiple regression models calculated levels ranging from 1146 to 5966 mg/kg at alkalinities ranging from 571 to 2286 mg bicarbonate/litre.

The addition of humic acid reduced the acute toxicity of copper to daphnids. The mean 72-h LC_{50} increased from 28.3 µg Cu/litre in water containing no added humic acid to 53.2 µg Cu/litre in water to which 1.5 mg humic acid/litre had been added (Winner, 1984). Giesy et al. (1983) showed a positive correlation between LC_{50} values for exposure of *Simocephalus serrulatus* to copper and total dissolved carbon concentration. Pantani et al. (1995) reported similar results for the amphipod *Echinogammarus tibaldii* with the acute toxicity of copper being reduced by the addition of humic acid, fluvial sediment or bentonite. Humic acid significantly reduced the acute toxicity of copper to *Daphnia pulex* at all levels of water hardness tested (58, 115, and 230 mg $CaCO_3$/litre) (Winner, 1985). Winner (1984) exposed *Daphnia pulex* to 30 µg Cu/litre in the presence of varying amounts of humic acid for up to 30 days. Daphnids showed significantly better survival in water containing 0.38, 0.75 or 1.50 mg humic acid/litre than in water to which no humic acid had been added. In the absence of humic acid only one brood of young was produced because of premature deaths of females. The addition of 0.38 mg humic acid/litre produced 49 broods but the mean brood size was significantly smaller than humic acid controls. Daphnids maintained on 30 µg Cu/litre and 1.50 mg humic acid/litre produced 101 broods with brood sizes significantly larger than humic acid controls. Meador (1991) determined that humic acid decreased the toxicity of *Daphnia magna* on the basis of total copper, but toxicity was constant on the basis of cupric ion activity. In contrast, Borgmann & Ralph (1983) and Borgmann & Charlton (1984) found that free metal concentrations did not provide a constant measure of copper toxicity to *Daphnia magna* as organic matter concentrations changed.

McLeese & Ray (1986) found the toxicity of copper to the marine shrimps *Crangon septemspinosa* and *Pandalus montagui* to be reduced when complexed with EDTA. LC_{50}s (144-h) were 1400 and 50 µg $CuCl_2$/litre for the two species, respectively, whereas LC_{50}s for copper–EDTA complexes were > 30 000 µg/litre. However, 144-h LC_{50}s for the clam *Macoma balthica* were 6000 µg/litre regardless of the form of copper exposure. In solutions containing nitrilotriacetic acid (NTA) or glycine, uncomplexed copper(II) ions were found to be the most acutely toxic form of copper to the freshwater shrimp *Paratya australiensis*. However, although the copper–NTA complex did not contribute to the toxic effect, the copper–glycine complex appears to be mildly toxic (Daly et al., 1990a). The acute toxicity of copper to *P. australiensis* was shown to decrease in solutions of increasing alkalinity. Additional experiments revealed that the tolerance to copper at higher alkalinities was caused by a combination of physiological effects associated with increased ionic strength of the test waters and changes in metal speciation (Daly et al., 1990b). The presence of natural organic matter significantly reduced the toxicity of copper to *P. australiensis* (Daly et al., 1990c). Daly et al. (1992) found that post-moult shrimps were more sensitive to acute copper toxicity than individuals at other stages of the moult cycle.

Baird et al. (1991) found that the 48-h EC_{50} for different clones of *Daphnia magna* ranged from 10.5 to 70.7 µg Cu/litre. Pre-exposure of daphnids (*Daphnia magna*) to 10 µg Cu/litre resulted in a significant reduction in the subsequent acute toxicity: 48-h LC_{50}s in pre-exposed animals ranged from 58 to 80 µg Cu/litre whereas those of unexposed daphnids ranged from 23 to 27 µg Cu/litre (LeBlanc, 1982).

Collyard et al. (1994) found little effect of age class (0.2–22 days) on the acute (96-h) toxicity of copper to the amphipod *Hyallela azteca*. With the exception of the 6–8-day age class, which appeared to be the most sensitive to copper, the 95% confidence limits overlapped for the different age groups. The 48-h LC_{50}s for the penaeid shrimp *Metapenaeus ensis* at different developmental stages were 160 µg Cu/litre (protozoaeal), 1580 µg Cu/litre (mysid) and 4760 µg Cu/litre (post-larval), showing that tolerance to acute copper toxicity increased with age (Wong et al., 1993).

Sosnowski et al. (1979) found that the sensitivity (72-h LC_{50}) of the copepod *Acartia tonsa* was strongly correlated with field population density and food ration. The 72-h LC_{50}s ranged from 9.0 to 78.0 µg Cu/litre. There was an inverse correlation between the log LC_{50} and adult *A. tonsa* density at the time of collection. The log LC_{50} increased with increasing food ration. Lewis (1983) exposed *Daphnia magna* to copper in 48-h static toxicity tests at six different loading densities. There was a trend of increasing toxicity at the lower density levels. However, the differences in LC_{50} values were not biologically significant, with the maximum difference being approximately threefold.

Dave (1984) found the 48-h EC_{50}, based on immobilization, for unfed and fed daphnids (*Daphnia magna*) to be 6.5 and 18.5 µg Cu/litre, respectively. Neonates of *Ceriodaphnia dubia* from mothers reared on an algal diet were 1.4–1.5 times more resistant to copper in acute toxicity tests than those reared on a synthetic diet (Belanger et al., 1989).

Nell & Chvojka (1992) reported that copper concentrations of 8 µg/litre significantly reduced the growth of Sydney rock oysters (*Saccostrea commercialis*) in 4-week studies. Exposure of oysters to copper concentrations ranging from 8 to 64 µg/litre and 20 ng trihutyl tin oxide/litre showed an additive effect on growth.

Bodar et al. (1989) exposed parthenogenetic eggs of *Daphnia magna* to copper concentrations of 1.0, 10 and 25 mg/litre. Copper exposure at concentrations exceeding 1.0 mg/litre significantly reduced the total development of daphnid eggs. However, stages 1 and 2 (which take about half of the development time from egg to juvenile) showed only a slight decrease in the mean lifetime of individuals in these stages at copper concentrations of 10 and 25 mg/litre. Therefore the toxicity of copper, apparent in the total developmental effect, is exerted at stages 3–6.

Hutchinson et al. (1994) exposed copepods (*Tisbe battagliai*) to copper for 7 days. NOECs for nauplius survival, adult survival and reproduction were 10, 18 and 6 µg Cu/litre, respectively; LOECs were 18, 32 and 10 µg Cu/litre, respectively. A subchronic value was calculated as the geometric mean of the highest NOEC and the lowest

LOEC; these were 13 µg Cu/litre for nauplius survival, 24 µg Cu/litre for adult survival and 8 µg Cu/litre for reproduction.

Long-term and reproductive toxicity

Ringwood (1992) exposed gametes and early life stages of sea urchins (*Echinometra mathaei*) and the bivalve *Isognomon californicum* to copper. EC_{50}s, based on fertilization, for sea urchins (1 h) and bivalves (2 h) were 14 and 55 µg Cu/litre, respectively; NOECs were 5.0 and 20 µg Cu/litre, respectively. A 48-h EC_{50} (embryo survival) was 7.0 µg Cu/litre with a NOEC of 1.0 µg Cu/litre. A NOEC for bivalve growth (96 h) was 1.0 µg Cu/litre.

Strømgren & Nielsen (1991) studied the effect of copper on spawning, growth and mortality in larval, juvenile and mature common mussel (*Mytilus edulis*). EC_{50}s, based on larval growth (10 days) and adult spawning frequency (30 days), were 5–6 and 2 µg Cu/litre, respectively. The larval 10-day LC_{50} was estimated to be approximately 10 µg Cu/litre.

Macdonald et al. (1988) exposed yellow crab (*Cancer anthonyi*) embryos to copper in 7-day tests. Copper concentrations $\geq$ 1000 µg/litre significantly reduced survival. Hatching of embryos and larval survival were significantly reduced at 10 µg Cu/litre; no embryos hatched at copper concentrations of 100 µg/litre or more.

Biesinger & Christensen (1972) exposed *Daphnia magna* to copper for 3 weeks. A 3-week LC_{50} of 44 µg Cu/litre was found; the 3-week EC_{50}, based on reproductive impairment, was 35 µg Cu/litre. Dave (1984) reports a 21-day EC_{50} (immobilization) of 1.4 µg Cu/litre.

Cowgill & Milazzo (1991) carried out a three-brood toxicity test on *Ceriodaphnia dubia*. EC_{50}s based on total progeny, mean brood number and mean brood size were found to be 357, 348 and 326 µg Cu/litre for metallic copper, and 305, 341 and 304 µg Cu/litre for copper nitrate.

Oris et al. (1991) studied the effects of copper on the reproduction of the cladoceran *Ceriodaphnia dubia*. Chronic survival values, which were calculated as the geometric mean between NOEC and LOEC,

were 34.6 and 24.5–34.6 µg Cu/litre for 4- and 7-day tests, respectively. EC_{50}s, based on mean total young per female, were 38.2–40.4 and 30.7–30.8 µg Cu/litre for the two tests, respectively.

In 21-day tests *Daphnia magna* showed 100% mortality at 110 µg Cu/litre. No significant effect on the intrinsic rate of natural increase was observed up to and including 36.8 µg Cu/litre. The carapace length was significantly reduced at 36.8 µg Cu/litre (Van Leeuwen et al., 1988).

LeBlanc (1985) studied the competitive interactions between *Daphnia magna* and *Daphnia pulex* in 28 day exposures to copper (10 and 30 µg Cu/litre). *D. pulex* populations consistently exceeded *D. magna* populations when cocultured in the absence of copper or temporary exposures to 10 µg Cu/litre. Exposure to 30 µg Cu/litre severely reduced initial population growth of *D. pulex* without affecting *D. magna*. By day 14 *D. magna* populations were dominant; however, *D. pulex* population growth was not completely suppressed and by the end of the experiment (28 days) *D. pulex* had gained population dominance. The reduced size of *D. magna* suggested that *D. pulex* was out-competing *D. magna* for available food.

Ingersoll & Winner (1982) carried out 70-day toxicity tests with *Daphnia pulex*. There was no significant effect on reproduction but survival was significantly reduced at 10 µg Cu/litre giving an NOEC of 5 µg Cu/litre. However, in pulse toxicity tests daphnids were exposed to 20 µg Cu/litre for 360 min/day (an average water concentration of 5 µg Cu/litre). Pulse exposures resulting in significant decreases in survival, brood size and body length, and delays in the age at which young were first produced.

Winner & Farrell (1976) exposed four species of daphnid to copper at concentrations ranging from 20 to 100 µg Cu/litre for up to 130 days. All four species exhibited reductions in survival at concentrations > 40 µg Cu/litre. *Daphnia magna* exhibited a decrease in the instantaneous rate of population growth at 60 µg Cu/litre; whilst the same parameter was affected at > 40 µg Cu/litre for *D. pulex*, *D. parvula* and *D. ambigua*. *Daphnia ambigua* produced significantly smaller broods at concentrations > 40 µg Cu/litre whereas mean brood size did not decrease in *D. pulex* and *D. parvula* until the concentration

exceeded 60 µg Cu/litre. Mean brood size in *D. magna* was unaffected by copper exposure.

De Nicola Giudici & Migliore (1988) studied the long-term toxicity of copper on the freshwater isopod *Asellus aquaticus*. In 30-day tests copper (5 µg Cu/litre) had no significant effect on female survival or birth rate. There was no significant effect on growth during embryonic development; however, copper treatment during juvenile development reduced body growth in 90-day exposures.

The development and hatchability of midge (*Chironomus decorus*) eggs were unaffected by copper (as copper sulfate) concentrations ranging from 100 to 5000 µg/litre. All larvae survived a 72-h exposure except those at 5000 µg Cu/litre which died after only partial emergence. The growth of larvae was significantly reduced when they were reared in copper-spiked food-substrate. An EC_{50} based on growth was 1602 mg Cu/kg (Kosalwat & Knight, 1987b).

Hatakeyama (1988) studied the effects of copper on the reproduction of chironomids (*Polypedilum nubifer*) through water (10–40 µg Cu/litre) and food (22–5180 µg Cu/g dry weight). Emergence success decreased to 74%, 38%, 16% and 2% of control values at 10, 20, 30 and 40 µg Cu/litre, respectively. The number of egg clusters produced by adults also decreased in accordance with the increase in copper concentration from 242 in controls to 31 at 30 µg Cu/litre; at 40 µg Cu/litre eggs were not oviposited. A significant decrease in emergence success occurred with food contaminated with 1770 mg Cu/kg: no emergence occurred at 5200 mg Cu/kg.

In flow-through life cycle tests with caddisfly (*Clistoronia magnifica*) concentrations of ≥ 17 µg Cu/litre prevented completion of the life cycle, and a significant reduction in adult emergence occurred at 13 µg Cu/litre. The NOEC was found to be 8.3 µg Cu/litre (Nebeker et al., 1984b).

Arthur & Leonard (1970) exposed the amphipod *Gammarus pseudolimnaeus* and the snails *Physa integra* and *Campeloma decisum* for 6 weeks to concentrations of 2.9–28.0 µg Cu/litre. For all three species, reduced survival and other significant adverse effects occurred at concentrations of 14.8 µg Cu/litre and above, but no effects were

noted at concentrations of 8.0 µg Cu/litre and below. In 100-day exposure to copper, *Gammarus pulex* populations densities were not affected at concentrations up to 11.0 µg Cu/litre, but were reduced at concentrations of 14.6 µg Cu/litre and above (Maund et al., 1992).

Phipps et al. (1995) determined 10-day LC_{50}s for the amphipod *Hyalella azteca*, the dipteran larva *Chironomus tentans*, and the oligochaete *Lumbriculus variegatus* to be 31, 54, and 35 µg Cu/litre, respectively, in Lake Superior (Canada) water (21–24 °C). Nebeker et al. (1986) reported 30-day LC_{50}s for the snails *Juga plicifera* and *Lithoglyphus virens* to be less than 8 µg Cu/litre. The 11-week LC_{50} for zebra mussel (*Dreissena polymorpha*) was reported to be 130 µg Cu/litre (Kraak et al., 1992). The marine copepod (*Tisbe furcata*) was determined to have a 96-h LC_{50} of 178 µg Cu/litre, but concentrations as low as 56 µg Cu/litre were estimated to significantly reduce the intrinsic rate of population increase (Bechmann, 1994).

Biochemical, physiological and behavioural effects

Lin et al. (1992) exposed Pacific oysters (*Crassostrea gigas*) to copper; 8–16 mg Cu/litre caused a significant increase in filtration rates whereas concentrations > 32 mg/litre reduced filtration rates. Glycine uptake rate was inhibited and the volume specific glycine transport declined in the presence of copper.

Krishnakumar et al. (1990) exposed green mussels (*Perna viridis*) to 25 µg Cu/litre for 2 weeks. Copper decreased ammonia-nitrogen excretion and significantly decreased filtration rate, O : N ratio, the scope for growth and growth efficiency; there was a nonsignificant increase in oxygen uptake. Microscopic examination of digestive glands revealed a significant increase in lysosomal lipofuscin content and percentage incidence of tubule dilation. Digestive cells showed extensive vacuolation of the cytoplasm. Copper exposure caused almost 100% cilia loss and tubule dilation.

Kraak et al. (1992) studied the effect of copper on the filtration rate in zebra mussel (*Dreissena polymorpha*) over a 9–11 week period. Filtration rate was unaffected at concentrations of 13 µg Cu/litre. Expressed as a percentage of the controls the average filtration rates of mussels exposed to 53, 72 and 90 µg Cu/litre were 44%, 33% and

27%, respectively. The EC_{50}, based on filtration rate, did not differ significantly from the 48-h EC_{50} (41 µg Cu/litre; Kraak et al., 1994) during 9 weeks (43 µg Cu/litre). The NOEC for the same parameter over 48 h was 16 µg Cu/litre (Kraak et al., 1994).

Ferrando & Andreu (1993) calculated 24-h EC_{50}s, based on filtration and ingestion rates, to be 43 and 53 µg Cu/litre for *Brachionus calyciflorus* and 59 and 90 µg Cu/litre for *Daphnia magna*.

Redpath & Davenport (1988) studied the action of three metals on pumping rate in the common mussel (*Mytilus edulis*); they found that pumping was stopped by shell valve adduction at copper concentrations in the range 20.8–25.6 µg Cu/litre.

Mussels (*Mytilus galloprovincialis*) exposed to 40 µg Cu/litre showed a significant increase in the levels of malondialdehyde (indicative of the peroxidative process) and a decrease in the concentration of glutathione in gills and digestive gland. The lipofuscin content in lysosome of the digestive gland was significantly increased (Viarengo et al., 1990).

Gill and hepatopancreas glycogen levels were significantly reduced in freshwater mussels (*Lamellidens corrianus*) exposed to concentrations of 100, 200 or 400 µg Cu/litre for up to 168 h (Rajalekshmi & Mohandas, 1993).

Ferrando et al. (1993) studied the feeding rates of rotifers (*Brachionus calyciflorus*) fed on the microalgae. A 5-h EC_{50}, based on feeding rate, was calculated to he 32 µg Cu/litre.

Weeks (1993) found a significant reduction in the feeding rate of the talitrid amphipod *Orchestia gammarellus* at dietary concentrations of 688 mg Cu/kg during 48-h tests. However, no significant effect was found at concentrations up to and including 817 mg Cu/kg in 20-day exposures.

Phelps et al. (1983) studied the effects of copper-enriched sediment on the burrowing behaviour of littleneck clams (*Protothaca staminea*). Above a threshold of 5.8 mg Cu/kg added to dry sediment, the time for 50% of the clams to burrow (ET_{50}) increased

logarithmically with increasing sediment copper concentration. Clams exposed to sediment mixed with a strong chelating agent and copper showed no significant change in burrowing time.

Interactions with other chemicals

Konar & Mullick (1993) studied the toxicity of different metal mixtures on zooplankton (*Diaptomus forbesi*) in 48-h acute tests. Zinc and iron individually were found to behave antagonistically in combination with copper but synergistically when all three metals were in combination. Copper in combination with lead alone, zinc and lead, iron and lead, and a combination of all four metals showed a synergistic interaction.

Vranken et al. (1988) exposed free-living marine nematodes (*Monhystera disjuncta*) to copper in metal mixtures (mercury, zinc and nickel). In 96-h tests, based on survival, all paired metal mixtures acted in a less than additive manner. However, in EC_{50} tests, based on developmental inhibition, the response was not as clear cut: the joint effect of copper with zinc, and copper with nickel was synergistic. Copper–mercury combinations did not reveal a clear mode of interaction.

Kaitala (1988) found that the presence of copper ions stimulated the accumulation of zinc and magnesium in mussels (*Mytilus edulis*). Zinc concentrations were 25% higher and zinc 100% higher than in the absence of copper. Copper did not influence the uptake of magnesium in burrowing clams (*Macoma baltica*) and zinc was not accumulated at all.

9.3.2.3 Vertebrates

Lethality and growth effects

The acute toxicity of copper to freshwater and marine fish is summarized in Tables 21 and 22, respectively. The 96-h LC_{50}s for freshwater fish range from 2.58 µg Cu/litre (Arctic grayling) to 7340 µg Cu/litre (bluegill). For marine fish, 96-h LC_{50} values range from 60 µg Cu/litre for chinook salmon to 1690 µg Cu/litre for killifish. However, a 48-h LC_{50} for killifish was calculated to be

Table 21. Acute toxicity of copper to freshwater fish (48-h and 96-h LC_{50}s)

Organism	Size/age	Conditions[a]	Temperature (°C)	Hardness (mg $CaCO_3$/litre)[b]	pH	Copper salt	Parameter	Concentration (µg/litre)	Reference
Chinook salmon *Oncorhynchus tshawytscha*	0.66 g	stat	11–13	211	7.4–8.3	sulfate (25.3%)	96-h LC_{50}	58	Hamilton & Buhl (1990)
	0.87 g	stat	11–13	211	7.4–8.3	sulfate (25.3%)	96-h LC_{50}	54	
	alevin	flow	12	24	7.1	ND	96-h LC_{50}	26	Chapman (1978)
	swim-up	flow	12	24	7.1	ND	96-h LC_{50}	19	
	parr	flow	12	24	7.1	ND	96-h LC_{50}	38	
	smolt	flow	12	24	7.1	ND	96-h LC_{50}	26	
Coho salmon *Oncorhynchus kisutch*	0.41 g	stat	12	41.3	7.1–8.0	sulfate	96-h LC_{50}	15	Buhl & Hamilton (1990)
	6 g	stat$	13.5	33	7.0–7.5	ND	96-h LC_{50}	17 (Cu^{2+})	Buckley (1983)
	6 g	stat$	13.5	33	7.0–7.5	ND	96-h LC_{50}	164 (total Cu)	
	2.7 kg	flow	9.4	20	7.29	chloride	96-h LC_{50}	46	Chapman & Stevens (1978)
Rainbow trout *Oncorhynchus mykiss*	0.60 g	stat	12	41.3	7.1–8.0	sulfate	96-h LC_{50}	13.8	Buhl & Hamilton (1990)
	1 g	stat	12	44	7.1	Count-N*	96-h LC_{50}	20.4	Mayer & Ellersieck (1986)
	1 g	stat	12	44	7.1	Count-NS*	96-h LC_{50}	121	
	1.6 g	stat	13	44	7.1	sulfate (98%)	96-h LC_{50}	135	
	alevin	flow	12	24	7.1	ND	96-h LC_{50}	28	Chapman (1978)
	swim-up	flow	12	24	7.1	ND	96-h LC_{50}	17	

Table 21 (contd).

