Guidelines for drinking-water quality

SECOND EDITION

Volume 2

Health criteria and other supporting information

World Health Organization
Geneva
Guidelines for drinking-water quality

SECOND EDITION

Volume 2
Health criteria and other supporting information

World Health Organization
Geneva
1996
## Contents

Preface xi
Acknowledgements xiii
Acronyms and abbreviations used in the text xiv

1. **Introduction** 1
   1.1 General considerations 2
   1.2 The nature of the guideline values 4
   1.3 Criteria for the selection of health-related drinking-water contaminants 6

Part 1. Microbiological aspects 7

2. **Microbiological aspects: introduction** 9
   2.1 Agents of significance 9
      2.1.1 Agents of high health significance 11
      2.1.2 Opportunistic pathogens 11
      2.1.3 Nuisance organisms 12
   2.2 Routes of exposure 12
   2.3 Persistence in water 13
   2.4 Infective dose 13

References 15

3. **Bacteria** 18
   3.1 Pathogens excreted 18
      3.1.1 *Salmonella* 18
      3.1.2 *Yersinia* 20
      3.1.3 *Campylobacter* 22
      3.1.4 *Escherichia coli* 23
      3.1.5 *Vibrio cholerae* 25
      3.1.6 *Shigella* 27
3.2 Pathogens that grow in supply systems
3.2.1 Legionella
3.2.2 Aeromonas
3.2.3 Pseudomonas aeruginosa
3.2.4 Mycobacterium

References

4. Viruses
4.1 General description
4.1.1 The nature of viruses
4.1.2 Classification of animal viruses
4.1.3 Virus families occurring in water

4.2 Routes of exposure
4.2.1 General considerations
4.2.2 Specific families of viruses

4.3 Health effects

References

5. Protozoa
5.1 Giardia
5.1.1 General description
5.1.2 Routes of exposure
5.1.3 Health effects

5.2 Cryptosporidium spp.
5.2.1 General description
5.2.2 Routes of exposure
5.2.3 Health effects

5.3 Entamoeba histolytica
5.3.1 General description
5.3.2 Routes of exposure
5.3.3 Health effects

5.4 Balantidium coli
5.4.1 General description
5.4.2 Route of exposure
5.4.3 Health effects
5.5 Naegleria and Acanthamoeba

5.5.1 General description
5.5.2 Routes of exposure
5.5.3 Health effects

References

6. Helminths

6.1 Dracunculus medinensis
6.1.1 General description
6.1.2 Routes of exposure
6.1.3 Health effects

6.2 Schistosoma
6.2.1 General description
6.2.2 Routes of exposure
6.2.3 Health effects

6.3 Other helminths

References

7. Toxins from cyanobacteria

References

8. Nuisance organisms

8.1 Microbiological problems
8.2 Problems caused by invertebrate animals

References

9. Microbial indicators of water quality

9.1 Rationale
9.2 Indicators of faecal contamination
9.2.1 Escherichia coli
9.2.2 Thermotolerant (faecal) coliform organisms
9.2.3 Coliform organisms (total coliforms)
9.2.4 Faecal streptococci
9.2.5 Sulfite-reducing clostridia
9.2.6 Bacteriophages
9.2.7 Miscellaneous indicators

9.3 Indicators of water quality and treatment efficacy
9.3.1 Heterotrophic plate counts (colony counts)
9.3.2 Aeromonas spp. and Pseudomonas aeruginosa
9.4 Methods

9.4.1 Standard methods
9.4.2 Methods for pathogenic bacteria, protozoa, and cytopathic enteroviruses

References

10. Microbiological criteria

10.1 Rationale

10.1.1 Overall strategy
10.1.2 Treatment objectives and microbiological criteria
10.1.3 Water supplies for small remote communities

10.2 Bacteriological quality

10.3 Virological quality

10.3.1 Rationale
10.3.2 Guidelines for groundwaters
10.3.3 Guidelines for surface water sources

10.4 Parasitological quality

10.5 Monitoring

10.5.1 Approaches and strategies
10.5.2 Sampling frequencies and procedures
10.5.3 Surveillance programme requirements

10.6 Action to be taken when contamination is detected

References

11. Protection and improvement of water quality

11.1 Water sources

11.1.1 Selection of sources
11.1.2 Source protection

11.2 Treatment processes

11.2.1 Storage
11.2.2 Preseccimation
11.2.3 Prechlorination
11.2.4 Coagulation and flocculation
11.2.5 Sedimentation or flotation
11.2.6 Rapid filtration
11.2.7 Slow sand filtration
11.2.8 Infiltration
11.2.9 Disinfection
CONTENTS

11.3 Choice of treatment
  11.3.1 Microbiological conditions
  11.3.2 Treatment of groundwater
  11.3.3 Treatment of surface water
  11.3.4 Small-scale treatment of surface water
11.4 Distribution networks
References

Part 2. Chemical and physical aspects

12. Chemical and physical aspects: introduction
  12.1 Background information used
  12.2 Drinking-water consumption and body weight
  12.3 Inhalation and dermal absorption
  12.4 Health risk assessment
  12.5 Mixtures
  12.6 Format of monographs for chemical substances
References