Organism	Size/age	Conditions[a]	Temperature (°C)	Hardness (mg $CaCO_3$/litre)[b]	pH	Copper salt	Parameter	Concentration (µg/litre)	Reference
O. mykiss (contd).	parr	flow	12	24	7.1	ND	96-h LC_{50}	18	Chapman (1978)
	smolt	flow	12	24	7.1	ND	96-h LC_{50}	29	
	adult	flow	9.2	42	7.57	chloride	96-h LC_{50}	57	Chapman & Stevens (1978)
Brook trout *Salvelinus fontinalis*	juvenile	flow	12	ND	ND	sulfate	96-h LC_{50}	110	McKim & Benoit (1971)
Cutthroat trout *Salmo clarki*	2.1 g	flow	13	194	7.8	chloride	96-h LC_{50}	83.3	Chakoumakos et al (1979)
	9.4 g	flow	13	194	7.8	chloride	96-h LC_{50}	221	
	25.6 g	flow	13	194	7.8	chloride	96-h LC_{50}	243	
Arctic grayling *Thymallus arcticus*	alevin	stat	12	41.3	7.1–8.0	sulfate	96-h LC_{50}	23.9–131[c]	Buhl & Hamilton (1990)
	fry	stat	12	41.3	7.1–8.0	sulfate	96-h LC_{50}	9.6	
	0.34 g	stat	12	41.3	7.1–8.0	sulfate	96-h LC_{50}	2.58	
Fathead minnow *Pimephales promelas*	1 g	stat	17	44	7.1	Count-N*	96-h LC_{50}	35.9	Mayer & Ellersieck (1986)
	1.1 g	stat	17	44	7.1	Count-NS*	96-h LC_{50}	154	
	1.2 g	stat	18	272	7.4	sulfate (98%)	96-h LC_{50}	838	
	22 mm	flow	20–26	202	7.5–8.2	sulfate	96-h LC_{50}	490	Pickering et al. (1977)
	55 mm	flow	20–26	202	7.5–8.2	sulfate	96-h LC_{50}	460	

Table 21 (contd).

	3.2–4.2 cm	stat	22	40–48	7.2–7.9	acetate	96-h LC_{50}	390	Curtis et al. (1979)
	47 mm	flow	24	200 (154)	8.0	ND	96-h LC_{50}	490	Geckler et al.
	56 mm	flow	24	200 (154)	8.0	ND	96-h LC_{50}	440	(1976)
Bluntnose minnow *Pimephales notatus*	15–16 mm	flow	25	200	7.9 8.3	sulfate	96-h LC_{50}	230	Horning & Neiheisel (1979)
	84 mm	flow	24	200 (154)	8.0		96-h LC_{50}	340	Geckler et al. (1976)
Bluegill	1.2 g	stat	17	44	7.1	Count-N*	96-h LC_{50}	3280	Mayer &
Lepomis	1.2 g	stat	17	44	7.1	Count-N*	96-h LC_{50}	13 700	Ellersieck
macrochirus	1 g	stat	24	44	7.4	oxychloride (99%)	96-h LC_{50}	980	(1986)
	1.5 g	stat	18	44	7.1	sulfate (98%)	96-h LC_{50}	884	Mayer &
	1.5 g	stat	18	272	7.4	sulfate (98%)	96-h LC_{50}	7340	Ellersieck (1986)
	18.6 g	flow	24	200 (154)	8.0	ND	96-h LC_{50}	8300	Geckler
	19.2 g	flow	24	200 (154)	8.0	ND	96-h LC_{50}	10 000	et al. (1976)
Green sunfish	1.1 g	stat	18	44	7.1	sulfate (98%)	96-h LC_{50}	3510	Mayer &
Lepomis cyanellus	1.1 g	stat	18	272	7.4	sulfate (98%)	96-h LC_{50}	3400	Ellersieck (1986)

Table 21 (contd).

Organism	Size/age	Conditions[a]	Temperature (°C)	Hardness (mg $CaCO_3$/litre)[b]	pH	Copper salt	Parameter	Concentration (µg/litre)	Reference
Pumpkinseed *Lepomis gibbosus*	ND	stat	28	55	8.0	ND	96-h LC_{50}	2700	Rehwoldt et al. (1972)
Goldfish *Carassius auratus*	0.9 g	stat	18	272	7.4	sulfate (98%)	96-h LC_{50}	13 800	Mayer & Ellersieck (1986)
Golden shiner *Notemigonus crysoleucas*	ND	flow	ND	72.2	7.5	chloride	96-h LC_{50}	8460	Hartwell et al. (1989)
Banded killifish *Fundulus diaphanus*	ND	stat	28	55	8.0	ND	96-h LC_{50}	840	Rehwoldt et al. (1972)
Striped bass *Roccus saxatilis*	ND	stat	28	55	8.0	ND	96-h LC_{50}	4000	Rehwoldt et al. (1972)
White perch *Roccus americanus*	ND	stat	28	55	8.0	ND	96-h LC_{50}	6400	Rehwoldt et al. (1972)

Table 21 (contd).

American eel *Aguilla rostrata*	ND	stat	28	55	8.0	ND	96-h LC_{50}	6000	Rehwoldt et al. (1972)
Carp *Cyprinus carpio*		stat	28	55	8.0	ND	96-h LC_{50}	800	Rehwoldt et al. (1972)
	3.5–5.5 g	stat	20	50	7.5	ND	48-h LC_{50}	118	Peres &
	3.5–5.5 g	stat	20	100	7.5	ND	48-h LC_{50}	289	Pihan
	3.5–5.5 g	stat	20	300	7.5	ND	48-h LC_{50}	751	(1991a)
	3.5 cm	stat$	20	ND	7.1	sulfate	96-h LC_{50}	300	Alam &
	6.5 cm	stat$	15	ND	7.1	sulfate	96-h LC_{50}	1000	Maughan (1992)
Fantail *Etheostoma flabellare*	36.8 mm	stat	19–21	ND	ND	sulfate	96-h LC_{50}	333[c]	Lydy &
	36.8 mm	stat	19–21	ND	ND	sulfate	96-h LC_{50}	385[c]	Wissing (1988)
Johnny darter *Etheostoma nigrum*	39.2 mm	stat	19–21	ND	ND	sulfate	96-h LC_{50}	489[c]	Lydy &
	39.2 mm	stat	19–21	ND	ND	sulfate	96-h LC_{50}	569[c]	Wissing (1988)

Table 21 (contd).

Organism	Size/age	Conditions[a]	Temperature (°C)	Hardness (mg $CaCO_3$/litre)[a]	pH	Copper salt	Parameter	Concentration (µg/litre)	Reference
Rainbow darter *Etheostoma caeruleum*	41 mm	flow	24	200 (154)	8.0	ND	96-h LC_{50}	320	Geckler et al. (1976)
Orangethroat darter *Etheostoma spectabile*	55 mm	flow	24	200 (154)	8.0	ND	96-h LC_{50}	850	Geckler et al. (1976)
Stoneroller *Campostoma anomalum*	60 mm	flow	24	200 (154)	8.0	ND	96-h LC_{50}	290	Geckler et al. (1976)
Creek chub *Semotilus atromachulatus*	64 mm	flow	24	200 (154)	8.0	ND	96 h LC_{50}	310	Geckler et al. (1976)
Blacknose dace *Rhinichthys atratulus*	47 mm	flow	24	200 (154)	8.0	ND	96-h LC_{50}	320	Geckler et al. (1976)

Table 21 (contd)

Brown bullhead *Ictalurus nebulosus*	39 mm	flow	24	200 (154)	8.0	ND	96-h LC_{50}	540	Geckler et al. (1976)
Striped shiner *Notropis chrysocephalus*	55 mm	flow	24	200 (154)	8.0	ND	96-h LC_{50}	790	Geckler et al. (1976)
	55 mm (1.7 g)	flow	24	200 (154)	8.0	ND	96-h LC_{50}	1900	
Mudfish *Clarias anguillaris*	27.1 g	stat	ND	ND	ND	sulfate	96-h LC_{50}	4301	Ebele et al. (1990)
Cichlid *Oreochromis niloticus*	82 g	stat	22	ND	7.24	ND	96-h LC_{50}	1059	Al-Akel (1987)

[a] Stat = static conditions (water unchanged for duration of test); stat$ = static renewal conditions (water changed at regular intervals) flow – flow-through conditions (copper concentration in water continuously maintained); ND = no data available.
[b] Alkalinity in parentheses (mg/litre).
[c] Range of LC_{50}s for fish from different sources
[d] Fish collected in winter
[e] Fish collected in summer.

Table 22. Acute toxicity of copper to marine fish[a]

Organism	Size/age	Conditions	Temperature (°C)	Salinity (‰)	pH	Copper salt	Parameter	Concentration (µg/litre)	Reference
Chinook salmon *Oncorhynchus tshawytscha*	1.6 g	stat	11–13	brackish	7.6–8.1	sulfate (25.3%)	96-h LC_{50}	60	Hamilton & Buhl (1990)
Coho salmon *Oncorhynchus kisutch*	smolt	flow	13	28.6	8.1	chloride	96-h LC_{50}	601	Dinnell et al. (1989)
Topsmelt *Atherinops affinis*	larvae	stat	21	33	ND	chloride	96-h LC_{50}	238	Anderson et al. (1991)
Tidewater silverside *Menidia peninsulae*	19 days	stat	25	20	ND	nitrate (34%)	96-h LC_{50}	140	Mayer (1987)
Spot *Leiostomus xanthurus*	adult	stat	25	20	ND	nitrate (34%)	96-h LC_{50}	280	Mayer (1987)
Sheepshead minnow *Cyprinodon variegatus*	larvae	stat§	25	34–35	6.2–8.2	nitrate	96-h LC_{50}	> 220 fed	Hutchinson et al. (1994)

Table 22 (contd).

Rivulus marmoratus	0.03–0.1 g	flow	26–27	14	ND	ND	96-h LC_{50}	1250–1610	Lin & Dunson (1993)
Killifish	0.02–0.13 g	flow	26–27	14	ND	ND	96-h LC_{50}	1690	Lin & Dunson (1993)
Fundulus heteroclitus	juvenile	stat$	20	10	ND	ND	48-h LC_{50}	19 000	Burton & Fisher (1990)
Dab *Limanda limanda*	16.9 g	flow	12	34.6	7.7	nitrate	96-h LC_{50}	300	Taylor et al. (1985)
Grey mullet *Chelon labrosus*	0.87 g	flow	12	34.6	7.7	nitrate	96-h LC_{50}	1400	Taylor et al. (1985)
Shiner perch *Cymatogaster aggregata*	adult	flow	13.2	29.5	7.8	chloride	96-h LC_{50}	418	Dinnell et al. (1989)

[a] Stat = static conditions (water unchanged for duration of test); stat$ = static renewal conditions (water changed at regular intervals); flow = flow-through conditions (copper concentration in water continuously maintained); ND = no data available.

19 000 µg Cu/litre. The toxicity of copper to amphibia is summarized in Table 23. For larvae of *Bufo melanostictus* and *Xenopus laevis*, respectively, 48-h LC_{50}s of 446 and 1700 µg Cu/litre were found.

Erickson et al. (1996) found the acute toxicity of copper to fathead minnow (*Pimephales promelas*) to vary widely depending on the chemical characteristics of the water. Increased pH, hardness, sodium, dissolved organic matter and suspended solids each caused toxicity to decrease on the basis of total copper concentrations, and 96-h LC_{50}s, based on total copper, ranged from 7 to 305 µg Cu/litre (0.11 to 4.8 µmol/litre) over the whole range of conditions tested in flow-through tests. The results did not show a particularly good correlation of toxicity to cupric-ion-specific electrode measurements. The authors concluded that the effects of the different test conditions on copper speciation have an important role in determining toxicity; however, factors unrelated to chemical speciation also influenced toxicity.

Smith & Heath (1979) studied the effect of temperature on the acute (24-h) toxicity of copper to five species of freshwater fish. There was considerable variation between species. There was a tendency for a higher sensitivity at higher temperatures in goldfish, channel catfish and rainbow trout; the converse was found for bluegill. However, the differences caused by temperature were a factor of 2 or less whereas the interspecies differences were as much as sixfold.

Chakoumakos et al. (1979) found the acute toxicity of copper to cutthroat trout (*Salmo clarki*) to be inversely correlated with both water hardness and alkalinity. The 96-h LC_{50}s ranged from 15.7 µg Cu/litre at low alkalinity (20.1 mg $CaCO_3$/litre) and hardness (26.4 mg/litre) to 367 µg Cu/litre at high alkalinity (178 mg/litre) and hardness (205 mg/litre). The most important copper species causing toxicity within the pH range tested were Cu^{2+}, $Cu(OH)^{-}$ and $Cu(OH)_2^0$. The concentration of each of these species varies with pH and alkalinity. Lower pHs favour Cu^{2+}; higher pHs favour $Cu(OH)^{-}$ and $Cu(OH)_2^0$. Lower alkalinities favour all three species.

Peres & Pihan (1991a) reported a similar relationship between acute copper toxicity and hardness for both carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*): 48-h LC_{50}s for trout ranged from 25 µg Cu/litre to 560 µg Cu/litre at a water hardness ranging from

Table 23. Acute toxicity of copper to amphibians

Organism	Size/age	Conditions[a]	Temperature (°C)	Hardness (mg $CaCO_3$/litre)	pH	Copper salt	Parameter	Concentration (µg/litre)	Reference
Clawed toad *Xenopus laevis*	larvae	stat	20	ND	ND	sulfate	48-h LC_{50}	1700	DeZwart & Sloof (1987)
Toad *Bufo melanostictus*	larvae	stat	31	185	7.4	sulfate	48-h LC_{50}	446	Khangarot & Ray (1987)
	larvae	stat	31	185	7.4	sulfate	96-h LC_{50}	320	

[a] Stat = static conditions (water unchanged for duration of test); ND = no data available.

10 to 300 mg/litre. For carp, LC_{50}s ranged from 118 to 751 µg/litre whilst the hardness ranged from 50 to 300 mg/litre. The toxicity of copper to rainbow trout decreased with increasing hardness. LC_{50}s (15 days) ranged from 18 µg Cu/litre at a hardness of 12 mg $CaCO_3$/litre to 96 µg Cu/litre at a hardness of 97 mg/litre.

Miller & Mackay (1980) tested the effects of different hardness (12–99 mg $CaCO_3$/litre) and alkalinity (10–51 mg $CaCO_3$/litre) on acute toxicity of copper to rainbow trout. Toxicity was inversely related to hardness at all alkalinities and to alkalinity at higher hardness. Incipient median lethal concentrations ranged from 18 to 96 µg Cu/litre. Howarth & Sprague (1978) evaluated the acute toxicity of copper to rainbow trout in waters of various hardness, alkalinity and pH and reported 96-h LC_{50}s to range from 20 to 516 µg Cu/litre. Cusimano et al. (1986) reported the 96-h LC_{50} for rainbow trout to vary from 66 µg Cu/litre at pH 4.7 (alkalinity –0.2 mg $CaCO_3$/litre) to 2.8 µg Cu/litre at pH 7.0 (alkalinity 11 mg $CaCO_3$/litre).

Welsh et al. (1993) determined the acute toxicity of copper to larval fathead minnows in waters of varying pH and dissolved organic carbon (DOC). Toxicity was inversely related to both parameters, with 96-h LC_{50}s ranging from 2.0 µg Cu/litre at pH 5.6 and 0.2 mg DOC/litre to 182 µg/litre at pH 7 and 16 mg DOC/litre. Empirical regression equations were derived that could be useful for predicting toxicity in different waters and the slopes of these equations were similar to those reported by Erickson et al. (1987, 1996).

Anderson et al. (1994) reported 7-day LC_{50}s and NOECs from three different laboratories for larval topsmelt. LC_{50}s were 162 and 274 µg Cu/litre and NOECs were 100 µg Cu/litre at a salinity of 34‰. LC_{50}s were 55.7 and 58.4 µg Cu/litre and NOECs were 32 µg Cu/litre at a salinity of 20‰. LC_{50}s and NOECs were calculated for topsmelt which were spawned at different times of the year over a 2-year period (1990–1991). NOECs for copper were 100 µg Cu/litre except two 180 µg Cu/litre (November 1990) and 56 µg Cu/litre (May 1991); LC_{50}s for these tests ranged from 131 to 240 µg Cu/litre.

McNulty et al. (1994) carried out a series of 7-day growth and survival experiments with larval topsmelt (*Atherinops affinis*) of different ages. Fish aged 0, 3 and 5 days were less sensitive to copper

than fish ≥ 7 days old. LC_{50}s ranged from 365 µg Cu/litre in 0-day larvae to 137 µg Cu/litre in 20-day larvae. NOECs were constant for all age groups at 180 µg Cu/litre for fish 1 and 3 days old and 100 µg Cu/litre for all other groups. Pickering & Lazorchak (1995) exposed fathead minnow (*Pimephales promelas*) to copper in a 7-day larval survival and growth test. The NOEC and LOEC, based on growth, were 25 and 50 µg Cu/litre, respectively, for larvae 1, 4 and 7 days old. For survival the NOEC and LOEC were 200 and 400 µg Cu/litre, respectively, for 1-day-old and 4-day-old larvae; however, for 7-day-old larvae the NOEC and LOEC were 100 and 200 µg Cu/litre. The subchronic value (geometric mean of NOEC and LOEC) was 35 µg Cu/litre. The authors found the test to be relatively insensitive to changes in test conditions.

Hutchinson et al. (1994) exposed sheepshead minnow larvae (*Cyprinodon variegatus*) to copper for 7 days. NOECs for survival and growth were 120 and 220 µg Cu/litre, respectively; LOECs were 220 and > 220 µg Cu/litre, respectively. A subchronic value was calculated as the geometric mean of the highest NOEC and the lowest LOEC; these were 160 µg Cu/litre for survival and > 220 µg Cu/litre for growth.

Seven-day survival tests with coho salmon (*Oncorhynchus kisutch*) carried out following 16 weeks exposure indicate that the exposed fish became significantly more tolerant of copper. The 168-h LC_{50} for previously unexposed fish was 220 µg Cu/litre whereas fish exposed to 140 µg Cu/litre were 2.5 times less sensitive at 550 µg Cu/litre (Buckley et al., 1982).

Collvin (1985) studied the effect of copper (1–81 µg Cu/litre) on the maximal growth rate of perch (*Perca fluviatilis*) over a 30-day period. Copper reduced maximal growth rate at concentrations > 22 µg Cu/litre. Reduced growth rate was mainly an effect of reduced food conversion efficiency attributed to an increased metabolism caused by detoxification.

Lanno et al. (1985) fed rainbow trout (*Oncorhynchus mykiss*) on a diet containing copper at concentrations ranging from 9 to 3088 mg Cu/kg for 8 weeks, and from 8.5 to 664 mg Cu/kg for up to 24 weeks. After 8 weeks reduced weight gain and feed intake, increased

feed : gain ratios and mortalities were observed in trout reared on test diets containing > 730 mg Cu/kg. Trout receiving 1585 or 3088 mg Cu/kg showed pronounced food refusal. After 16 weeks trout reared on a diet containing 664 mg Cu/kg had significantly lower live body weights, although there was no significant difference after 24 weeks.

Mount et al. (1994) fed rainbow trout (*Oncorhynchus mykiss*) on a brine shrimp (*Artemia* sp.) diet containing 440, 830 or 1000 mg Cu/kg (dry weight) for up to 60 days. Fish fed the 830 or 1000 mg Cu/kg diets showed 30% mortality during the experiment. No significant effect of copper on growth was observed. The authors conclude that waterborne copper released from *Artemia* may have contributed to the mortality.

Effects on reproduction and early life-stages

Stouthart et al (1996) exposed newly fertilized common carp (*Cyprinus carpio*) eggs to copper (19.1 and 50.8 µg/litre; 0.3 and 0.8 µmol/litre) at pH 6.3 and 7.6. No significant effect of copper on egg mortality, larval heart rate and tail movement or whole-body potassium and magnesium content was observed at pH 7.6. However, whole-body sodium and calcium were significantly decreased and larval mortality and larval deformation were significantly increased at the higher copper exposure. At pH 6.3, exposure to 50.8 µg Cu/litre (0.8 µmol/litre) significantly increased egg mortality and decreased heart rate and tail movements; premature hatching and a concentration-dependent increase in larval mortality and larval deformation were also observed. The whole-body content of potassium, sodium, magnesium, and calcium were all significantly decreased by both copper exposures at pH 6.3.

Anderson et al. (1991) carried out both fertilization tests and embryo development tests on topsmelt (*Atherinops affinis*). In fertilization tests percentage fertilization was measured following exposure of sperm to copper. The NOEC values for four fertilization tests ranged from 32 to ≥ 90 µg Cu/litre; EC_{50}s ranged from 24 to 163 µg Cu/litre. In embryo tests embryos were checked for up to 12 days for viability, abnormalities, mortality and hatching success. The NOEC for embryo abnormalities ranged from 55 to 123 µg Cu/litre; EC_{50}s ranged from 115 to 180 µg Cu/litre. The NOEC for

larval hatching success and for larval abnormalities ranged from 55 to 123 µg Cu/litre, and from 55 to 68 µg Cu/litre for the two parameters, respectively; EC_{50}s ranged from 108 to 182 µg Cu/litre, and from 75 to 190 µg Cu/litre.

Pickering et al. (1977) exposed fathead minnow (*Pimephales promelas*) to copper (8–100 µg Cu/litre) at 6, 3 and 0 months prior to spawning. The prespawning exposure time had no significant effect on reproduction. However, egg production was significantly lower at concentrations of 37 µg Cu/litre or more. The maximum acceptable toxicant concentration (MATC) was estimated to be 32 µg Cu/litre.

Dave & Xiu (1991) monitored the effects of copper chloride on hatching and survival of zebrafish (*Brachydanio rerio*) exposed from post-fertilization for 16 days. Significant mortality of embryos occurred at concentrations of 32 µg Cu/litre and above during the first day of exposure. For exposures of 1–16 µg Cu/litre, hatch was significantly delayed relative to the control value of 4 days and embryo mortality exceeded 50%. Delayed hatching was also reported at concentrations < 1 µg Cu/litre, but effect levels are very uncertain because exposure concentrations were unmeasured and background concentrations are unknown.

Scudder et al. (1988) exposed embryos of fathead minnow (*Pimephales promelas*) to total copper concentrations ranging from 0.6 to 621 µg Cu/litre from 5 to 10 h post-fertilization to 2 days post-hatch. Significant declines in percentage survival and percentage total hatch were observed at 621 µg Cu/litre but not at 338 µg Cu/litre. A significant increase in the number of embryos with abnormalities was observed at ≥ 338 µg Cu/litre. Larval fish were exposed to copper at the same concentrations for 28 days post-hatch. Fish growth was significantly reduced and percentage abnormalities increased at the lowest treatment concentration (61 µg Cu/litre) and effects increased with increasing concentration. Percent survival was significantly reduced at concentrations of 113 µg Cu/litre and above and the 28-day LC_{50} was estimated to be 128 µg Cu/litre.

Mount (1968) conducted an 11-month, full-life-cycle exposure of fathead minnows to copper in a hard water (hardness 200 mg $CaCO_3$/litre, alkalinity 160 mg $CaCO_3$/litre, pH 7.9). Growth and

survival were significantly reduced at 95 µg Cu/litre and reproduction was completely suppressed at 32–34 µg Cu/litre, but unaffected at 14–15 µg Cu/litre. In a softer water (hardness 31 mg $CaCO_3$/litre, alkalinity 30 mg $CaCO_3$/litre, pH 7.0), Mount & Stephen (1969) reported survival, growth, aud reproduction to be significantly affected at 18.4 µg Cu/litre, but not at 10.6 µg Cu/litre.

McKim et al. (1978) tested the effects of copper on the growth and survival of embryos and larvae of eight fish species. The standing crop of fish after 30–70 days post-hatch was significantly reduced at exposure concentrations of 32 µg Cu/litre for rainbow trout, 34 µg Cu/litre for white sucker, 44 µg Cu/litre for brook trout, 42 µg Cu/litre for lake trout, 46 µg Cu/litre for brown trout, 103 µg Cu/litre for lake herring, 104 µg Cu/litre for northern pike, and 104 µg Cu/litre for smallmouth bass.

Seim et al. (1984) contrasted the effects of continuous and intermittent (4.5 h/day) exposure of steelhead trout to copper for 78 days. The EC_{50} for growth reduction on the basis of average copper concentrations was 46 µg Cu/litre for continuous exposure, but only 27 µg Cu/litre for intermittent exposure.

Sayer et al. (1989) exposed yolk-sac fry of brown trout (*Salmo trutta*) to copper concentrations of 1.2, 2.5 and 5.1 µg Cu/litre (20, 40 and 80 nmol/litre) at pH 4.5 and at calcium concentrations of 20 or 200 µmol for 30 days. Mortalities were high (70–100%) at the lower calcium concentration for all three copper concentrations. Only one death was observed for copper and high calcium exposures. At high calcium levels, impaired sodium, potassium and calcium uptake were observed for all three copper concentrations.

Horning & Neiheisel (1979) exposed bluntnose minnow (*Pimephales notatus*) to copper concentrations ranging from 4.3 (control) to 119.4 µg Cu/litre. Minnows exposed to 119.4 µg Cu/litre for 60 days were significantly smaller than the other groups. Survival of parental bluntnose minnows was not affected by any copper concentrations during the 60-day exposure. Copper concentrations ≥ 18 µg Cu/litre significantly reduced the number of spawnings, the total number of eggs produced and the number of eggs per female. Therefore, the MATC based on reproductive impairment was between

4.3 and 18 µg Cu/litre. Minnows held in "clean" water for 9 months ceased to spawn on exposure to 119.4 µg Cu/litre. Fish exposed to 119.4 µg Cu/litre for the same 9-month period began to spawn 60 days after being transferred to "clean" water.

McKim & Benoit (1971) exposed brook trout (*Salvelinus fontinalis*) to copper(II) concentrations ranging from 1.9 to 32.5 µg Cu/litre for 22 months. The highest concentration decreased survival and growth in adnlt fish, and reduced the number of viable eggs produced and hatchability. No effects on adult survival, growth or reprodnction were observed at copper concentrations of 17.4 µg/litre or less. Concentrations of 17.4 and 32.5 µg Cu/litre had marked adverse effects on survival and growth of alevins and juvenile fish. Therefore, the MATC for brook trout exposed to copper (hardness 45 mg $CaCO_3$/litre; pH 7.5) was between 9.5 and 17.4 µg Cu/litre.

Benoit (1975) exposed bluegills (*Lepomis macrochirus*) to copper concentrations ranging from 12 to 162 µg Cu/litre for a period of 22 months. Adult bluegill survival and reproduction were significantly affected only at the highest copper concentration of 162 µg Cu/litre. A 90-day exposure of larvae transferred at hatch revealed a significant reduction in survival at > 40 µg Cu/litre; larval growth was not significantly reduced at 77 µg Cu/litre and below.

Metabolic, biochemical and physiological effects

Beckman & Zaugg (1988) exposed chinook salmon (*Oncorhynchus tshawtscha*) to natural springwater with an elevated copper concentration (48 µg/litre). In parr, gill Na^+, K^+-ATPase activity was unaffected by an 18-h exposure, whereas in smolt there was significant inhibition. In both parr and smolt there were significant increases in haematocrit and plasma glucose.

Arillo et al. (1984) investigated the effect of copper at levels of 30–100 µg Cu/litre on a wide variety of biochemical parameters in the rainbow trout (*Oncorhynchus mykiss*). The exposure period was 4 months. Copper significantly reduced ALAD (aminolaevulinic acid dehydratase) activity in liver, carbonic anhydrase activity in blood, gill sialic acid content and the respiratory control ratio and oxygen consumption in liver mitochondria.

Heath (1991) exposed bluegill (*Lepomis macrochirus*) to a free copper concentration of 261 µg Cu/litre for 7 days. Copper caused an elevation in plasma glucose of approximately 100% and a significant reduction in liver ATP. Acute hypoxia stress responses such as hyperglycaemia, lower plasma sodium, lower liver ATP and higher plasma potassium were accentuated by prior exposure to copper.

Nemcsók & Hughes (1988) exposed rainbow trout to concentrations of 200 or 2000 µg Cu/litre for up to 48 h. Activities of blood glucose, ASAT and ALAT were significantly increased after 24 h at 2000 µg Cu/litre and after 48 h at the lower concentration. Significant decreases in acetylcholinesterase activity were observed over the same time periods at each of the copper concentrations. Copper sulfate (200 µg/litre) had only a slight damaging effect on tissues after 24 h as indicated by biochemical and haematological parameters. However, the addition of sulfuric acid (pH 6.5) significantly increased blood glucose, ASAT, ALAT and lactate dehydrogenase, and significantly decreased acetyl cholinesterase activity.

Muñoz et al. (1991) exposed juvenile rainbow trout to copper (50 µg Cu/litre) for 21 days. Copper caused rapid and significant elevations of plasma cortisol levels; plasma sodium showed a significant decrease for 7–15 days.

Lydy & Wissing (1988) studied the thermal resistance of fantail darters (*Etheostoma flabellare*) and johnny darters (*E. nigrum*) exposed to copper at sublethal concentrations for 96 h. Thermal resistance was determined using the critical thermal maxima (CT_{MAX}) with loss of equilibrium as the end-point. The mean CT_{MAX} value for fantail darters exposed to 149 µg Cu/litre was 7.6 °C below that of the control means. In johnny darters, a concentration of 292 µg Cu/litre depressed the CT_{MAX} value by 5.2 °C. The NOEC for the fantail darter was 42 µg Cu/litre and for the johnny darter 128 µg Cu/litre.

Structural effects and malformations

Benedetti et al. (1989) exposed brown bullhead (*Ictalurus nebulosus*) to concentrations of 5000 µg Cu/litre for 24 h and 300 µg Cu/litre for 40 days. All fish reared for more than 1 month in 300 µg Cu/litre showed epidermal changes such as an increase in mucus cell

numbers and a tendency for the cells to become superficial. In fish most severely impaired by copper poisoning the epidermis appeared thinner in patches compared with controls. The gills of fish exposed to 5 mg Cu/litre were markedly damaged with swollen and hyperaemic lamellae, necrosis and disaggregation of the epithelium, whereas fish in the 300 μg Cu/litre group showed more variable gill damage. Histomorphological analysis of livers from both groups did not reveal diffuse changes in the hepatic parenchyma. However, areas of patchy degeneration and isolated degenerated elements located within areas of normal hepatocytes were observed. Histochemical staining revealed that all treated fish had lower liver glycogen content than controls.

Khangarot (1992) investigated ultrastructural alterations in the liver of snake-headed fish (*Channa punctatus*) following exposure to 50 and 100 μg Cu/litre using transmission electron microscopy. After 4 days at 50 μg Cu/litre there is a marked proliferation of the smooth endoplasmic reticulum (SER), complete degeneration of rough endoplasmic reticulum (RER), loss of ribosomes from the surface of RER, a random distribution of ribosomes throughout the cytoplasm and an increase in the number and size of SER cisternae. Mitochondrial swelling, and the loss of internal and external mitochondrial membranes, were observed. A large number of vacuoles and lysosome having dense bodies were observed after 7 days exposure. The lysosomal matrix frequently displayed crystalline structures of various sizes and the nuclear size was reduced with chromatin material clumped within the nucleus. Prominent changes in nuclei of fish hepatocytes were observed after exposure to 100 μg Cu/litre. Rupture of nuclear membranes and clumping of chromatin in necrotic cell nuclei with the aggregation of interchromatin material at the centre of the nucleus were recorded. More dilation and vesiculation were observed in RER after 7 days. Aggregation of SER and RER, rupturing of mitochondrial membrane, a decrease in the number of mitochondria and an increase in the number of Golgi complexes were also observed.

Kirk & Lewis (1993) studied copper-induced changes in gills of rainbow trout (*Oncorhynchus mykiss*) by scanning electron microscopy. Trout exposed to 500 μg Cu/litre for 2 h showed collapse of lamellae and considerable secretory activity of mucous cells. Filament tips were swollen and bent, and had an increase in the number of mucous cells which extruded copious amounts of mucus. Exposure

of fish to 1000 µg Cu/litre caused the gills to be covered in mucus and cellular debris. There were many ruptured and exhausted mucous cells, lamellar fusion occurred and epithelial cells were extremely swollen throwing the gill surface into swellings and ridges. There was a greater proliferation of chloride and mucous cells, and increased mucus secretion compared with the lower copper exposure.

Behavioural effects

Pedder & Maly (1985) studied the attraction–avoidance response of rainbow trout at concentrations ranging from 500 to 4000 µg Cu/litre. There was an initial attraction response at all copper concentrations which led to high mortality at 3000 and 4000 µg Cu/litre. Avoidance of copper was observed, following the initial period of attraction, at > 500 µg Cu/litre; maximum avoidance at 1.0 mg Cu/litre. The 96-h EC_{50}, based on avoidance, was between 500 and 750 µg Cu/litre. Hartwell et al. (1989) found the avoidance threshold for golden shiner (*Notemigonus crysoleucas*) to be 26 µg Cu/litre in flow-through tests.

Ellgaard & Guillot (1988) observed that exposure to copper elicited a hypoactive response in bluegill at all concentrations tested (40, 80 and 400 µg Cu/litre) and that the effect was concentration-dependent. At all concentrations, locomotor activity appeared to fall rapidly during the first 4 days following exposure and then tended to plateau for the rest of the 8-day exposure.

Steele (1989) studied the effect of sublethal exposures of copper (50, 100 and 200 µg Cu/litre) on the daily activity of sea catfish (*Arius felis*) after a 72-h static exposure. Fish exposed to 0.1 or 0.2 mg Cu/litre showed significant hyperactivity and a loss of the normal daily activity pattern of this species; the same two exposure groups showed significantly less variability in activity.

9.3.2.4 *Model ecosystems and community effects*

Havens (1994a) dosed mesocosms with copper concentrations ranging from 2 to 200 µg Cu/litre for 5 days. There was a significant negative relationship between total algal biovolume and copper dose. The decline in algal biovolume at higher doses (> 50 µg Cu/litre)

reflected the loss of *Rhodomonas*, *Aphanizomeron*, *Chlamydomonas* and *Ceratium*. The assemblage that survived was dominated by diatoms. There was a significant negative exponential relationship between zooplankton biomass and copper dose. The most sensitive species were the calanoid copepods with a biomass reduction of ≥ 50% at 20 µg Cu/litre; the cyclopoids were the most tolerant with more than 50% of the biomass of cyclopoid copepodids surviving the highest dose.

Havens (1994b) exposed a freshwater plankton community to copper (140 µg Cu/litre) for 14 days. Copper significantly reduced the dry weight biomass of zooplankton, ciliates, flagellates and autotrophic phytoplankton. Bacterial biomass was significantly increased; however, this resource went virtually unexploited because the most effective bacterial grazers (large cladocerans and protozoans) were greatly reduced by copper exposure.

Hedtke (1984) exposed an aquatic microcosm to copper for 32 weeks under flow-through conditions. No significaut effects on material cycling and biological structure were observed at 4.0 µg Cu/litre. At 9.3 µg Cu/litre primary production levels were significantly reduced by the end of the experiment and dissolved organic carbon production was substantially lower than controls. Copper concentrations > 30 µg Cu/litre significantly lowered primary production and macroalgal growth, and there were substantial structural changes increasingly shifting from autotrophic to heterotrophic systems with increasing copper levels.

Scanferlato & Cairns (1990) spiked sediment with copper concentrations of 10, 100 or 1000 mg Cu/kg dry sediment in an aquatic microcosm. Most of the added copper remained bound to sediment particles during the 8-week experiment. The lowest concentration had no effect on the structure or function of the microcosm. In microcosms exposed to 100 mg Cu/kg (500 µg Cu/litre in overlying water) both chlorophyll *a* content and respiration were significantly decreased. Other structural and functional attributes were rather variable. Significant decreases in production, respiration, respiration/biomass ratio, ATP and chlorophyll *a*, and significant increases in assimilation ratio and autotrophic index were observed at 1000 mg Cu/kg (overlying water concentration = 20 mg Cu/litre).

Hart et al. (1992) evaluated the effects of copper on phytoplankton communities in 5-m diameter (40-m^3 volume) enclosures in Island billabong (floodplain on Magela Creek, northern Australia). Copper (1.3 g) was added to the enclosure at a rate thought to be 10× the normal wet season values for Island billabong. Concentrations of total copper over the 10-week experiment ranged from 2.2 to 51 μg Cu/litre in the treatment and 3.9 to 26 μg Cu/litre in the control. The addition of copper had little effect on the phytoplankton populations.

Winner et al. (1990) evaluated the seasonal responses of planktonic and benthic communities exposed to copper concentrations of 20 or 40 μg Cu/litre in oligotrophic ponds for 5-week periods. Phytoplankton and zooplankton were more sensitive to copper in the spring than in the summer or autumn. Zooplankton exhibited a 43% and an 86% reduction in density in the 20 and 40 μg Cu/litre enclosures, respectively. The authors suggest that this is related to seasonal changes in the dissolved organic carbon content of the ponds.

Winner & Owen (1991a) evaluated the toxicity of copper to daphnids in 7-day chronic tests (*Ceriodaphnia dubia*) and algae in 4-day cell reproduction tests (*Chlamydomonas reinhardii*) using filtered pond water from Brandenburg Pond, Ohio, USA. The studies were performed numerous times over a 6-month period. *C. reinhardii* NOECs were typically 20–40 μg Cu/litre whereas for *C. dubia* they were 50–80 μg Cu/litre. For both species, NOECs increased with increasing alkalinity and hardness. *C. reinhardii* NOECs based on cell growth also declined with increasing dissolved organic carbon values.

Winner & Owen (1991b) examined the sensitivity of freshwater phytoplankton communities to chronic copper exposure in 100-litre polyethylene enclosures in Brandenburg Pond, Ohio, USA. The studies were conducted in four 5-week exposures over the course of 2 years. Nominal exposure levels of 0, 20 and 40 μg Cu/litre were used and verified analytically. Over the course of the experiment 82 taxa of phototrophs were identified in the enclosures. Seasonal variation in algal population density was observed with the largest depression of the algal populations in the two spring exposures at both the 20 and 40 μg Cu/litre concentrations. Summer and fall population responses to copper at the 20 and 40 μg Cu/litre level were minimal, although individual taxa were effected. During the first spring

exposure, effects on zooplankton and benthos were also measured (Moore & Winner, 1989). Small mayflies and chironomids were effected at 40 µg Cu/litre and no effects on benthos were observed at 20 µg Cu/litre. Rotifers and copepods exhibited significant reduction in density at both 20 and 40 µg Cu/litre. Daphnids were not affected at either treatment level, which was consistent with laboratory toxicity tests with *Ceriodaphnia dubia*.

Moore & Winner (1989) studied the effect of copper (20 and 40 µg Cu/litre) on zooplankton and benthos in enclosure experiments. During 5-week exposures copper caused significant decreases in the alga *Uroglena*, rotifers, cyclopoids and calanoid copepods. The density of small mayflies and chironomids was significantly decreased at the higher copper exposnre. Other benthic organisms such as fingernail clams, larger midges and mayflies were not affected by copper but rather by fish predation and/or adult emergence. *Daphnia* achieved significantly higher densities at 20 µg Cu/litre than in the controls.

Clements et al. (1989) conducted experiments in artificial streams to examine the influence of water quality on the macroinvertebrate responses to copper. The effects of copper on the reduction of macroinvertebrate abundance was greater in streams of low hardness (53–60 mg/litre) and alkalinity (49–61 mg/litre) at a copper concentration of 6 µg Cu/litre compared with streams of higher hardness (150–157 mg/litre) and alkalinity (137–146 mg/litre), and a copper concentration of 15 µg/litre. However, the responses to copper were highly variable among taxa. Tanytarsini chironomids and the mayflies *Baetis brunneicolor* and *Isonychia bicolor* were particularly sensitive, whereas Orthocladiini chironomids and net-spinning caddisflies were quite tolerant of copper in experimental streams.

Clements et al. (1988, 1990) conducted a series of macroinvertebrate community toxicity tests with copper in laboratory- and field-constructed aquatic streams using water from the New River in Giles County, Virginia, USA. Rock-filled trays were colonized by macroinvertebrates in the New River for 30 days and then placed in either the laboratory- or the field-constructed streams. The results of the 1988 laboratory experiments indicated that 96-h exposures to copper (low dose = 15–32 µg Cu/litre) resulted in a reduction in the number of taxa present by 24–36%. In 1990, 10-day exposures were

performed with copper and laboratory and field results compared. The field-constructed streams showed significantly less response to copper than the macroinvertebrates in laboratory-housed streams. The low dose (11.3 µg Cu/litre) resulted in a 10% reduction of taxa and 44% reduction in species abundance in field constructed streams as compared to 56 and 75% reduction in laboratory streams, respectively. Species of the order Ephemeroptera (mayflies) were the most sensitive in both studies.

A field study was conducted at Shayler Run, a natural stream near Clermont County, Ohio, USA, to determine the effects of copper on stream biota (Geckler et al., 1976). A single nominal concentration of 120 µg Cu/litre was chosen as the test level. This value was selected because it was thought that it would be high enough to affect sensitive fish populations, based on laboratory chronic fish studies performed earlier. This stream was also known to receive sewage from a small waste treatment plant 6.5 km (4 miles) upstream from the test area. Measured concentrations in the treated portion of the stream during the study ranged from 44.1 to 96.3 µg Cu/litre. All but one abundant fish species in the stream and four of the five abundant macroinvertebrates were adversely affected by the exposure to copper.

Leland et al. (1989) exposed oligotrophic streams to copper concentrations ranging from 2.5 to 15 µg total Cu/litre (12–75 ng Cu^{2+} activity/litre calculated by computer modelling). Declines in population density of species representing all major orders (Ephemeroptera, Plecoptera, Coleoptera, Trichoptera and Diptera) occurred at 5 and/or 10 µg total Cu/litre. Herbivores were more sensitive to copper toxicity than predators.

Saward et al. (1975) exposed a marine food chain, comprised of phytoplankton, bivalves (*Tellina tenuis*) and fish (plaice *Pleuronectes platessa*), to copper at concentrations of 10, 30 and 100 µg Cu/litre for 100 days. All copper exposures reduced the standing crop and the rate of photosynthesis per unit of chlorophyll *a* in phytoplankton. Copper adversely affected bivalve condition by means of a reduction in carbohydrate reserves and nitrogen levels. Fish showed reduced growth; however, no significant change in condition or biochemical composition was reported.

9.3.3 Terrestrial organisms

9.3.3.1 Plants

Generally visible symptoms of copper toxicity are small chlorotic leaves and early leaf fall. Growth is stunted, and initiation of roots and development of root laterals are poor. Reduced root development may result in a lowered water and nutrient uptake which leads to disturbances in the metabolism and growth retardation (Balsberg Påhlsson, 1989).

Toxicity to plants grown hydroponically

Beckett & Davis (1977) stated that yield alone is a poor index of toxicity since the height of the plateau depends on many other factors. The toxic effects of a given concentration also depend on many factors. However, the effect on yield of a potentially harmful element depends mainly on its concentration in the tissue. Therefore, the tissue concentration of the element at the upper critical level should be relatively independent of other factors. Davis & Beckett (1978) grew barley (*Hordeum vulgare*), lettuce (*Lactuca sativa*), rape (*Brassica napus*) and wheat (*Triticum aestivum*) in a nutrient solution containing copper. The dry matter yield of these plants was independent of the copper concentrations in their photosynthesizing tissues, up to a critical concentration (upper critical level). The upper critical concentrations of copper for barley, lettuce, rape and wheat were, respectively, 19, 16, 21 and 21 mg Cu/litre. Beckett & Davis (1978) exposed young barley plants to combinations of copper, zinc and nickel. At tissue concentrations in excess of their respective upper critical levels the toxic effects of copper and nickel appear to be additive, but the combination of copper and zinc appears to be antagonistic.

Taylor & Foy (1985) observed that plants of *Triticum aestivum* exposed to 3 mg Cu/litre (50 µmol/litre), as copper sulfate, showed acute signs of copper toxicity, including mild necrosis and symptoms of induced iron deficiency in the leaves, and inhibition of root growth and lateral root initiation. Plants exposed to 50 mg Cu/litre (800 µmol/litre), as copper–EDTA, showed systemic toxicity symptoms probably reflecting iron deficiency as the primary toxic effect. Leaves showed mild necrosis and symptoms of iron deficiency;

root growth, although depressed, was not as severely affected as with copper sulfate, and lateral root initiation was unaffected.

Wong & Bradshaw (1982) grew perennial rye grass (*Lolium perenne*) from seed in solutions of copper sulfate for 14 days. Copper concentrations of 0.02 mg Cu/litre (0.3 µmol/litre) caused a 50% reduction in normal root growth.

Alva & Chen (1995) examined the effects of 6.35, 317.5, 635 and 1270 µg Cu/litre (0.1, 5, 10 or 20 µmol Cu/litre) in nutrient solution at pH 5.5 on growth, uptake and partitioning of copper by seedlings of mandarin *Cleopatra* and citrumelo *Swingle* rootstock. There was a significant exposure-dependent decrease in both shoot and root dry weight. The concentration of copper in both shoots and roots increased with increasing exposure concentration. The increase in tissue copper concentration was more marked in roots than in shoots. The pronounced effect of copper on iron uptake could, in part, be explained by the development of iron chlorosis symptoms.

Winter wheat plants (*Triticum aestivum* L cv. Starke II) were grown for 7 days in split-root chambers containing nutrient solutions with various copper chloride coucentrations (32 controls) to 635 µg $CuCl_2$/litre; 0.5 to 10 µmol/litre). Average root length and dry weight of the root parts exposed to 127–635 µg Cu/litre (2–10 µmol/litre) decreased, and lateral root initiation was delayed; dry weight of root parts increased in control plants. Copper was not exported from the roots to the other plant parts (Adalsteinsson, 1994).

Schmidt (1988) reported IC_{50}s for inhibition of root growth from germinated seeds at 1.8 mg Cu/litre for *Lupinus albus* and 0.274 mg Cu/litre for *Cicer arietinum*.

Burton et al. (1986) studied the interactive effects of copper, cadmium and nickel on seedlings of Sitka spruce (*Picea sitchensis*) grown in nutrient solution for 42 days. Copper concentrations of 5 and 10 mg/litre significantly reduced seedling yield. There were no significant interactive effects on yield even where copper and cadmium individually affected yields, but nickel and copper did interact.

Huber et al. (1989) treated 3-year-old white, Scots and Austrian pine seedlings with copper (80 mg/litre, 500 µmol/litre) for up to 90 days. Copper significantly affected energy homoeostasis and oleoresin production, and induced a loss of tolerance in Scots pine and loss of resistance to nematodes in Austrian pine.

Sela et al. (1988) exposed roots and shoots of *Azolla filiculoides* to copper (10 mg/litre) for 1 day. Copper exposure caused losses of potassium, chloride and magnesium from *Azolla* roots.

Root growth of seedlings of the *Agrostis capillaris* cultivars Parys (copper tolerant), Gognian (lead/zinc tolerant) and Highland (non-tolerant) was measured after 14 days growth in solutions containing 64–762 µg Cu/litre (1–12 µmol/litre). Highland cultivars showed a sharp negative exponential decline in root growth at concentrations > 64 µg Cu/litre (1 µmol/litre). Gognian and Parys cultivars are unaffected by copper levels of 64 and 254 µg Cu/litre (1 and 4 µmol/litre); however, at higher concentrations Parys cultivars are less affected by copper than Gognian (Symeonidis et al., 1985).

Wong et al. (1994) reported that a zinc/lead tolerant cultivar of *Festuca rubra* was tolerant of copper. The cultivar showed a high tolerance index (80.33%) at 50 mg Cu/litre. A treatment of 50 mg Cu/litre appeared to cause little damage to root and shoot elongation. Fresh weights of roots were significantly reduced at copper concentrations of 10 mg/litre; there was no effect on fresh weight of shoots. Metal transport to the shoot was minimal, indicating that the root may play a major role in preventing transportation of copper to the upper portion of the plant.

Toxicity to plants grown in soil

Toxicity of copper to terrestrial plants grown in soil is summarized in Table 24.

Jarvis (1978) grew perennial ryegrass (*Lolium perenne*) in a loam soil amended with copper at concentrations of 9.5, 95.3 and 953 mg/kg (dry weight). Significant reductions in dry weight of shoots and roots over 4 harvests were observed only at the highest concentration.

Table 24. Toxicity of copper to terrestrial plants grown in soil

Organism	Parameter	End-point	Concentration	Reference
Perennial grass (*Lolium perenne*)	4 harvests	significant reduction in dry weight of shoots and roots	953 mg/kg	Jarvis et al. (1978)
Snap beans (*Phaseolus vulgaris*)	2 years	yield	significant decrease at concentrations > 20 mg/kg extracted with HCl or DTPA or at 15 mg/kg when extracted with EDTA	Walsh et al. (1972)

Graham et al. (1986) grew carrizo citrange seedlings in sandy soil amended with copper at concentrations ranging from 25 to 300 mg/kg as basic copper sulfate. The growth of seedlings and the colonization by the mycorrhizal fungus *Glomus intraadices* were reduced logarithmically with copper exposure. Copper-induced reductions in seedling phosphorus uptake were more closely related to the inhibition of hyphal development outside the root than to the development of vesicles and arbuscules in the root.

Walsh et al. (1972) applied copper sulfate and hydroxide to a loamy sand at rates of 15–486 kg Cu/ha for 2 years. Rates of up to 54 kg Cu/ha had no adverse effect on the yield of snap beans (*Phaseolus vulgaris*). Slight decreases were noted at copper concentrations in excess of 130 kg/ha and marked reductions in yield were observed at 405 kg/ha of the hydroxide and 486 kg/ha of the sulfate. Soil copper concentrations and yield were highly correlated. Significant reductions in the yield of snap beans were noted when more than 20 mg Cu/kg was extracted from soil with HCl or DTPA and when more than 15 mg Cu/kg was extracted with EDTA.

Gettier et al. (1988) studied the response of corn (*Zea mays*) grown in fields amended with six annual applications of copper-enriched manure or copper sulfate at application rates ranging from 48 to 198 kg

Cu/ha. No significant effect on grain yield was observed in a fine sandy loam and clay loam soils. However, applications of copper sulfate (60 and 198 kg/ha) caused significant increases in grain yield on silt loam soil. There was no significant accumulation of copper by corn plants during the experiment.

Cineraria maritima L and *Centauria moschata* L were tested for their tolerance/sensitivity in metal-rich soils, which contained high levels of copper and were derived from iron ore from Lalitpur, Girar, India. The two plant species growing in the mineralized soil showed higher accumulations of copper than those grown in non-mineralized soils. The rate of photosynthesis and chlorophyll content were reduced in *C. maritima* but not in *C. moschata*, indicating that *C. maritima* is more sensitive to mineralized soil (Farooqui et al., 1995).

9.3.3.2 *Invertebrates*

Neuhauser et al. (1985) exposed earthworms (*Eisenia fetida*) to copper in both contact and artificial soil toxicity tests. In 48-h contact tests LC_{50}s were 6.7 μg/cm^2 for copper acetate, 4.9 μg/cm^2 for copper chloride, 7.4 μg/cm^2 for copper nitrate and 6.3 μg/cm ^{2} for copper sulfate. There was no significant difference between the toxicity of the different copper salts. In an artificial soil test (2 weeks) the LC_{50} was found to be 643 mg Cu/kg. Spurgeon et al. (1994), using the OECD recommended protocol, reported the 14-day LC_{50} for *E. fetida* to be 683 mg Cu/kg. The 56-day LC_{50} and NOEC were 555 and 210 mg Cu/kg, respectively; the EC_{50} and NOEC, based on cocoon production, were 53.3 and 32 mg Cu/kg, respectively. Ma (1984) estimated that the 6-week LC_{50} for the earthworm *Lumbricus rubellus* was 1000 mg Cu/kg (dry weight soil).

Martin (1986) maintained earthworms (*Allolobophora caligniosa*) in soil containing copper concentrations ranging from 5 to 1000 mg/kg for 14 days. All worms died at 1000 mg Cu/kg; copper significantly reduced growth at 500 mg/kg and reduced the number of egg capsules per worm at 100 mg/kg.

Van Gestel et al. (1989) exposed earthworms (*Eisenia fetida andrei*) to copper for a 1-week preconditioning period followed by a further 3 weeks. EC_{50}s, based on cocoon production, were 62 mg

Cu/kg for the pre-conditioning period (1 week) and 191 mg Cu/kg for the following 3-week exposure. A NOEL was derived for the whole of the exposure period of 60–120 mg Cu/kg. Cocoon hatchability was not affected by copper exposure. In 12-week exposures copper concentrations of 10 and 18 mg/kg stimulated growth; the EC_{50} and NOEC for growth reduction were > 100 and 56 mg Cu/kg, respectively (Van Gestel et al., 1991).

Neuhauser et al. (1984) exposed earthworms (*Eisenia fetida*) to 500, 1000, 2000 and 4000 mg Cu/kg of manure (dry weight) for 6 weeks. Copper significantly reduced growth and cocoon production. Similar results were obtained with four different copper salts (acetate, chloride, nitrate and sulfate). The growth rate and reproduction had returned to normal after a 6-week period without copper.

Ma (1984) studied the effects of copper on growth, reproduction and litter breakdown in earthworms (*Lumbricus rubellus*) during 6-week exposure periods in sandy or loam soils. Copper concentrations of up to 373 mg/kg did not cause significant mortality. In sandy soil the number of cocoons and litter breakdown were significantly reduced at 131 mg Cu/kg; body weight gain was significantly reduced at 372 mg Cu/kg. In loam soil the number of cocoons were significantly reduced at 63 mg Cu/kg, litter breakdown at 136 mg Cu/kg and body weight gain was unaffected at concentrations up to 373 mg Cu/kg. Ma (1988) calculated 4-week EC_{50}s, based on cocoon production, to be 122, 68 and 51 mg Cu/kg for the earthworms *Lumbricus rubellus*, *Aporrectodea caliginosa* and *Allolobophora chlorotica*, respectively.

Malecki et al. (1982) exposed earthworms (*Eisenia fetida*) to six different copper salts for 8 weeks. Significant reductions in growth and reproduction (cocoon production) were observed at copper nitrate concentrations of 100 mg/kg (dry weight). Copper sulfate caused significant reductions in reproduction at 100 mg/kg and copper chloride reduced growth at 500 mg/kg. The other salts tested affected growth and reproduction at copper concentrations of > 1000 mg/kg. The least toxic salt was copper oxide which significantly affected growth and reproduction at > 20 000 mg/kg. Long-term studies (20 weeks) with copper acetate revealed significant reductions in cocoon production at 5000 mg Cu/kg.

Bengtsson et al. (1986) exposed earthworms (*Dendrobaena rubida*) to copper concentrations of 10, 100 and 500 mg/kg in soil at varying levels of acidity over a 3-month period. Survival of adults, cocoon production and hatching success decreased with increasing acidity; the reduction was even greater when low pH (pH 4.5) was combined with copper. Irrespective of pH, 500 mg Cu/kg in the soil rapidly caused a collapse of the worm population; survival and cocoon production were significantly lower than in controls and hatching failed entirely.

Streit (1984) exposed orbatid mites and earthworms (*Octolasium cyaneum*) to copper in soil-filled plastic containers for 6 weeks. Copper concentrations of up to 200 mg/kg had no significant effect on the numbers of the seven predominating orbatid mite species. However, at copper concentrations of 40 mg/kg the earthworms either had been killed or had migrated to the noncontaminated half of the container. The authors state that the toxicity of copper to earthworms depends on the particular soil type and in particular the organic carbon content of the soil. For example, 4-day LC_{50}s were reported at 181 mg Cu/kg in poor organic soil (3.2% carbon) and 2760 mg Cu/kg in peat soil (42.6% carbon).

Denneman & Van Straalen (1991) exposed the oribatid mite (*Platynothrus peltifer*) to dietary copper in 3-month reproduction tests. Copper concentrations of up to 2000 mg/kg (dry weight) had no significant effect on survival. The NOECs for growth and reproduction were found to be 9.42 and 2.65 µmol Cu/g, respectively.

Donkin & Dusenbery (1993) exposed nematodes (*Caenorhabditis elegans*) to copper in different soil types in 24-h toxicity tests. LC_{50}s were 70, 534, 413, 1061 and 629 mg Cu/kg for sand, sandy loam (66% sand), sandy loam (55% sand), loam and clay loam, respectively. An LC_{50} for nematodes exposed in water only was 105 mg Cu/litre.

Parmelee et al. (1993) incubated forest soil treated with copper (100, 200, 400 and 600 mg/kg) for 7 days. Omnivore-predator nematodes and mesostigmatid and orbatid mites were the groups most sensitive to copper and were significantly reduced at copper levels of 100 mg/kg. Total nematode and microarthropod numbers declined significantly at copper concentrations above 200 mg/kg. Trophic

structure analysis revealed that the high sensitivity of nematode predators reduced predation and resulted in significantly increased numbers of nematodes at 200 mg Cu/kg.

Marigomez et al. (1986) fed terrestrial slugs (*Arion ater*) on a diet containing copper concentrations ranging from 10 to 1000 mg/kg for 27 days. No treatment-related effect on mortality was observed. Copper concentrations of 100 mg Cu/kg or more showed an exponential change in feeding with treated slugs eating less than controls. Initially the feeding behaviour of slugs on a diet of 50 mg Cu/kg was unaffected, but by the end of the 278-day treatment they showed the same reduction in feeding as the higher exposures.

Bayley et al. (1995) exposed larvae of the carabid beetle (*Pterostichus cupreus*) to copper in both the soil (500 mg Cu/kg) and in their food (500 mg Cu/kg fresh weight; 1357 mg Cu/kg dry weight). Larval mortality due to copper exposure was 69% when adjusted for control mortality and mainly occurred during larval metamorphosis and pupation. The locomotor behaviour of male and female adult beetles surviving the exposure to elevated copper during larval development was significantly impaired.

Gintenreiter et al. (1993) reared gypsy moth (*Lymantria dispar*) larvae on an artificial medium contaminated with copper (10, 50, 250 and 1250 mg/kg) from hatching or the fourth instar stage to pupation. All larvae died at 1250 mg Cu/kg and larval survival was significantly reduced at 250 mg Cu/kg. Contamination from hatching generally resulted in a decrease in headcapsule width and this was significant at a copper concentration of 50 mg/kg. The number of larvae hatched per egg cluster was significantly reduced at 50 mg Cu/kg. NOECs were 10 mg Cu/kg for development rate, growth and reproduction, and 50 mg Cu/kg for mortality in larvae exposed from the first instar. NOECs were 10 mg Cu/kg for reproduction, and 50 mg Cu/kg for development rate, mortality and growth in larvae exposed from the fourth instar stage. Ortel et al. (1993) reared moth larvae on diets containing copper at 10 or 50 mg/kg. No correlation was found between the extent of copper contamination and parasitization success by the braconid wasp (*Glyptapanteles liparidis*).

Nectoux & Bounias (1988) dosed honeybees (*Apis mellifera*) with sucrose solutions containing copper at concentrations ranging from 250 to 2000 mg/litre. Controls gave an LT_{50} of 27 days with a daily percentage mortality at 3%. LT_{50}s for dosed bees ranged from 5.1 to 14.2 days with the daily percentage mortality ranging from 5.78% per day to 30.22% per day.

9.3.3.3 *Vertebrates*

Dodds-Smith et al. (1992a,b) maintained shrews (*Sorex araneus*) on a diet containing copper at an intake of 2.13 mg/day for 12 weeks. There was no significant effect on growth rate during the feeding trial and no relationship between copper intake and mortality.

Aulerich et al. (1982) fed young mink on a diet containing 0, 20, 50, 100 or 200 mg Cu/kg for 153 or 357 days. The shorter exposure did not significantly affect haemoglobin or haematocrit levels. Reproduction performance was not adversely affected, although greater mortality in young mink and reduced litter mass were a result of the higher copper exposures. Intraperitoneal LD_{50}s for adult mink were 7.5 mg/kg for copper sulfate and 5.0 mg/kg for copper acetate.

A summary of the toxicity of copper in domestic animals was published by the committee on animal nutrition of the National Research Council (1980). The information presented indicates that sheep are more sensitive to copper than other domestic animals, and horses appear to be more tolerant than cattle, pigs, sheep or poultry. This is in agreement with the results presented by Smith et al. (1975) who fed yearling ponies a pelleted diet up to 791 mg Cu/kg with no visible effects after 6 months.

The results of Hill & Williams (1965) showed that a dietary concentration of 266 μg Cu/g (dry weight) slightly reduced the live-weight gain in lamb. At 40.7 μg Cu/g the reduction in the rate of live-weight gain was statistically significant.

The use of copper as a feed additive for growth stimulation has attracted interest in its toxic effects. Combs et al. (1966) studied the effect of the level of dietary protein in pigs fed high copper rations. Their results indicated that pigs fed a diet containing 250 ppm (mg/kg)

copper and either 14 or 22% protein had daily gains of 0.80 and 0.78 kg, respectively, while those given diets containing 500 ppm (mg/kg) copper and either 14 or 22% protein gained 0.48 and 0.55 kg daily, respectively.

9.4 Field observations

9.4.1 Microorganisms

Mathur et al. (1979) studied a 2-ha field comprising an organic muck soil which had been cultivated for 45 years, having residual fertilizer copper applied to a distinct site for the last 15 years. The soil copper content ranged from 150 to 260 mg/kg (dry weight). The rate of carbon dioxide evolution was significantly negatively correlated with both the total and extractable copper contents of the soil. Acid phosphatase activity significantly decreased as copper content increased. Dumontet et al. (1992) reported a significant negative correlation between both copper and cadmium, and soil respiration in the 0–15-cm layer of contaminated soil from the vicinity of a copper–zinc smelter. However, the authors did not find a significant correlation between soil copper and acid phosphatase activity.

Minnich & McBride (1986) studied five soils which had received anthropogenic copper inputs for many years: recent sludge, aged sludge, spillsite, vineyard and muck. The copper concentrations in the soils ranged from 33 to 1445 mg/kg soil (dry weight). No significant effects due to copper on carbon mineralization were detected. Nitrogen metabolism was significantly increased only in the soil which had received recent sludge additions.

Burton (1987) surveyed river water and sediment samples and one soil sample for bacterial resistance to metals. Between 0 and 2.4% of bacterial populations sampled were resistant to 1 mmol Cu/litre. The highest resistance was found at Crater Lake, Colorado, USA. However, sediment copper concentrations were much higher at several other sites.

9.4.2 Aquatic organisms

Wood (1983) found that naturally occurring marine phytoplankton populations show a tolerance to added cupric ions which far exceeds

the physiological limits of phytoplankton cultures grown in chemically defined media. The tolerance appears to be due to regulation of bioavailability of added copper by an abundance of copper-complexing agents. Coastal phytoplankton were less sensitive than continental shelf or oceanic communities. The toxicity of copper correlated more with copper-complexing capacity than with biotic species composition or community structure.

Effler et al. (1980) monitored the impact of low-level copper applications to Cazenovia Lake, New York, USA. The application caused only small increases (up to 5 µg Cu/litre) for 2–5-day periods. The treatment did not achieve the desired algicidal action on the target phytoplankton. There were short-term alterations in the seasonal succession processes within phytoplankton populations. No significant effects on zooplankton or submerged macrophytes were observed.

Hanson & Stefan (1984) summarized the effects of nearly 60 years of copper use as an algicide on some lakes in Minnesota, USA. Copper treatments have been as high as 250 µg Cu/litre averaged over the entire lake. Algal mortality has led to hypolimnetic oxygen depletion and affected nutrient dynamics. Phytoplankton populations have shifted to greater dominance of blue-green algae. Several major fish kills related to transiently high copper concentrations have been documented, and fish populations have shifted to less desired species. Sediment concentrations as high as 5600 mg Cu/kg have developed, affecting development of macrophytes and benthic invertebrate populations.

Carlson et al. (1986) investigated the effects of copper on the Naugatuck River, Connecticut, USA, which received multiple discharges from domestic and industrial sources. Downstream from the major effluents, there were severe effects on fish, periphyton, and benthic invertebrates. Average total copper concentrations at sampling stations in the affected area ranged from 50 µg Cu/litre to over 400 µg Cu/litre, whereas stations with little or no impact had average concentrations < 20 µg Cu/litre. Toxicity tests using river water showed reduced survival of *Ceriodaphnia dubia* in samples from affected areas, but little or no effects from samples from unimpacted areas. The US EPA water quality criteria for the river varied from 5 to 12 µg Cu/litre for 4-day average concentrations and from 7 to 18 µg

Cu/litre for 1-h average concentrations. These concentrations were exceeded even in the unaffected area. However, toxicity tests using dilution water from the river showed copper to be 3–8-fold less toxic than did dilution water typical of laboratory tests used to establish the criteria. Therefore, site-specific criteria were estimated to be 24–43 µg Cu/litre for the 4-day average and 34–61 µg Cu/litre for the 1-h average. These concentrations were rarely exceeded upstream from the major discharges, but were routinely exceeded downstream, consistent with the observed biological impact.

Grant et al. (1989) studied the tolerance of polychaete worms (*Nereis diversicolor*) to copper. Polychaete worms collected from a site with surface sediment levels of 1733 mg Cu/kg were more tolerant when exposed to 500 µg Cu/litre in acute tests than those from a low-metal site (19 mg Cu/kg). LT_{50} values were 70 h for worms from a low-metal site and 1407 h for those from the contaminated site. Laboratory-bred worms still retained the tolerance. Worms showed a graded level of tolerance depending on field exposure.

Han & Hung (1990) reported the case of green oysters (*Crassostrea gigas*) in the Charting mariculture area of southwestern Taiwan in January 1986. The green colouration was found to be due to high copper content of the oyster tissue. A survey of the area revealed total dissolved copper levels ranging from 5 to 23.6 µg/litre, particulate copper levels ranging from 1 to 5.5 µg/litre and oyster tissue levels with a mean of 4401 mg Cu/kg (dry weight). Green oysters have occasionally been observed in other areas.

Copper-rich granules have been reported to occur in a wide variety of invertebrates inhabiting copper-polluted habitats. Weeks (1992) observed copper-rich granules in the ventral caeca of talitrid amphipods (*Orchestia gammarellus*) revealed by transmission electron microscopy. In addition, copper deposits also appear in the physodes of *Fucus vesiculosis* and *F. serratus* (Smith et al., 1986). The occurrence of intracellular deposits containing copper were reported in a copper-tolerant isolate of the green alga *Scenedesmus* (Silverberg et al., 1976). In the latter case the metal appeared mainly in the nucleus, although similar structures were observed in the cytoplasm. Silverberg and co-workers concluded that the occurrence of these inclusions could be regarded as a detoxifying mechanism because they

were absent in the non-tolerant strains. Copper has been detected in polyphosphate bodies in the green alga *Chlorella furca* (Wong et al., 1994) and in the fouling diatoms *Amphora* and *Navicula* (Daniel & Chamberlain, 1981).

9.4.3 Terrestrial organisms

9.4.3.1 Tolerance

Duvigneau & Denaeyer-De Smet (1963) studying the copper content in leaves of plant species growing on soils containing 500 mg Cu/kg (dry soil) emphasize that there is more than one tolerance mechanism operating. They found that some species avoid copper toxicity by excluding the metal, some species accumulate the metal to very high concentrations and other species occupy an intermediate position. They conclude that there may be both exclusion and accumulation mechanisms evolving in different species of the same genus.

Wu & Kruckeberg (1985) fonnd that two legume plants *Lupinus bicolor* and *Lotus purshianus* growing on copper mine waste in northern California, USA, with a mean soil copper content of 460 mg/kg exhibited considerably greater copper tolerance at 0.2 mg/litre in nutrient solution than plants from an adjacent meadow where copper levels were 0.1–1.5 mg/kg. The tolerance index of the field-collected plants was positively correlated with the copper concentration of the soil from which the plants were collected. Wu & Lin (1990) isolated the nitrogen-fixing bacterium *Rhizobium loti* from root nodules of *L. purshianus* growing on the copper mine and found it to have greater copper tolerance than rhizobium isolated from plants in a nearby field. No difference was detected in uptake pattern or concentration of copper in tolerant and nontolerant *L. purshianus*. However, a copper accumulation mechanism associated with tolerance was found in the symbiotic rhizobium. Effective nitrogen fixation was seen in copper-enriched soils.

Kruckeberg & Wu (1992) investigated the tolerance of herbaceous plants colonizing copper mine waste sites in northern California, USA. Five of the seven species tested showed elevated copper tolerance. The copper-tolerant species were found at more than one copper mine. The mines were geographically isolated, so tolerance in these plant

species probably evolved independently. In *Arenaria douglasii*, *Bromous mollis* and *Vulpia microstachya* the exclusion of copper from the shoots partly by immobilization at the roots may be a mechanism of tolerance. However, in some species there were no differences between the uptake of copper into tissues of tolerant and nontolerant species. Therefore, it would appear that different mechanisms of copper tolerance have evolved among the plant species colonizing California copper mine waste sites.

Dickinson et al. (1991) studied the survival of sycamore (*Acer pseudoplatanus*) trees at a metal-contaminated site (copper refinery) in northwest England where populations of the herbaceous flora have evolved metal tolerance. Cell culture growth experiments on explant material from shoot meristems of mature trees showed increased tolerance to copper. Some of these trees predated the establishment of the refinery. However, tolerance tests on tree seedlings showed no evidence that the trees produce tolerant offspring. The tolerance of the mature trees is ascribed to phenotypic adaptation induced during the life of the tree as site contamination occurred.

Taylor & Crowder (1984) did not find copper tolerance in the cattail rush (*Typha latifolia*) collected from the vicinity of a copper smelter near Sudbury, Ontario, Canada, with soils contaminated with 3738 mg Cu/kg and 9372 mg Ni/kg. Growth of both contaminated and non-contaminated plants was inhibited by 100 mg/kg copper-EDTA. No particular *in vivo* copper tolerance was found in the clones from the heavily contaminated site. The metals at this site are believed not to be bioavailable owing to the strongly anaerobic waterlogged conditions or to the presence of high sulfide levels in the mud.

Rauser & Winterhalder (1985) collected several grass species from the vicinity of the Sudbury copper smelter (Ontario, Canada). They found clones of the grass species *Deschampsia caespitosa*, *Agrostis gigantea* and *Poa compressa* to be tolerant to copper. *Hordeum jubatum* plants showed no tolerance to copper exposure.

Frenckell-Insam & Hutchinson (1993) found copper tolerance in populations of the grass *Deschampsia cespitosa* collected at seven Canadian or German mine sites, with some Sudbury area plants performing better in the presence of normally toxic levels of copper.

Schultz & Hutchinson (1988) showed that this copper tolerance in *D. cespitosa* was not due to a metallothionein-like protein.

Wainwright & Woolhouse (1977) studied three clones of *Agrostis tenuis* with respect to the effects of copper (64, 6.4 and 0.64 mg/litre; 10^{-1}, 10^{-2} and 10^{-3} mmol/dm^3) in nutrient solution on growth of root segments excised from the zone of cell elongation. Growth (24 h) of copper-tolerant and zinc-tolerant clones was less inhibited by copper than was the growth of a non-tolerant clone. Concentrations of copper ions which inhibited root growth also caused leakage of potassium ions from cells. The authors suggest that the loss of potassium ions from roots is due to the toxic effect of copper ions on the plasmalemma.

9.4.3.2 *Copper fungicides and fertilizers*

During perennial tree production, amendments to soil of trace elements essential for growth are often necessary. However, application of such amendments results in the accumulation of elements in topsoil. Copper can also build up as a result of routine application of copper at relatively high rates with fertilizers. In addition, the routine spraying of copper-based fungicides can result in copper accumulation. Reuther & Smith (1953) reported an increasing number of Florida, USA, citrus orchards on sandy, acid well-drained soils affected with a chlorotic disorder of the foliage. The authors linked this disorder with possible effects of high copper levels.

Paoletti et al. (1988) reported that spring/summer fungicide (Bordeaux mixture) treatment of vineyards in Italy caused a decrease in the local population of earthworms. In particular, a decrease in the number of juvenile *Allolobophora* was recorded. Other macro-invertebrates were unaffected, both in terms of biomass and number of species, by fungicide applications.

10. EVALUATION OF HUMAN HEALTH RISKS AND EFFECTS ON THE ENVIRONMENT

10.1 Concepts and principles to assess risk of adverse effects of essential elements such as copper

10.1.1 Human health risks

There are risks associated with low intakes as well as high intakes of essential elements. The relationship between intake/exposure level and risk therefore has a U-shaped curve, with risks from deficiency at low intakes and risk of toxicity at high intakes (see Fig. 2). There is a need to define an intake range that prevents both deficiency and toxicity for the general population. The range of acceptable intakes to meet the biological requirement, as well as prevent risk of toxicity, may be extremely narrow. A balanced and comparable scientific approach to assess risk from deficit as well as excess is needed when evaluating essential elements such as copper.

10.1.2 Homoeostatic model

The homoeostatic model describes an acceptable range of exposures or intake (AROI, acceptable range of oral intake) for essential trace elements that permits optimum health (Fig. 2). Environmental levels of copper within the acceptable range of exposure do not produce adverse health effects among members of the general population. However, there are individuals or groups with disorders in homoeostatic mechanisms that experience health effects, either deficiency or toxicity, from exposures within the acceptable range. These disorders may be of genetic origin or from acquired disease.

10.2 Evaluation of risks to human health

10.2.1 Exposure of general population

For healthy humans who are not occupationally exposed the major route of exposure to copper is oral. The mean daily dietary intake of copper in adults ranges between 0.9 and 2.2 mg (see section 5.4). A

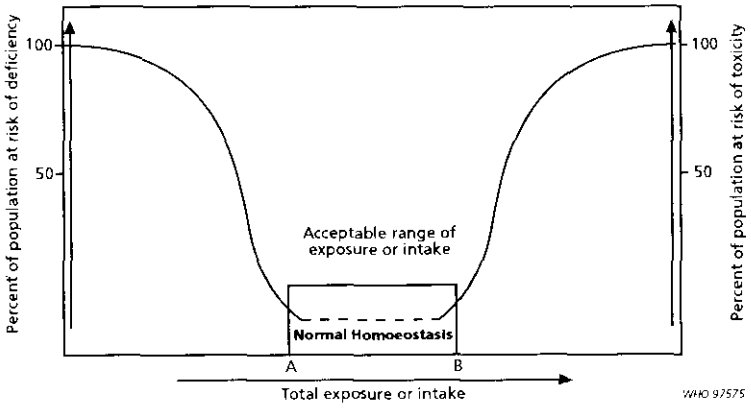

Fig. 2. Percentage of population subjected to deficiency and toxicity effects according to exposure/intake. As intake drops below A for risk deficiency increases; at extremely low exposures or intakes all subjects will manifest deficiency. As exposures or intakes increase beyond B a progressively larger proportion of subjects will exhibit effects of toxicity.

majority of studies have found intakes to be at the lower end of that range. The variation reflects different dietary habits, as well as different agricultural and food-processing practices used throughout the world. Drinking-water may make a substantial additional contribution to the total daily intake of copper, particularly in households where corrosive waters have stood in copper pipes. In homes without copper piping, or with noncorrosive water, copper intake from drinking-water seldom exceeds 0.1 mg/day, although intakes greater than a few mg per day can result from corrosive water distributed through copper pipes. In general, total daily oral intakes are between 1 and 2 mg Cu/day, although they may occasionally exceed 5 mg Cu/day. All other routes of copper exposure (inhalation and dermal) are insignificant in comparison to the oral route. Inhalation adds between 0.3 and 2.0 μg Cu/day from dusts and smoke. Women using copper IUDs are exposed to only 80 μg Cu/day or less from this source.

10.2.2 Occupational exposures

Under occupational conditions where exposure to airborne copper is controlled at or below the widely accepted standard of 1 mg Cu/m^3, and assuming a shift inspiratory volume of 10 m^3 and a usual workplace distribution of particle sizes, an estimated intake of 8.5 mg Cu/day can be calculated for occupational sources. This may be an important contribution to daily intake; however, this is a worst-case estimate using default values and present occupational conditions rarely lead to this level of exposure.

10.3 Essentiality versus toxicity in humans

10.3.1 Risk of copper deficiency

Clinically evident copper deficiency in adults is rarely found in the general population. However, recent dietary surveys show that the mean population intake is suboptimal. In some regions of the world, such as Europe and the USA, intakes are about 20% below the recommended levels. The health consequences of marginally adequate intakes remain to be determined.

Infants with low birth weight (up to 15% of children worldwide) are particularly at risk for deficiency. Frequent episodes of diarrhoea are another risk factor leading to copper deficiency. Copper deficiency commonly occurs during the recovery from protein energy malnutrition, since these infants grow rapidly and are usually fed diets that supply inadequate copper.

Based on dietary interactions, individuals taking supplements of zinc and ascorbic acid are at risk of developing copper deficiency.

Malabsorption states associated with copper deficiency include chronic diarrhoea, short bowel syndrome, partial gastrectomy, coeliac disease, sprue and cystic fibrosis.

Patients receiving prolonged intravenous nutrient mixtures which lack sufficient copper may develop symptomatic evidence of copper deficiency.

Menkes disease (see chapter 8) is a rare (~ 1 : 200 000) X-linked recessive disorder which results in a defect in the intestinal absorption of copper. This disorder leads to a severe, symptomatic, fatal deficiency state even at copper intakes above the AROI.

Copper deficiency has been implicated as a possible risk factor in the pathogenesis of cardiovascular disease.

10.3.2 Risk from excess copper intake

10.3.2.1 General population

When copper homoeostatic control is defective and/or copper intake is excessive, copper toxicity may occur. Ingestion of excess copper is infrequent in humans and is usually a consequence of the contamination of beverages (including drinking-water) or from accidental or deliberate ingestion of high quantities of copper salts. Effects which occur at lowest levels are those on the gastrointestinal tract; for example, nausea, vomiting and diarrhoea. Doses which induce such effects have not been well characterized and confounders such as microbiological quality of water supplies or other potential causes of the symptoms have not been adequately considered. On the basis of available data, gastrointestinal illness appears to be associated with consumption of drinking-water containing several mg/litre of copper, but it is not possible to provide a precise number. Symptoms disappear following a change of water supply.

Wilson disease (hepatolenticular degeneration; see chapter 8) is the most common (~ 1 : 30 000) inherited disorder of copper metabolism. The mode of inheritance is an autosomal recessive trait which results in decreased biliary excretion of copper and in hepatic accumulation of the metal.

Other extremely rare conditions such as ICC and ICT are characterized by copper accumulation in early childhood. The relative contribution of genetic factors and/or elevated environmental exposure to copper remains undefined.

A more common cause of copper accumulation in the liver is chronic liver disease associated with chronic cholestasis. These disorders include primary biliary cirrhosis, primary sclerosing

cholangitis, extrahepatic biliary obstruction or atresia and intrahepatic cholestasis of childhood. In these conditions, copper accumulation does not appear to be primarily responsible for hepatic injury.

Copper or copper salts may induce allergic contact dermatitis in susceptible individuals.

There is no convincing evidence of an association between increased dietary copper intake and cardiovascular disease.

Available data in humans and animals are inadequate to assess the reproductive/developmental effects of copper compounds.

There is no convincing evidence that copper plays an aetiological role in the development of cancer in humans, on the basis of available epidemiological data and limited experimental data in animals. The weight of evidence from *in vitro* and *in vivo* assays indicates that copper sulfate is not genotoxic.

10.3.2.2 Occupational risks

Studies of populations of copper workers have failed to demonstrate systemic copper toxicity or significant excess of cancer. No occupational studies were found to indicate that copper exposures resulted in reproductive or developmental effects.

In occupational settings, acute effects are limited to metal fume fever. This condition has been produced by inhalation of fresh copper fume at air concentrations above 0.1 mg Cu/m^3. Similar responses to very finely divided copper metal and oxide dusts have been reported where conditions probably resulted in unusually high dust concentrations.

Chronic effects involving the liver have been reported in workers whose exposures were uncontrolled and likely to have been high.

10.4 Evaluation of effects on the environment

10.4.1 Concept of environmental risk assessment

The science of performing environmetal risk assessments has evolved rapidly in recent years, with standardized techniques being

adopted in both the USA and Europe (US EPA, 1992; OECD, 1995). The key components of environmental risk assessment paradigms include problem formulation, analysis (which includes both exposure and effects analysis), and risk characterization.

Problem formulation consists of defining the risk problem, assessing the population, community, or ecosystem at risk, establishing the model for evaluating the potential for risk and selecting the biological end-points and environmental media to analyse. The analysis phase consists of performing detailed studies designed to characterize the spatial and temporal concentrations of the chemical of interest. Additionally, a series of standardized laboratory and, in some cases field studies, are performed to evaluate the toxicity dose–response curve for selected end-points and species of interest. The risk characterization phase integrates the exposure and effects data, determines the potential for co-occurrence between organism and contaminant and comes to a conclusion about the potential for risk. The risk statement can be made in terms of a probability statement, frequency of time effects are expected to occur or number of species to be affected. Risk is assessed by determination of the adequacy of the margin of safety between effects and exposure concentrations, and expert judgement is typically used to determine the acceptability of the perceived margin of safety. There is a general consensus that the larger the margin of safety the lower is the environmental risk. Margins of safety less than 1.0 are usually indicative of a higher potential for risk and may require further evaluation.

10.4.2 Components of risk assessment process for copper

The principal components of risk assessment are exposure and effects characterization. The environmental exposure has been assessed by reviewing the fate (transport, distribution and behaviour) from the point of release into and through the environmental compartments of air, water soil/sediment and biota. Toxicity tests with copper have been done on representative species of the trophic levels in the ecological community of interest, including algae and plants (primary producers), aquatic and terrestrial invertebrates (secondary producers) and fish and terrestrial animals (consumers).

Since copper is an essential micronutrient, a lower limit exists below which deficiency will occur (see section 10.1). Thus the use of large safety factors in procedures to limit exposures to below toxic levels might result in target concentrations below essential levels. This potential problem has been addressed in the deficiency toxicity optimum concentration band for essential elements (DT-OCEE) concept (van Tilborg, 1996). Because copper is a ubiquitous trace metal in the natural environment, it is unlikely except in some terrestrial regions where copper concentrations are very low, or where antagonistic molybdenum interactions occur, that deficiency will be a significant issue in the environment. In view of these concepts, the environmental risk assessment paradigm for essential elements such as copper must (as for humans, see section 10.1.1) be expressed as a deficiency–toxicity model which describes an acceptable concentration range for copper in the environment.

One of the key questions in ecotoxicology is to what extent laboratory tests in defined media under carefully controlled conditions are predictive of effects that will be seen in the environment. Traditional toxicity testing has in the past focused on the acute (mortality) and chronic (e.g. growth and reproduction) effects of chemicals on the life stages of representative aquatic and terrestrial organisms. In recent years it has been realized that the environmental chemistry, especially in relation to metal speciation and complexation, will have a signficant influence on and be a determinant of the outcome of laboratory toxicity tests as well as the effects actually seen in the environment. Several papers cited in chapter 9 report this circumstance (see section 9.1), which has been generalized in an hypothesis which describes the bioavailability of copper. This has led to the now accepted view that the total copper in the environmental medium is not a good predictor of its bioavailability. Acceptance of this concept also leads to the logical conclusion that the risk assessment of copper should ideally be made on a site-specific basis.

Organisms may also become adapted at a local scale by physiological acclimation and possibly genetic changes. Because of such adaptations the test-derived toxicity values will be elevated compared to the values for the same species from a nonadapted population. On this basis it is essential that the risk assessment of copper should be made on a site-specific basis.

10.5 Environmental risk assessment for copper

For the purposes of characterizing the potential risk of copper to the environment there are limited data available for perfoming a detailed risk assessment for each environmental medium (air, water, soil, sediment). The largest data set is available for the aquatic environment. The intent of this section is to evaluate the available biological effects and exposure data for various organisms and media consistent with this risk paradigm, and describe ranges of concentrations where the potential for risk increases.

10.5.1 Aquatic biota

10.5.1.1 Overview of exposure data

Natural freshwater streams normally have total dissolved copper concentrations in the range of 1.0–20 µg/litre. Open ocean surface waters contain 0.02–0.2 µg Cu/litre, although near-shore seawater may have copper concentrations as high as 1.0 µg/litre. In the ocean, copper concentration increases with depth. These natural copper levels can be increased by anthropogenic input; for example, acid mine drainage increased the copper concentration up to 600 µg/litre in Restronquet creek, United Kingdom, and Chesapeake bay, USA, can have copper levels as high as 80 µg/litre as a result of shipping activity.

The toxic effect of copper on aquatic biota is critically dependent on the bioavailability of copper in water, which in turn depends on the physicochemical form (i.e. speciation) of the copper. The bioavailability is decreased by the complexation and adsorption of copper by natural organic matter, iron and manganese hydrated oxides, and chelating agents excreted by algae and other aquatic organisms. Toxicity can also be affected by pH and hardness. For these reasons, total copper is rarely useful as a predictor of toxicity. Studies have shown that in natural seawater more than 98% of copper is bound by organic matter and in rivers a high percentage is often organically bound, but the actual percentage depends on the dissolved organic concentration of the river water and its pH.

10.5.1.2 Overview of toxicity data

Copper exhibits significant toxicity to some aquatic organisms, although the degree of toxicity is highly variable and the bioavailability of copper dictates its toxicity to a large extent.

Some algal species are very sensitive to copper. EC_{50} values as low as 47 µg/litre total dissolved copper have been reported for 96-h growth rate experiments, but for other algal species EC_{50} values up to 481 µg/litre have been found. However, it is possible that many of the high EC_{50} values in the literature are the result of the growth rate experiments being carried out in culture media containing copper-complexing agents such as silicate, iron, manganese and EDTA, which reduce the bioavailability of copper.

Acutely lethal copper concentrations to aquatic invertebrates range from several µg/litre to several mg/litre. The 48–96-h LC_{50}s of copper ranged from 7 to 54 µg/litre for *Daphnia magna*, 37 to 183 µg/litre for amphipods, 58 to 112 µg/litre for gastropods and 50 to 100 µg/litre for crab larvae. Sublethal effects and effects on longer-term survival have been reported in a variety of invertebrate species for copper concentrations from about 1 µg/litre to a few hundred µg/litre. For high bioavailability waters, effect concentrations for several sensitive taxa can be < 10 µg Cu/litre.

Acutely lethal copper concentrations for fish range from a few µg/litre to several mg/litre, depending greatly both on the test species and exposure conditions. Acute LC_{50}s less than 50 µg Cu/litre for fish generally are associated with test waters with low DOC, low hardness, and neutral to slightly acidic pH. Sublethal effects and effects on longer-term survival have been reported from 1 µg/litre to a few hundred µg/litre, with effects less than 50 µg Cu/litre being reported for several species. Again, lower effect concentrations are generally associated with test waters of high bioavailability.

Because of the variability of toxic effects concentrations among different biological taxa and exposure conditions, the expected response of aquatic communities will be highly site specific. Table 25 provides a general summary of the nature of response expected for various concentration ranges at sites with moderate to high bioavailability similar to water used in most toxicity tests.

Table 25. Responses expected for various concentration ranges of copper[a]

Total dissolved Cu concentration range (µg/litre)	Effects of high bioavailability in water
1–10	significant effects are expected for diatoms and sensitive invertebrates, notably cladocerans. Effects on fish could be significant in freshwaters with low pH and hardness
10–100	significant effects are expected on various species of microalgae, some species of macroalgae, and a range of invertebrates, including crustaceans, gastropods and sea urchins. Survival of sensitive fish will be affected and a variety of fish should show sublethal effects
100–1000	most taxonomic groups of macroalgae and invertebrates will be severely affected. Lethal levels for most fish species will be reached
> 1000	lethal concentrations for the most tolerant organisms are reached

[a] Sites chosen have moderate to high bioavailability similar to water used in most toxicity tests.

10.5.2 Terrestrial biota

10.5.2.1 *Overview of exposure data*

Copper in uncontaminated soils in Europe, USA, Canada and elsewhere has been measured as total and extractable and with depth in soils. The range of copper concentrations in such soils varies between 0.3 and 250 mg/kg (Bowen, 1985; Adriano, 1992) with soil type being a factor in determining the levels found. Peaty and organic soils are at the upper end of this range, as are loams; sandy soils are at the low end.

Any anthropogenic addition to the surface of such soils, whether by fertilizer or fungicide applications or from highway dust or airborne deposition from urban and industrial sources, causes sharp increases in

the copper levels of such soils. Copper added to provide adequate levels for citrus crops or in orchards and vineyards from fungicide and insecticide application causes a buildup in soils (150–400 mg Cu/kg).

Mining and smelting activities, especially from copper or copper–zinc smelters, often cause surface soil levels to exceed 1000 mg Cu/kg.

10.5.2.2 *Plant foliar levels*

Generally, vegetation rooted in soils reflects the soil copper levels in its foliage. This is dependent upon the bioavailability of the copper, and the physiological requirements of species concerned. On uncontaminated soils foliar levels vary broadly in the range 6.1–25 mg/kg. For grazing animals and for much of the food chain, plant foliar levels are of concern.

On soil contaminated by copper additions (in the range of 150–450 mg Cu/kg), the foliar levels may reach 80 mg Cu/kg, and in mining and smelting area the copper level in foliage can reach 300 mg/kg. Specific hyperaccummulator species can have foliar levels to 17 000 mg Cu/kg without adverse symptoms.

On normal forest soils the nonrooted plants can have higher copper concentrations. These species include mosses and lichens. The fruiting bodies and mycorrhizal sheaths of soil fungi associated with higher plants in forests often accumulate copper to much higher levels than the higher plants at the same site, e.g. Lepp (1992) reports copper concentrations in fungi up 469 mg/kg while foliage levels of 5–20 mg Cu/kg were measured at the same site.

10.5.2.3 *Assessment of toxicity of copper in soil*

Leaving aside the question of copper surface mineralization, at the normal soil concentrations reported (0.3–250 mg Cu/kg) plants rarely if ever show symptoms of toxicity or of adverse growth effects. Crops are often more sensitive to copper than the native flora, so protection levels for agricultural crops range from 25 mg Cu/kg to several hundred mg/kg, depending on the country. Chronic and or acute effects on sensitive species do occur at copper levels occurring in some

soils as a result of human activities, e.g. copper fertilizer addition, fungicide spraying, sludge additions (50–150 mg Cu/kg).

When soil levels rise above 150 mg Cu/kg we begin to find more and more native and agricultural species showing chronic effects. Soils in the range 500–1000 mg Cu/kg act in a strongly selective way, allowing only survival of copper-tolerant species or strains. A reduction in species diversity occurs. By the time soil levels reach 2000 mg Cu/kg a high number of species cannot survive. By 3500 mg Cu/kg areas are largely devoid of vegetation cover. Exceptions again are the old-established copper-tolerant flora on major mineralizations, e.g. in Zaire, Zimbabwe and Borneo.

Effects of copper in soil on terrestrial biota are reported at concentrations ranging from approximately 4 to 7000 mg Cu/kg (chapter 9). With the exception of one study reporting a decrease in yield for snap beans at 15 mg/kg (copper extracted with EDTA) (Walsh et al., 1972) the most sensitive end-points were related to soil microbial metabolism, measured as enzymatic activity and soil respiration. On the basis of the data reviewed in this assessment, the organic content of the soil appears to be a key factor affecting the bioavailability of copper, thus strongly influencing its toxicity.

11. CONCLUSIONS AND RECOMMENDATIONS FOR PROTECTION OF HUMAN HEALTH AND THE ENVIRONMENT

11.1 Human health

The lower limit of the AROI is 20 μg Cu/kg per day. This figure is arrived at from the adult basal requirement with an allowance for variations in copper absorption, retention and storage (WHO, 1996). In infancy, this figure is 50 μg Cu/kg per day.

The upper limit of the AROI in adults is uncertain, but it is most likely in the range of several but not many mg per day in adults (more than 2 or 3 mg/day). This evaluation is based solely on studies of gastrointestinal effects of copper-contaminated drinking-water. A more specific value for the upper AROI could not be confirmed for any segment of the general population. We have limited information on the level of ingestion of copper from food that would provoke adverse health effects.

The available data on toxicity in animals were considered unhelpful in establishing the upper limit of the AROI, owing to uncertainty about an appropriate model for humans. Moreover, traditional methodology for safety assessment, based on application of uncertainty factor to data in animals, does not adequately address the special attributes of essential elements such as copper.

From available data on human exposures worldwide, but particularly in Europe and the Americas, there is greater risk of health effects from deficiency of copper intake than from excess copper intake.

To increase the level of public health protection worldwide, it is recommended that:

1. National and international nutritional guidelines are adhered to in order to address potential copper deficiency.
2. Increased monitoring of the concentration of copper in drinking-water and food should be carried out.

3. There should be increased awareness of the possibility that high copper exposure of newborns may result in adverse health effects.
4. The development of population-based liver disease registries for infant and childhood disease should be encouraged.

11.2 Environmental protection

Protection of aquatic life in waters with high bioavailability will require limiting total dissolved copper to some concentration less than 10 µg/litre (see Table 25); however, the appropriate concentration limit will depend on the biota and exposure conditions at sites of concern and should be set based on further evaluation of relevant data.

At many sites, physicochemical factors limiting bioavailability will warrant higher copper limits. Regulatory criteria should take into account the speciation of copper if dischargers can demonstrate that the bioavailability of copper in the receiving water can be measured reliably.

When sampling and analysing environmental media for copper, it is essential that clean techiques be employed.

Because copper is an essential element, procedures to prevent toxic levels of copper should not incorporate safety factors that result in desired concentrations being below natural levels.

12. FURTHER RESEARCH

12.1 Health protection

1. Determine the bioavailability of dietary copper, particularly in vegetarian diets.

2. In human populations develop the methodology for identifying adverse effects of marginal copper deficiency and of intakes in excess of recommended levels. This should include an evaluation of stable isotope technology to define bioavailability and body stores of copper.

3. Determine the concentrations of copper and the other quality parameters of drinking-water that produce toxicity from single and chronic exposures (e.g. gastrointestinal effects).

4. Characterize the mechanisms that influence copper homoeostasis including placental transfer of copper.

5. Studies on ICC populations to determine:

   a) genetic component
   b) relationship to ICT
   c) mechanisms related to basic defect
   d) methods for early diagnosis of ICC and ICT

12.2 Environmental protection

1. More research is needed to validate existing physicochemical speciation techniques for copper and to develop improved methods. These methods should be calibrated against suitable bioassays. There is also a need for the development of more sensitive, rapid bioassays for copper.

2. Predictive models should be developed for relating bioaccumulation and toxic response to copper speciation and other physicochemical factors that affect bioavailability and toxicity.

3. Insufficient data are available on the toxicity of copper to benthic organisms and more studies are needed in this area.

4. Considerations should be given to the development of more realistic soil toxicity tests that utilize "real" soils; possibly of national relevance. Alternative and more appropriate invertebrate test species should be investigated. Studies correlating measures of copper bioavailability to body burden should be undertaken.

13. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES

The International Agency for Research on Cancer evaluated copper 8-hydroxyquinoline in 1977 (IARC, 1977) and re-evaluated it in 1987 (IARC, 1987). The conclusions were that there are no data on the carcinogenicity of copper 8-hydroxyquinoline in humans and insufficient data in animals. It was, therefore, put into Group 3 – cannot be classified as to its carcinogenic risk to humans.

At the twenty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives and Food Contaminants, the previous recommendation of 0.5 mg/kg body weight as an acceptable daily load for copper was tentatively reconfirmed (WHO, 1982). A provisional tolerable daily intake (PTDI) from all sources was established as 0.5 mg Cu/kg body weight.

During the revisions of the WHO Drinking-water Guidelines (WHO, 1989), copper was re-evaluated. Using the PTDI for copper developed by JECFA (WHO, 1982) a provisional guideline value of 2 mg Cu/litre was proposed (WHO, 1993).

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RÉSUMÉ ET CONCLUSIONS

1. Résumé

1.1 Identité, propriétés physiques et chimiques

Le cuivre est un métal ductile et malléable, de couleur brun rougeâtre. Il appartient au groupe IB de la Classification périodique. Il est généralement présent dans l'environnement au degré d'oxydation +2 mais peut également exister au degré 0, c'est-à-dire à l'état métallique, ainsi qu'aux degrés +1 et +3.A l'état naturel, il se présente sous la forme de sels minéraux et de composés organiques très divers ou encore sous forme métallique. Le métal est à peine soluble dans l'eau et les solutions salines ou légèrement acides mais il est dissous par l'acide nitrique et l'acide sulfurique ainsi que par les solutions basiques d'hydroxyde ou de carbonate d'ammonium.

Le cuivre présente une forte conductivité électrique et thermique et il est résistant à la corrosion.

1.2 Méthodes d'analyse

La grande diversité des dérivés du cuivre, qu'ils soient minéraux ou organiques, a conduit à la mise au point de tout un arsenal de techniques d'échantillonnage, de préparation et d'analyse en vue du dosage de cet élément dans les échantillons biologiques ou ceux qui proviennent de l'environnement. Il est essentiel de mettre en oeuvre des techniques "propres" car la contamination des échantillons par du cuivre provenant de l'air, de la poussière, des récipients ou des réactifs est une source importante d'erreurs d'analyse.

Le dosage colorimétrique ou gravimétrique du cuivre est bon marché et d'exécution simple. Son intérêt se limite cependant aux cas où il n'est pas essentiel d'avoir une sensibilité extrêmement élevée. Pour doser de faibles concentrations de cuivre dans des matrices diverses, on fait le plus souvent appel à la spectrophotométrie d'absorption atomique. En opérant par la même méthode mais avec un four à électrodes de graphite, le gain de sensibilité est considérable par rapport à la spectrophotométrie de flamme. En fonction du traitement

préalable subi par l'échantillon ainsi que des techniques de séparation et de concentration utilisées, la limite de détection dans l'eau atteint environ 1 µg/litre par absorption atomique avec électrodes de graphite (GF-AAS) et 20 µg/litre par absorption atomique classique; on a pu aller jusqu'à 0,05–0,2 µg/g de tissu biologique par GF-AAS. On peut parvenir à une sensibilité encore meilleure en ayant recours à des techniques d'émission comme par exemple le plasma d'argon à couplage inductif associé à la spectroscopie d'émission atomique ou à la spectrométrie de masse. Il existe encore d'autres méthodes comme la fluorescence X, l'utilisation d'électrodes à membrane sélective ou la voltampérométrie avec redissolution anodique ou cathodique.

1.3 Sources d'exposition humaine et environnementale

Parmi les sources d'exposition au cuivre on peut citer la poussière soulevée par le vent, les volcans, les végétaux en décomposition, les feux de forêt et les embruns marins. Parmi les sources d'émission anthropogéniques, on compte les fours de fusion, les fonderies de fonte, les centrales thermiques ainsi que les sources de combustion telles que les installations municipales d'incinération. Les rejets de cuivre dans le sol proviennent essentiellement des résidus et terres de recouvrement des exploitations minières et des boues d'égouts. Les produits agricoles à base de cuivre représentent 2% des rejets de cuivre dans le sol.

L'extraction, la fusion et le raffinage des minerais de cuivre débouchent sur la fabrication d'un grand nombre de produits industriels et commerciaux. Le cuivre est très utilisé pour la fabrication d'ustensiles de cuisine, dans les réseaux de distribution d'eau, ainsi que sous la forme d'engrais, de bactéricides, d'algicides et de peintures antisalissures. Il sert également à la préparation d'additifs pour l'alimentation des bestiaux et de produits favorisant la croissance, et il entre dans la composition de substances permettant de lutter contre les maladies du bétail et de la volaille. Dans l'industrie, on l'utilise comme activateur pour la flottation des minerais sulfurés, pour la production d'agents de protection du bois, en galvanoplastie, dans la fabrication des colorants azoïques, comme mordant pour les colorants des tissus, dans le raffinage du pétrole et enfin pour la préparation de composés divers.

1.4 Transport, distribution et transformation dans l'environnement

Le cuivre est libéré dans l'atmosphère en association avec des particules de matière. Il s'élimine par gravité, dépôt à sec, lessivage et entraînement par les précipitations. La vitesse d'élimination et la distance parcourue depuis la source dépendent des caractéristiques de cette dernière, de la granulométrie des particules et de la vitesse du vent.

Le lessivage naturel du sol par les intempéries et les décharges provenant de l'industrie et des stations d'épuration sont à l'origine du cuivre présent dans l'eau. Il est également possible que des composés cupriques soient volontairement introduits dans l'eau pour détruire les algues. Le devenir du cuivre dans le milieu aquatique est tributaire d'un certain nombre de processus. Il s'agit notamment de la formation de complexes, de la sorption par des oxydes métalliques hydratés, des argiles et des substances organiques ou encore de la bioaccumulation. La connaissance de la forme physicochimique sous laquelle se trouve le cuivre (l'espèce chimique en cause) apporte plus de renseignements que la concentration totale de l'élément. Une grande partie du cuivre rejeté dans l'eau se trouve sous forme particulaire et il a tendance à se déposer, à précipiter ou à s'adsorber à des matières organiques, à des oxydes de fer et de manganèse hydratés ou aux argiles présents dans les sédiments ou dans la couche aqueuse. Dans l'environnement aquatique, la concentration du cuivre dépend de facteurs tels que la dureté et l'alcalinité de l'eau, sa force ionique, son pH et son potentiel redox, la préseuce de substances complexantes, de matières en suspension et de carbone et enfin, des interactions entre l'eau et les sédiments.

C'est dans le sol que le cuivre est rejeté en majeure partie; les sources principales en sont les exploitations minières, l'agriculture et les déchets solides ou les boues provenant des stations d'épuration. La plus grande partie du cuivre déposé dans le sol est fortement adsorbée et demeure dans les premiers centimètres de la couche supérieure. Le cuivre s'adsorbe aux matières organiques, aux carbonates, aux argiles ainsi qu'aux oxydes hydratés de fer et de manganèse. C'est dans les sols sableux acides que le lessivage est le plus important. Dans

l'environnement terrestre, un certain nombre de facteurs importants conditionnent le devenir du cuivre. Il s'agit notamment de la nature du sol lui-même, de la présence d'oxydes, du potentiel redox, des surfaces porteuses de charges électriques, des matières organiques et des échanges de cations.

Il peut y avoir bioaccumulation du cuivre présent dans l'environnement s'il est biologiquement disponible. La valeur du facteur de bioaccumulation varie beaucoup d'un organisme à l'autre, mais il a tendance à être plus élevé en cas d'exposition à de faibles concentrations. Par accumulation, il peut arriver que certains animaux (par exemple, les bivalves) ou certaines plantes terrestres (comme celles qui poussent sur des sols contaminés) se chargent d'une quantité exceptionnellement élevée de cuivre. Néanmoins, de nombreux organismes sont capables de réguler leur concentration totale de cuivre.

1.5 Concentrations dans l'environnement et exposition humaine

La concentration du cuivre dans l'air d'un site est liée à la proximité de sources polluantes importantes comme les fours, les centrales électriques et les installations d'incinération. Le cuivre étant un élément naturel, il est largement disséminé dans l'eau. Il faut cependant être prudent lorsqu'on cherche à interpréter la teneur en cuivre d'un environnement aquatique donné. En effet, dans un système aquatique, la quantité de cuivre qui est mesurée correspond généralement soit au cuivre total, soit au cuivre dissous, ce dernier étant plus représentatif de la biodisponibilité du métal.

En milieu rural, la concentration moyenne de fond dans l'air va de 5 à 50 ng/m^3. Dans les zones non contaminées, la concentration est de 0,15 µg/litre dans l'eau de mer et de 1 à 20 µg/litre dans l'eau douce. Les sédiments constituent un important réservoir et milieu récepteur pour le cuivre. La concentration de fond dans les sédiments naturels en eau douce va de 16 à 5000 mg/kg de poids sec. Dans les sédiments marins, la teneur en cuivre va de 2 à 740 mg/kg de poids sec. En milieu dépourvu d'oxygène, le cuivre présent dans les sédiments est fortement lié sous la forme de sulfure et il n'est donc pas

biodisponible. Dans des sols non contaminés, on a relevé une concentration médiane en cuivre de 30 mg/kg (limites: 2–250 mg/kg). Le cuivre s'accumule dans les végétaux, les invertébrés et les poissons. La teneur de divers organismes en cuivre est plus élevée dans les zones contaminées que dans celles qui ne le sont pas.

Chez les personnes en bonne santé qui ne sont pas soumis à une exposition professionnelle, la principale voie d'exposition est la voie buccale. L'apport journalier moyen par l'alimentation est de 0,9 à 2,2 mg pour un adulte. La plupart des études montrent que dans la majorité des cas, cet apport est voisin de l'extrémité inférieure de la fourchette. Les variations que l'on peut constater traduisent la diversité des habitudes alimentaires et des pratiques agricoles ou culinaires de par le monde. Parfois, l'eau de boisson peut contribuer de façon substantielle à l'apport journalier total de cuivre, en particulier dans les habitations dont la tuyauterie est au contact d'une eau corrosive. Dans les habitations dont la tuyauterie n'est pas en cuivre ou qui ne sont pas alimentées par une eau corrosive, l'apport de cuivre par l'eau de boisson ne dépasse que rarement 0,1 mg/jour, alors que si l'eau distribuée est corrosive, cet apport peut excéder plusieurs mg par jour. En général, l'apport journalier total par la voie buccale (alimentation et eau de boisson) se situe entre 1 et 2 mg par jour, avec des pointes occasionnelles à plus de 5 mg/jour. L'apport de cuivre par les autres voies (respiratoire ou percutanée) est négligeable par rapport à la voie buccale. L'inhalation de poussières et de fumée ajoute quelque 0,3–2,0 mg de Cu par jour. Les femmes qui portent des DIU en cuivre ne sont exposées de ce fait qu'à un apport supplémentaire de 80 µg au maximum.

1.6 Cinétique et métabolisme chez les animaux de laboratoire et l'Homme

L'homéostase du cuivre est liée à la dualité du cuivre, élément à la fois essentiel et toxique. Son caractère essentiel tient au fait qu'il intervient dans un grand nombre de protéines, tant comme élément structural que comme catalyseur. Les mêmes processus cellulaires de fixation et d'incorporation dans les protéines ainsi que les sorties de cuivre se retrouvent chez tous les mammifères et sont modulés par le métal lui-même.

Le cuivre est principalement absorbé dans les voies digestives. Le taux de résorption du cuivre alimentaire est de 20 à 60%, le reste étant excrété dans les matières fécales. Après être passé à travers la membrane basolatérale, le cuivre se fixe à l'albumine qui le transporte jusqu'au foie. Cet organe joue un rôle déterminant dans l'homéostase du cuivre. Le cuivre se répartit ensuite en deux fractions, l'une qui est excrétée par la bile, et l'autre qui est incorporée aux protéines intra- et extracellulaires. La bile constitue la principale voie d'excrétion. Le transport du cuivre vers les tissus périphériques est assuré par l'albumine plasmatique, la céruléoplasmine et des complexes de faible masse moléculaire.

Parmi les méthodes utilisées pour étudier l'homéostase du cuivre chez les mammifères figurent les analyses de rations alimentaires et les études de bilan. Il est essentiel de recourir à des méthodes isotopiques et à des analyses biochimiques standardisées pour bien établir l'existence de carences ou d'excès de cuivre.

La toxicité biochimique du cuivre, lorsque la régulation homéostatique devient inopérante, résulte de l'effet que cet élément exerce sur la structure et la fonction des biomolécules comme l'ADN, les membranes et les protéines, soit directement, soit par l'intermédiaire de mécanismes faisant intervenir des radicaux oxygénés.

1.7 *Effets sur les animaux de laboratoire et les systèmes d'épreuve* in vitro

La toxicité d'une dose unique de cuivre varie largement selon l'espèce en cause (DL_{50} comprise entre 15 et 1664 mg Cu/kg de poids corporel). Parmi les sels de cuivre, ceux qui présentent une bonne solubilité (sulfate de Cu(II), chlorure de Cu (II)), sont généralement plus toxiques que par exemple l'hydroxyde de Cu(II) ou l'oxyde de Cu (II), moins solubles. Des symptômes tels qu'hémorragie gastrique, tachycardie, hypotension,crise hémolytique, convulsions et paralysie précèdent l'issue fatale. Pour la DL_{50} en exposition percutanée, on a fait état de valeurs > 1124 et >2058 mg Cu/kg p.c. respectivement chez le rat et chez le lapin. La CL_{50} pour une exposition par inhalation (durée non précisée) a été trouvée > 1303 mg Cu/kg p.c. chez des

lapins et on a constaté une détérioration de la fonction respiratoire chez des cobayes exposés à une dose de 1,3 mg Cu/m^3 pendant 1 h.

Des rats qui avaient reçu quotidiennement pendant 15 jours 305 mg Cu/kg dans leur nourriture, sous la forme de sulfate de Cu (II), on présenté des modifications de leurs paramètres biochimiques sanguins accompagnées d'autres anomalies hématologiques (anémie en particulier) et l'on a également observé des effets nocifs au niveau du foie, des reins et des poumons. Ces effets étaient de même nature que ceux observés chez d'autres espèces avec d'autres dérivés du cuivre. La dose sans effet observable (NOEL) a été évaluée dans cette étude à 23 mg Cu/kg p.c. par jour. On a cependant relevé que les moutons étaient particulièrement sensibles et des doses de Cu (II) réitérées correspondant à 1,5–7,5 mg Cu/kg p.c. administrées chaque jour sous la forme de sulfate ou d'acétate ont entraîné des lésions hépatiques progressives, une crise hémolytique et la mort.

Exposés pendant une longue période, des rats et des souris n'ont pas présenté de signes manifestes de toxicité autres qu'une réduction de croissance liée à la dose, après ingestion de doses quotidienne équivalant à 138 mg Cu/kg p.c. (rats) et 1000 mg Cu /kg p.c. (souris). La dose sans effet nocif observable (NOAEL) a été évaluée à 17 mg Cu/kg p.c. par jour pour les rats et à 44 et 126 mg Cu/kg p.c. par jour, respectivement pour les souris mâles et les souris femelles. Les effets observés consistaient notamment en une inflammation du foie et en une dégénérescence de l'épithélium tubulaire rénal.

Les études consacrées aux effets toxiques sur la reproduction et le développement sont limitées. On a constaté une certaine dégénérescence testiculaire et une réduction du poids du corps et des organes chez des rats nouveau-nés à des doses dépassant 30 mg Cu/kg p.c. par jour et administrées sur de longues périodes. On a également observé des malformations foetales et autres effets foetotoxiques à dose élevée (> 80 mg Cu/kg p.c. par jour).

Le sulfate de Cu (II) ne s'est pas révélé mutagène dans les épreuves sur bactéries. Toutefois, on a observé une synthèse non programmée de l'ADN qui augmentait en fonction de la dose dans des hépatocytes de rat. Lors du test des micronoyaux sur la souris, on a

observé- dans une étude tout du moins- une augmentation significative des cassures chromosomiques à la dose I.V. la plus élevée (1,7 mg Cu/kg p.c.), mais aucun effet n'a été constaté lors d'une autre étude à des doses allant jusqu'à 5,1 mg Cu/kg p.c.

Les études de neurotoxicité n'ont révélé aucun effet sur le comportement mais des modifications neurochimiques ont été signalées après administration par voie buccale de doses correspondant à 20–40 mg Cu/kg p.c. par jour. D'après un nombre limité d'études d'immunotoxicité, il y a eu une détérioration de la fonction immunitaire humorale et à médiation cellulaire après ingestion, avec l'eau de boisson, de doses équivalant à environ 10 mg Cu /kg p.c. par jour.

1.8 Effets sur l'Homme

Le cuivre est un élément essentiel et les effets indésirables qui lui sont imputables peuvent provenir d'une carence comme d'un excès. La carence en cuivre est à l'origine d'anémies, de neutropénies et d'anomalies osseuses mais il est rare qu'elle se manifeste cliniquement chez l'Homme. On peut faire un bilan cuprique pour essayer de prévoir certains effets cliniques ou encore procéder à un dosage du cuivre et de la céruléoplasmine sériques pour évaluer une carence modérée à forte, dosage qui n'offre toutefois pas autant de sensibilité dans le cas d'une carence limite.

Si l'on excepte les cas d'intoxication aiguë, on n'observe guère d'effets dans les populations normales. L'absorption d'une dose unique d'un dérivé du cuivre soit accidentellement, soit dans un but de suicide, donne lieu aux symptômes suivants: goût métallique, douleurs épigastriques, céphalées, nausées, étourdissements, vomissements et diarrhée, tachycardie, difficultés respiratoires, anémie hémolytique, hématurie, hémorragie gastrointestinale massive, insuffisance hépatique et rénale aboutissant finalement à la mort. On a observé des effets gastrointestinaux après ingestion unique ou répétée d'eau à forte teneur en cuivre et on a fait état d'insuffisance hépatique consécutive à l'absorption de cuivre pendant une longue période. Il ne semble pas qu'une exposition cutanée puisse entraîner une intoxication générale, mais le cuivre peut provoquer des réactions allergiques chez certains individus. On a mentionné des cas de fièvre des fondeurs consécutifs

à l'inhalation, sur le lieu de travail, d'air fortement chargé en cuivre mais, bien que d'autres effets respiratoires aient été attribués à l'inhalation de mélanges contenant du cuivre (par ex. bonillie bordelaise, travail à la mine, travail auprès des fours), la responsabilité du cuivre n'a pas été démontrée. Des ouvriers apparemment exposés à des concentrations atmosphériques correspondant à l'absorption d'une dose de 200 mg Cu/jour, ont présenté des signes évocateurs d'une intoxication cuprique (par ex. élévation du Cu sérique, hépatomégalie). Les données dont on dispose au sujet de la cancérogénicité et des effets toxiques du cuivre sur la reproduction sont insuffisantes pour permettre une évaluation du risque.

On a décrit un certain nombre de groupes que des troubles de l'homéostase cuprique semblent rendre plus sensibles que le reste de la population à une carence ou à un excès de cuivre. Certains troubles ont une origine génétique précise. Il s'agit notamment de la maladie de Menkes, une carence cuprique généralement mortelle, de la maladie de Wilson (dégénérescence hépatolenticulaire), une pathologie qui conduit à une accumulation progressive de cuivre et de l'acéruléoplasminémie héréditaire, qui s'accompagne des manifestations cliniques d'une surcharge martiale. La cirrhose infantile indienne et la cuprotoxicose idiopathique sont des affections liées à un excès de cuivre et peut-être associées à une sensibilité au cuivre d'origine génétique, encore que cette hypothèse n'ait pas été indiscutablement prouvée. Il s'agit là d'affections mortelles de la petite enfance dans lesquelles le cuivre s'accumule dans le foie. On a pu mettre ces maladies en parallèle avec une forte consommation de cuivre, tout du moins dans certains cas.

Parmi les autres groupes potentiellement sensibles à l'excès de cuivre on peut citer les personnes en hémodialyse et les malades atteints d'une affection hépatique chronique. Parmi les groupes exposés au risque de carence en cuivre figurent les nourrissons (notamment les enfants de faible poids de naissance et les prématurés, les enfants qui se remettent d'une malnutrition et les enfants nourris exclusivement au lait de vache), les sujets souffrant d'un syndrome de malabsorption (maladie coeliaque, sprue, mucoviscidose) et les malades nourris exclusivement par voie parentérale. On a également

incriminé une carence en cuivre dans la pathogénèse de certaines maladies cardiovasculaires.

1.9 Effets sur les autres êtres vivants au laboratoire et dans leur milieu naturel

Il faut mettre en balance les effets indésirables du cuivre et son caractère essentiel. Cet élément est en effet essentiel pour tout les êtres vivants et il faut veiller à ce que ces organismes reçoivent la quantité de cuivre qui correspond à leur besoins. Il y a au moins 12 protéines importantes dont le cuivre fait partie intégrante de la structure. Il joue un rôle essentiel dans l'utilisation du fer pour la formation de l'hémoglobine et la plupart des crustacés et des mollusques possèdent une protéine, l'hémocyanine, qui contient du cuivre et représente leur principal transporteur d'oxygène. Chez les végétaux, le cuivre entre dans la composition de plusieurs enzymes qui interviennent dans le métabolisme des sucres, de l'azote et de la paroi cellulaire.

Dans l'évaluation du risque imputable au cuivre, la biodisponibilité de cet élément joue un rôle déterminant. L'adsorption du cuivre à des particules de matière ou sa complexation par des substances organiques peuvent en limiter fortement l'accumulation et par voie de conséquence, les effets. Les autres cations ainsi que le pH peuvent également avoir une influence importante sur la biodisponibilité.

On a montré que le cuivre exerçait des effets nocifs sur la reproduction, les paramètres biochimiques, les fonctions physiologiques et le comportement chez divers organismes aquatiques. Ainsi, des effets toxiques se manifestent chez ces organismes à des concentrations ne dépassant pas 1–2 µg/litre. Il est vrai cependant qu'il faut prendre en considération les importantes variations de sensibilité et de biodisponibilité interspécifiques lorsque l'on se propose d'interpréter et d'appliquer ces données.

Daus des communautés naturelles de phytoplancton, on a constaté que la chlorophylle *a* et la fixation de l'azote étaient sensiblement réduites à des concentrations de cuivre ≥ 20 µg/litre et que la fixation du carbone était aussi notablement réduite à une concentration ≥ 10

µg/litre. Pour les algues, on a obtenue une CE_{50} basée sur l'inhibition de la croissance qui allait de 47 à 120 µg Cu/litre.

Chez les invertébrés dulçaquicoles, la valeur de la CL ou de la CE_{50} à 48 h varie de 5 µg Cu/litre pour une espèce de daphnie à 5300 µg Cu/litre pour un ostracode. Dans le cas des invertébrés marins, on a obtenu une CL_{50} à 96 h de 29 µg Cu/litre pour une coquille saint-jacques et de 9400 µg Cu/litre pour les crabes du genre *Uca*. La toxicité aiguë du cuivre pour les poissons d'eau douce et les poissons de mer est très variable. Pour les poissons d'eau douce, la valeur de la CL_{50} à 96 h va de 3 µg Cu/litre (ombre arctique *Thymallus signifer*) à 7340 µg Cu/litre (*Lepomis machrochirus*). Dans le cas des espèces marines, la Cl_{50} à 96 h va de 60 µg Cu/litre pour un saumon, *Onchorhynchus tschawtscha*, à 1400 µg Cu/litre pour le mulet.

Le cuivre joue le rôle d'oligoélément pour les plantes mais un sol trop riche en cuivre peut se révéler extrêmement toxique. En général, les signes d'une toxicité d'origine métallique consistent dans l'apparition de petites feuilles chlorotiques qui tombent prématurément. Il y a rabougrissement de la plante dont les racines démarrent mal et ne forment pas de départs latéraux. La réduction du développement des racines peut conduire à une moindre fixation d'eau et de nutriments par la plante avec perturbation du métabolisme et de la croissance. Au niveau cellulaire, le cuivre inhibe un grand nombre d'enzymes et perturbe plusieurs processus biochimiques (notamment la photosynthèse, la synthèse des pigments et l'intégrité des membranes) ou physiologiques (notamment le métabolisme des acides gras et des protéines avec également un effet inhibiteur sur la respiration et les processus de fixation de l'azote).

Des effets toxiques ont également été observés au laboratoire chez des lombrics placés dans une terre riche en cuivre; le paramètre le plus sensible qui ait été mesuré était la formation de cocons et des effets nocifs ont été notés à des concentrations de 50–60 mg Cu/kg.

Certains effets délétères observés chez des microorganismes terricoles ont pu être mis en corrélation avec la présence de fortes concentrations de cuivre dues à l'épandage d'engrais à base de cuivre ou à l'implantation de fonderies de zinc dans le voisinage. Dans des

plantations d'agrumes traitées par des fongicides à base de cuivre, on a constaté une chlorose foliaire en corrélation significative avec la teneur du sol en cuivre.

On a montré que dans le milieu naturel, le phytoplancton, les invertébrés aquatiques et terrestres, de même que les poissons et les plantes terrestres, faisaient preuve d'une certaine tolérance au cuivre. Parmi les mécanismes invoqués pour expliquer cette tolérance chez les plantes, on peut citer la fixation du métal à certains composants de la paroi cellulaire, la présence d'enzymes métallo-tolérantes, la formation de complexes avec des acides organiques suivie d'une élimination dans la vacuole et enfin, la combinaison avec des protéines spécialisées riches en thiols ou avec des phytochélatines.

2. Conclusions

2.1 Santé humaine

La limite inférieure de l'intervalle de dose acceptable par ingestion (AROI) est égale à 20 µg Cu/kg de poids corporel par jour. Pour obtenir cette valeur, on est parti de l'apport minimal requis pour un adulte en tenant compte des variations du taux d'absorption, de rétention et d'accumulation du cuivre (OMS, 1996). Pour les enfants en bas âge, ce chiffre est égal à 50 µg Cu/kg p.c. par jour.

La limite supérieure de l'intervalle précité n'est pas connue avec certitude chez l'adulte mais il est très probable qu'elle est de l'ordre de quelques mg par jour et pas davantage (par *quelques* on entend plus de 2 à 3 mg/jour). Cette évaluation ne repose que sur l'étude des effets gastrointestinaux d'une consommation d'eau contaminée par du cuivre. Il n'a pas été possible de donner une limite supérieure plus spécifique pour un groupe quelconque de population. Nous ne disposons que de données limitées sur la quantité de cuivre d'origine alimentaire qui serait susceptible de nuire à la santé.

On a estimé que les données toxicologiques obtenues sur l'animal n'étaient d'aucun secours pour l'établissement de la limite supérieure de l'intervalle de dose acceptable par ingestion chez l'Homme, du fait de l'incertitude quant à l'applicabilité à l'Homme des modèles utilisés.

En outre, les méthodes auxquelles on a habituellement recours pour évaluer l'innocuité d'une substance, méthodes qui impliquent l'application d'un coefficient de sécurité aux données obtenues sur l'animal, ne sauraient convenir dès lors que l'on doit prendre en considération des caractéristiques particulières qui sont celles d'éléments essentiels comme le cuivre.

A la lumière des données dont on dispose sur l'exposition humaine au cuivre dans l'ensemble du monde, mais plus spécialement en Europe et dans les Amériques, il semble que les dangers d'une carence en cuivre sont plus grands que ceux d'un excès de cet élément.

2.2 *Effets sur l'environnement*

Pour assurer la protection des organismes aquatiques dans les eaux où la biodisponibilité est forte, il faut que le cuivre total en solution reste en dessous de 10 µg/litre environ, la valeur la plus appropriée étant fonction des espèces présentes et des conditions d'exposition du site en cause; elle devra être fixée après étude approfondie de tous les paramètres à prendre en considération.

En de nombreux endroits, l'existence de facteurs physico-chimiques limitant la biodisponibilité permettra de relever les limites de concentration. La réglementation devra prendre en considération les espèces chimiques en présence si les auteurs de rejets sont à même de prouver que la biodisponibilité du cuivre dans les eaux réceptrices peut être mesurée avec une fiabilité suffisante.

Lors des prélèvements et des analyses effectués dans l'environnement en vue de la recherche et du dosage du cuivre, il est essentiel d'utiliser des techniques "propres".

Etant donné que le cuivre est un élément essentiel, il faut, lorsqu'on cherche à éviter l'absorption de quantités toxiques de cuivre, se garder d'introduire des coefficients de sécurité qui aboutissent finalement à des concentrations recommandées inférieures aux teneurs naturelles.

RESUMEN Y CONCLUSIONES

1. Resumen

1.1 Identidad, propiedades físicas y químicas

El cobre es un metal de color pardo rojizo, dúctil y maleable. Pertenece al grupo IB de la Tabla periódica. Se suele encontrar en el medio ambiente formando compuestos con valencia 2, pero pueden existir estados metálicos de valencia +1 y +3. Está presente en la naturaleza en una gran variedad de sales minerales y compuestos orgánicos, y en forma metálica. El metal es muy poco soluble en soluciones acuosas, salinas o ligeramente ácidas, pero se puede disolver en los ácidos nítrico y sulfúrico, así como en soluciones básicas de hidróxido o carbonato de amonio.

El cobre posee una elevada conductividad eléctrica y térmica y es resistente a la corrosión.

1.2 Métodos analíticos

La gran variedad de especies de cobre, inorgánicas y orgánicas, ha dado lugar a una serie de técnicas de muestreo, preparación y métodos analíticos para cuantificar el elemento en muestras del medio ambiente y biológicas. La contaminación de las muestras por cobre procedente del aire, el polvo, los recipientes o los reactivos durante la preparación y el muestreo es una fuente importante de errores analíticos, por lo que es fundamental el uso de técnicas "limpias".

Los métodos colorimétricos y gravimétricos para la medición del cobre son fáciles de usar y económicos; sin embargo, su utilidad se limita a las situaciones en las cuales no es indispensable una sensibilidad máxima. Para la medición de concentraciones bajas de cobre en diversas matrices, los métodos más utilizados son los de espectrofotometría de absorción atómica. La sensibilidad aumenta enormemente con la utilización de la espectrofotometría de absorción atómica en electrohorno de grafito, en lugar de la de llama. En función de los procedimientos de tratamiento previo, separación y concentración de la muestra, se han notificado límites de detección de

alrededor 1 µg/litro en agua mediante espectrofotometría en electrohorno de grafito y 20 µg/litro por la de llama y niveles de 0,05–0,2 µg/g de tejido con la primera. Se puede conseguir una sensibilidad mayor mediante el uso técnicas de emisión, como las técnicas de plasma de argon con acoplamiento inductivo de alta temperatura, seguidas de espectroscopia de emisión atómica o espectrometría de masas. Existen otras metodologías más sensibles y especializadas, como la fluorescencia por rayos X, los métodos de electrodos selectivos de iones y potenciométricos y la voltametría de descascarillado anódico y de descascarillado catódico.

1.3 Fuentes de exposición humana y ambiental

Las fuentes naturales de exposición al cobre son el polvo arrastrado por el viento, los volcanes, la vegetación en descomposición, los incendios forestales y la dispersión marina. Entre las emisiones antropogénicas cabe mencionar los hornos de fusión, las fundiciones de hierro, las centrales eléctricas y fuentes de combustión como los incineradores municipales. El desplazamiento principal del cobre a la tierra se produce a partir de las escorias y el manto de las minas de cobre y los fangos cloacales. El uso agrícola de productos de cobre representa el 2% de la liberación de cobre al suelo.

Los minerales de cobre se extraen, funden y refinan para la fabricación de numerosos productos industriales y comerciales. Se utiliza ampliamente en utensilios de cocina y sistemas de abastecimiento de agua, así como en fertilizantes, bactericidas, fungicidas, alguicidas y pinturas antiincrustantes. Se emplea asimismo en aditivos de piensos y estimulantes del crecimiento, así como en la lucha contra determinadas enfermedades del ganado vacuno y de las aves. El cobre se utiliza en la industria como activador en la flotación por espuma de los minerales sulfurosos, la producción de conservantes de la madera, la galvanoplastia, la fabricación de colorantes nitrogenados, como mordiente para tintes de tejidos, en el refinado del petróleo y en la fabricación de los compuestos de cobre.

1.4 Transporte, distribución y transformación en el medio ambiente

El cobre se libera en la atmósfera asociado con materia particulada. Se elimina mediante sedimentación gravitatoria, deposición seca, arrastre y lavado por la lluvia. La velocidad de eliminación y la distancia recorrida desde la fuente dependen de las características de ésta, del tamaño de las partículas y de la velocidad del viento.

El cobre se libera en el agua como consecuencia de la exposición natural a la intemperie del suelo y los vertidos de industrias y plantas de depuración de aguas residuales. Se pueden aplicar compuestos de cobre de manera intencionada al agua para destruir las algas. Hay varios procesos que influyen en el destino del cobre en el medio acuático. Son la formación de complejos, la sorción para formar óxidos metálicos hidratados, arcillas y materiales orgánicos y la bioacumulación. Los datos sobre las formas fisicoquímicas del cobre (especiación) son más informativos que las concentraciones totales de cobre. Gran parte del cobre vertido en el agua está en forma particulada y tiende a sedimentarse, precipitar o adsorberse en materia orgánica, hierro hidratado, óxidos de manganeso y arcilla en el sedimento o la columna de agua. En el medio acuático, la concentración de cobre y su biodisponibilidad dependen de factores como la dureza y la alcalinidad del agua, la fuerza iónica, el pH y el potencial redox, así como de la formación de ligandos complejos, la materia particulada y el carbón suspendidos y la interacción entre los sedimentos y el agua.

La liberación más importante de cobre se produce hacia la tierra; sus fuentes principales son las operaciones de extracción, la agricultura, los residuos sólidos y los fangos procedentes de las actividades de tratamiento. La mayor parte del cobre depositado en el suelo se adsorbe fuertemente y se mantiene en los centímetros más superficiales. El cobre se adsorbe en la materia orgánica, los minerales carbonados, los minerales arcillosos, el hierro hidratado y los óxidos de manganeso. La mayor parte de la lixiviación se produce a partir de suelos arenosos ácidos. En el medio ambiente terrestre hay varios factores importantes que influyen en el destino del cobre en el suelo.

Son las características del propio suelo, el pH, la presencia de óxidos, el potencial redox, las superficies cargadas, la materia orgánica y el intercambio de iones.

Se produce bioacumulación de cobre procedente del medio ambiente si el cobre está biológicamente disponible. Los factores de acumulación varían enormemente entre los distintos organismos, pero tienden a ser más elevados a concentraciones de exposición más bajas. La acumulación puede dar lugar a concentraciones corporales excepcionalmente altas en algunos animales (como por ejemplo los bivalvos) y en plantas terrestres (como las que crecen en suelos contaminados). Sin embargo, muchos organismos son capaces de regular su concentración interna de cobre.

1.5 Niveles medioambientales y exposición humana

La concentración de cobre en el aire depende de la proximidad del lugar a fuentes importantes, como hornos de fusión, centrales eléctricas e incineradores. El cobre esta ampliamente distribuido en el agua, porque se encuentra en ella de forma natural. Sin embargo, sus concentraciones en el medio acuático se deben interpretar con cautela. En sistemas acuáticos, los niveles ambientales de cobre se suelen medir como concentración total o disuelta, siendo esta última más representativa de la biodisponibilidad del metal.

El promedio de las concentraciones básicas de cobre en el aire de las zonas rurales oscila entre 5 y 50 ng/m^3. En las zonas no contaminadas, en el agua marina se encuentran concentraciones de 0,15 µg/litro y en el agua dulce de 1–20 µg/litro. Los sedimentos son un depósito y una reserva importantes de este metal. Las concentraciones básicas de cobre en sedimentos de agua dulce naturales oscilan entre 16 y 5000 mg/kg (peso seco). Las concentraciones en sedimentos marinos varían entre 2 y 740 mg/kg (peso seco). En sedimentos anóxicos, el cobre se une fuertemente mediante sulfuros, por lo que no está biodisponible. Se notificaron concentraciones medias de cobre de 30 mg/kg en suelos no contaminados (intervalo de 2–250 mg/kg). Este metal se acumula en las plantas, los invertebrados y los peces. En organismos de lugares contaminados por cobre se han

notificado concentraciones más elevadas que en los de zonas no contaminadas.

Para las personas sanas no expuestas al cobre en el puesto de trabajo la vía principal de exposición es la oral. La ingesta diaria media con los alimentos oscila en las personas adultas entre 0,9 y 2,2 mg. En la mayoría de los estudios se ha encontrado que los valores de la ingesta se sitúan en el extremo inferior del intervalo. La variación refleja los diferentes hábitos alimentarios, así como las distintas prácticas agrícolas y de preparación de alimentos utilizadas en todo el mundo. En algunos casos, el agua potable puede contribuir a un aumento importante del valor total de la ingesta diaria de cobre, sobre todo en hogares con aguas corrosivas y tuberías de cobre. En los hogares sin tuberías de cobre o con aguas no corrosivas, la ingesta de cobre a partir del agua potable raramente supera el valor de 0,1 mg/día, aunque puede haber valores superiores a unos pocos mg al día a causa de la distribución de agua corrosiva a través de tuberías de cobre. En general, la ingesta diaria total de cobre por vía oral (alimentos más agua potable) oscila entre 1 y 2 mg/día, aunque ocasionalmente puede alcanzar un valor superior a 5 mg/día. Todas las demás ingestas de cobre (inhalación y cutánea) son insignificantes en comparación con la vía oral. La inhalación añade 0,3–2,0 µg/día procedente del polvo y el humo. Las mujeres que utilizan DIU están expuestas sólo a 80 µg o menos de cobre al día a partir de esta fuente.

1.6 Cinética y metabolismo en animales de laboratorio y en el ser humano

La homeóstasis del cobre se debe al doble carácter del elemento, esencial y tóxico. El carácter esencial se deriva de su incorporación específica a un gran número de proteínas con fines catalíticos y estructurales. En los mamíferos se conservan las rutas celulares de absorción, incorporación a las proteínas y exportación del cobre, reguladas por el propio metal.

El cobre se absorbe fundamentalmente a través del tracto gastrointestinal. Se absorbe del 20% al 60% del cobre procedente de los alimentos, mientras que el resto se excreta a través de las heces. Una vez que el metal ha atravesado la membrana basolateral, es

transportado hasta el hígado unido a la seroalbúmina. El hígado es el órgano fundamental para la homeóstasis del cobre. El metal se reparte entre la excreción a través de la bilis y la incorporación a proteínas intracelulares y extracelulares. La vía de eliminación más importante es la biliar. El transporte de cobre hasta los tejidos periféricos se realiza a través del plasma, unido a seroalbúmina, ceruloplasmina o complejos de bajo peso molecular.

Los métodos utilizados para estudiar la homeóstasis del cobre en los mamíferos son el análisis de los alimentos y los estudios del balance. Para conocer la deficiencia y el exceso de cobre son imprescindibles los isótopos y los análisis bioquímicos normalizados de estos procesos.

La toxicidad bioquímica del cobre, cuando supera el control homeostático, se debe a sus efectos en la estructura y la función de biomoléculas como el ADN, las membranas y las proteínas, directamente o mediante mecanismos con intervención de radicales de oxígeno.

1.7 *Efectos en los animales de laboratorio y en los sistemas de prueba* in vitro

La toxicidad de una dosis oral única de cobre varía enormemente entre las especies (DL_{50} de 15–1664 mg de Cu/kg de peso corporal). Las sales más solubles de cobre (sulfato de cobre (II) y cloruro de cobre (II)) son generalmente más tóxicas que las menos solubles (hidróxido de cobre (II), óxido de cobre (II)). La muerte se produce tras la aparición de hemorragia gástrica, taquicardia, hipotensión, crisis hemolítica, convulsiones y parálisis. Se notificaron valores de la DL_{50} para la exposición cutánea > 1124 y > 2058 mg de Cu/kg de peso corporal en ratas y conejos, respectivamente. La DL_{50} por inhalación (duración de la exposición no especificada) fue >1303 mg de Cu/kg de peso corporal en conejos, y en los cobayas expuestos a concentraciones de 1,3 mg de Cu/m^3 durante una hora se observó insuficiencia respiratoria.

Las ratas que recibieron 305 mg de Cu/kg al día por vía oral con los alimentos en forma de sulfato de cobre (II) durante 15 días

mostraron alteraciones de la bioquímica sanguínea y los datos hematológicos (particularmente anemia) y efectos secundarios en el hígado, el riñón y los pulmones. Los efectos fueron cualitativamente semejantes a los de otros compuestos de cobre y en otras especies. La concentración sin efectos observados (NOEL) en este estudio fue de 23 mg de Cu/kg de peso corporal al día. Sin embargo, las ovejas fueron particularmente sensibles, y dosis repetidas de 1,5–7,5 mg de Cu/kg de peso corporal al día en forma de sulfato de cobre (II) o acetato de cobre (II) produjeron lesiones hepáticas progresivas, crisis hemolítica y por último la muerte.

La exposición prolongada de ratas y ratones no puso de manifiesto signos evidentes de toxicidad, salvo una reducción del crecimiento relacionada con la dosis tras la ingestión de 138 mg de Cu/kg de peso corporal al día (ratas) y 1000 mg de Cu/kg de peso corporal al día (ratones). La concentración sin efectos adversos observados (NOAEL) fue de 17 mg de Cu/kg de peso corporal al día en ratas y de 44 y 126 mg de Cu/kg de peso corporal al día en ratones machos y hembras, respectivamente. Los efectos fueron la inflamación del hígado y la degeneración del epitelio tubular del riñón

Los estudios de la toxicidad reproductiva y en el desarrollo fueron limitados. Se observó cierta degeneración testicular y una reducción del peso del cuerpo y de los órganos al nacer en ratas tratadas con dosis superiores a 30 mg de Cu/kg de peso corporal al día durante períodos prolongados de tiempo y efectos fetotóxicos y malformaciones con concentraciones altas (>80 mg de Cu/kg de peso corporal al día).

El sulfato de cobre (II) no fue mutagénico en valoraciones realizadas con bacterias. Sin embargo, se observó un aumento relacionado con la dosis de la síntesis de ADN no programado en hepatocitos de rata. En el ensayo del micronúcleo, un estudio puso de manifiesto un aumento significativo de las fracturas cromosómicas con la dosis intravenosa más alta (1,7 mg de Cu/kg de peso corporal al día), pero en otro estudio realizado con dosis intravenosas de hasta 5,1 mg de Cu/kg de peso corporal al día no se observó ningún efecto.

Los estudios de neurotoxicidad no han puesto de manifiesto efectos en el comportamiento, pero se han notificado cambios neuroquímicos tras la administración oral de 20–40 mg de Cu/kg de peso corporal al día. En un número limitado de estudios de inmunotoxicidad se ha observado un trastorno de la función inmunitaria humoral y mediada por células en ratones después de la ingesta oral con agua de bebida de unos 10 mg de Cu/kg de peso corporal al día.

1.8 Efectos en el ser humano

El cobre es un elemento esencial y hay efectos perjudiciales para la salud relacionados tanto con su deficiencia como con su exceso. La deficiencia de cobre está asociada con anemia, neutropenia y anomalías óseas, pero la deficiencia clínicamente manifiesta es relativamente poco frecuente en el ser humano. Se pueden utilizar los datos del balance para prever los efectos clínicos, mientras que las concentraciones de cobre en el suero y en la ceruloplasmina son medidas útiles de la deficiencia entre moderada y grave, pero son medidas menos sensibles de la deficiencia marginal.

Excepto en el caso de accidentes agudos ocasionales de intoxicación por cobre, se han observado pocos efectos en la población normal. Se han notificado efectos de una exposición única tras la ingestión oral con fines suicidas o accidental consistentes en sabor metálico, dolor epigástrico, dolor de cabeza, náuseas, desvanecimiento, vómitos y diarrea, taquicardia, dificultad respiratoria, anemia hemolítica, hematuria, hemorragia gastrointestinal masiva, insuficiencia hepática y renal y la muerte. También se han presentado efectos gastrointestinales por una ingestión única y repetida de agua de bebida con altas concentraciones de cobre y se ha notificado insuficiencia hepática tras la ingestión crónica de cobre. La exposición cutánea no se ha asociado con la toxicidad sistémica, pero el cobre puede inducir respuestas alérgicas en personas sensibles. Se han notificado casos de fiebre de los fundidores debidos a la inhalación de concentraciones elevadas en el aire en el puesto trabajo y, aunque se han atribuido otros efectos respiratorios a la exposición a mezclas que contenían cobre (por ejemplo, caldo bordelés, extracción y fundición), no se ha demostrado la función del cobre. Los trabajadores aparentemente expuestos a concentraciones elevadas en el aire que daban lugar a una

ingesta estimada de 200 mg de Cu/día mostraron signos que parecían indicar una intoxicación por cobre (por ejemplo, concentraciones elevadas de cobre en el suero, hepatomegalia). Los datos disponibles sobre la toxicidad reproductiva y la carcinogenicidad son inadecuados para la evaluación del riesgo.

Se describen varios grupos en los cuales los trastornos aparentes de la homeóstasis del cobre producen una sensibilidad mayor al déficit o el exceso de cobre que en la población general. Algunos trastornos tienen una base genética bien definida. Entre éstos figuran la enfermedad de Menkes, manifestación de la deficiencia de cobre generalmente fatal; la enfermedad de Wilson (degeneración hepatolenticular), enfermedad que lleva a una acumulación progresiva de cobre; y la aceruloplasminemia hereditaria, con síntomas clínicos de sobrecarga de hierro. La cirrosis infantil india y la toxicosis idiopática por cobre son enfermedades relacionadas con el exceso de cobre que pueden estar asociadas con una sensibilidad al cobre de base genética, aunque esto no se ha demostrado de manera inequívoca. Estas son enfermedades hepáticas fatales en la primera infancia, en las que el cobre se acumula en el hígado. Las incidencias de las enfermedades estaban relacionadas con un ingestión elevada de cobre, por lo menos en algunos casos.

Otros grupos potencialmente sensibles al exceso de cobre son los pacientes sometidos a hemodiálisis y las personas con enfermedades hepáticas crónicas. Los grupos con riesgo de deficiencia de cobre incluyen los niños pequeños (en particular los recién nacidos de bajo peso al nacer/prematuros, los niños que se están recuperando de una malnutrición, los niños pequeños alimentados exclusivamente con leche de vaca), las personas con síndrome de mala absorción (por ejemplo enfermedad celíaca, esprue, fibrosis cística) y los pacientes totalmente dependientes de una nutrición parenteral. Se ha relacionado la deficiencia de cobre con la patogénesis de las enfermedades cardiovasculares.

1.9 Efectos en otros organismos en el laboratorio y en el medio ambiente

Hay que buscar un equilibrio entre los efectos adversos del cobre y su carácter esencial. El cobre es un elemento esencial para toda la biota, y hay que tener cuidado para asegurar que queden cubiertas las necesidades nutricionales de cobre de los organismos. Este elemento forma parte integrante de la estructura de 12 proteínas importantes por lo menos. Es imprescindible para la utilización del hierro en la formación de la hemoglobina; en la mayor parte de los crustáceos y moluscos la principal proteína sanguínea transportadora de oxígeno es la hemocianina, en cuya estructura figura el cobre. En las plantas, el cobre forma parte de varias enzimas que intervienen en el metabolismo de los hidratos de carbono, del nitrógeno y de la pared celular.

Un factor decisivo en la evaluación del peligro del cobre es su biodisponibilidad. La adsorción de cobre en las partículas y la formación de complejos con la materia orgánica puede limitar mucho el grado de acumulación del metal y sus efectos. Su biodisponibilidad puede verse afectada también en gran medida por la presencia de otros cationes y por el pH.

Se ha demostrado que el cobre tiene efectos adversos en la reproducción, la bioquímica, la fisiología y el comportamiento de diversos organismos acuáticos. Se ha observado que concentraciones de cobre de apenas 1–2 µg/litro tienen efectos perjudiciales en organismos acuáticos; sin embargo, en la interpretación y aplicación de esta información se deben considerar grandes variaciones debidas a la sensibilidad y la biodisponibilidad de las especies.

En las comunidades naturales de fitoplancton se observó una reducción significativa de la clorofila á y de la fijación del nitrógeno con concentraciones de cobre ≥ 20 µg/litro, y la fijación del carbono disminuyó de manera considerable con concentraciones ≥ 10 µg/litro. La CE_{50} (72 horas) para las algas, basada en la inhibición del crecimiento, oscila entre 47 y 120 µg de Cn/litro.

En los invertebrados de agua dulce, la $C(E)L_{50}$ a las 48 horas oscila entre 5 µg de Cu/litro para una especie de dáfnidos y 5300 µg

de Cu/litro para un ostrácodo. La CL_{50} a las 96 horas en los invertebrados marinos varía entre 29 µg de Cu/litro para el peine caletero y 9600 µg de Cu/litro para el cangrejo violinista. La toxicidad aguda del cobre para los peces de agua dulce y marinos es enormemente variable. En los primeros, la CL_{50} oscila entre 3 µg de Cu/litro (tímalo ártico) y 7340 µg de Cu/litro para *Lepomis macrochirus*. En los segundos, la CL_{50} a las 96 horas oscila entre 60 µg de Cu/litro para el salmón real y 1400 µg de Cu/litro para el mujol.

Aunque las plantas necesitan cobre como elemento traza, la concentración elevada de este metal en el suelo puede ser muy tóxica. En general, los síntomas visibles de la toxicidad metálica son las hojas cloróticas pequeñas y la caída temprana de las hojas. También se produce un retraso del crecimiento y la iniciación de las raíces y el desarrollo de las laterales son escasos. El reducido crecimiento de las raíces puede dar lugar a una menor absorción de agua y de nutrientes, y esto provoca alteraciones en el metabolismo y retraso del crecimiento. A nivel celular, el cobre inhibe un gran número de enzimas e interfiere con varios aspectos de la bioquímica vegetal (por ejemplo la fotosíntesis, la síntesis de pigmentos y la integridad de la membrana) y la fisiología (en particular interfiere con los ácidos grasos y el metabolismo de las proteínas e inhibe la respiración y los procesos de fijación del nitrógeno).

Se han observado efectos tóxicos en estudios de laboratorio realizados con lombrices de tierra expuestas a cobre en el suelo; la formación del cocón es el parámetro más sensible medido, con efectos adversos importantes en presencia de 50–60 µg de Cu/litro.

En la naturaleza, los efectos adversos en los microorganismos del suelo se han relacionado con concentraciones más elevadas de cobre en zonas tratadas con fertilizantes que contenían este elemento y en lugares cercanos a fundiciones de cobre-zinc. En las zonas citrícolas en las cuales se han aplicado fungicidas con cobre se ha observado que la clorosis foliar está fuertemente relacionada con las concentraciones de cobre en el suelo.

Se ha demostrado tolerancia al cobre en el medio ambiente para el fitoplancton, los invertebrados acuáticos y terrestres, los peces y las

plantas terrestres. Los mecanismos de tolerancia propuestos en las plantas comprenden la unión del metal al material de la pared celular, la presencia de enzimas tolerantes al metal, la formación de complejos con ácidos orgánicos y la consiguiente eliminación en las vacuolas y la unión a proteínas específicas ricas en grupos tiol o a fitoquelatinas.

2. Conclusiones

2.1 Salud humana

El límite inferior de la gama aceptable de ingesta oral (AROI) es de 20 µg/kg de peso corporal al día. Esta cifra se obtiene a partir de las necesidades basales de una persona adulta con un margen para tener en cuenta las variaciones en la absorción, retención y almacenamiento del cobre (OMS, 1996). En la infancia, esta cifra es de 50 µg/kg de peso corporal.

El límite superior de la AROI en las personas adultas es incierto, pero muy probablemente es del orden de varios, pero no muchos, mg por día (por varios se entiende más de 2–3 mg/día). Esta evaluación se basa únicamente en los estudios de los efectos gastrointestinales del agua de bebida contaminada por cobre. No se pudo confirmar un valor más específico para el límite superior de la AROI con respecto a ningún sector de la población general. Es limitada la información disponible sobre el nivel de ingestión del cobre en los alimentos capaz de provocar efectos adversos para la salud.

Los datos disponibles sobre la toxicidad en los animales no se consideraron de ayuda para establecer el límite superior de la AROI, debido a la incertidumbre acerca del modelo apropiado para el ser humano. Además, la metodología tradicional para la evaluación de la inocuidad, basada en la aplicación de factores de incertidumbre a los datos de los animales, no aborda de manera adecuada las características especiales de elementos esenciales como el cobre.

De los datos disponibles sobre la exposición humana en todo el mundo, pero particularmente en Europa y en las Américas, se deduce que la deficiencia en la ingesta de cobre representa un riesgo de efectos en la salud mayor que el debido a un exceso.

2.2 Efectos en el medio ambiente

La protección de la vida acuática en las aguas con una elevada biodisponibilidad exigirá el mantenimiento de la concentración total de cobre disuelto en un valor inferior a 10 µg/litro; sin embargo, el límite adecuado de la concentración depende de la biota y de las condiciones de exposición en los lugares que despiertan preocupación y se debe establecer en función de una nueva evaluación de todos los datos pertinentes.

En muchos lugares, los factores fisicoquímicos que limitan la biodisponibilidad permitirán valores de cobre más elevados. En los criterios reglamentarios se debe tener en cuenta la especiación del cobre si los autores de los vertidos pueden demostrar que se puede medir de forma fidedigna la biodisponibilidad del cobre en las aguas receptoras.

En el muestreo y el análisis del cobre en el medio ambiente es fundamental la utilización de técnicas "limpias".

Habida cuenta de que el cobre es un elemento esencial, no se deben incorporar a los procedimientos para impedir niveles tóxicos de este metal factores de inocuidad que den lugar a concentraciones recomendadas inferiores a los niveles naturales.

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