13. Inorganic constituents and physical parameters
  13.1 Aluminium
  13.2 Ammonia
  13.3 Antimony
  13.4 Arsenic
  13.5 Asbestos
  13.6 Barium
  13.7 Beryllium
  13.8 Boron
  13.9 Cadmium
  13.10 Chloride
  13.11 Chromium
  13.12 Colour
  13.13 Copper
  13.14 Cyanide
  13.15 Fluoride
  13.16 Hardness
  13.17 Hydrogen sulfide
  13.18 Iron
  13.19 Lead
  13.20 Manganese
  13.21 Mercury
13.22 Molybdenum
13.23 Nickel
13.24 Nitrate and nitrite
13.25 Dissolved oxygen
13.26 pH
13.27 Selenium
13.28 Silver
13.29 Sodium
13.30 Sulfate
13.31 Taste and odour
13.32 Tin and inorganic tin compounds
13.33 Total dissolved solids
13.34 Turbidity
13.35 Uranium
13.36 Zinc

14. Organic constituents

14.1 Carbon tetrachloride
14.2 Dichloromethane
14.3 1,1-Dichloroethane
14.4 1,2-Dichloroethane
14.5 1,1,1-Trichloroethane
14.6 Vinyl chloride
14.7 1,1-Dichloroethene
14.8 1,2-Dichloroethene
14.9 Trichloroethene
14.10 Tetrachloroethene
14.11 Benzene
14.12 Toluene
14.13 Xylenes
14.14 Ethylbenzene
14.15 Styrene
14.16 Polynuclear aromatic hydrocarbons
14.17 Monochlorobenzene
14.18 Dichlorobenzene
14.19 Trichlorobenzene
14.20 Di(2-ethylhexyl)adipate
14.21 Di(2-ethylhexyl)phthalate
14.22 Acrylamide
14.23 Epichlorohydrin
14.24 Hexachlorobutadiene
14.25 Edetic acid

viii
14.26 Nitrilotriacetic acid 565
14.27 Organotins 573

15. Pesticides 586

15.1 Introduction 586
15.2 Alachlor 586
15.3 Aldicarb 593
15.4 Aldrin 602
15.5 Atrazine 608
15.6 Bentazone 614
15.7 Carbofuran 620
15.8 Chlordane 626
15.9 Chlorotoluron 633
15.10 DDT 638
15.11 1,2-Dibromo-3-chloropropane 645
15.12 2,4-Dichlorophenoxyacetic acid 653
15.13 1,2-Dichloropropane 664
15.14 1,3-Dichloropropane 670
15.15 1,3-Dichloropropene 673
15.16Ethylene dibromide 679
15.17Heptachlor 684
15.18Hexachlorobenzene 692
15.19Isoproturon 699
15.20Lindane 704
15.21MCPA 711
15.22Methoxychlor 717
15.23Metolachlor 725
15.24Molinate 729
15.25Pendimethalin 734
15.26Permethrin 738
15.27Propanil 744
15.28Pyridate 748
15.29Simazine 753
15.30Trifluralin 758
15.31Chlorophenoxy herbicides 763

16. Disinfectants and disinfectant by-products 788

Disinfectants 788

16.1 Introduction 788
16.2 Chloramines 789
16.3 Chlorine 796
16.4 Chlorine dioxide, chlorite, and chlorate 803
16.5 Iodine 816
Disinfectant by-products

16.6 Bromate
16.7 Chlorophenols
16.8 Formaldehyde
16.9 MX
16.10 Trihalomethanes

Other chlorination by-products

16.11 Chlorinated acetic acids
16.12 Chlortal hydrate (trichloroacetaldehyde)
16.13 Chloroacetones
16.14 Halogenated acetonitriles
16.15 Cyanogen chloride
16.16 Chloropicrin

Part 3. Radiological aspects

17. Radiological aspects

17.1 Introduction
17.2 Application of the reference level of dose

Annex 1. List of participants in preparatory meetings
Annex 2. Tables of guideline values

Index
Preface

In 1984 and 1985, the World Health Organization (WHO) published the first edition of Guidelines for drinking-water quality in three volumes. The development of these guidelines was organized and carried out jointly by WHO headquarters and the WHO Regional Office for Europe (EURO).

In 1988, the decision was made within WHO to initiate the revision of the guidelines. The work was again shared between WHO headquarters and EURO. Within headquarters, both the unit for the Prevention of Environmental Pollution (PEP) and the ILO/UNEP/WHO International Programme on Chemical Safety (IPCS) were involved, IPCS providing a major input to the health risk assessments of chemicals in drinking-water.

The revised guidelines are being published in three volumes. Guideline values for various constituents of drinking-water are given in Volume 1, Recommendations, together with essential information required to understand the basis for the values. Volume 2, Health criteria and other supporting information, contains the criteria monographs prepared for each substance or contaminant; the guideline values are based on these. Volume 3, Surveillance and control of community supplies, is intended to serve a very different purpose; it contains recommendations and information concerning what needs to be done in small communities, particularly in developing countries, to safeguard their water supplies.

The preparation of the current edition of the Guidelines for drinking-water quality covered a period of four years and involved the participation of numerous institutions, over 200 experts from nearly 40 different developing and developed countries and 18 meetings of the various coordination and review groups. The work of these institutions and scientists, whose names appear in Annex 1, was central to the completion of the guidelines and is much appreciated.

For each contaminant or substance considered, a lead country prepared a draft document evaluating the risks for human health from exposure to the contaminant in drinking-water. The following countries prepared such evaluation documents: Canada, Denmark, Finland, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom of Great Britain and Northern Ireland and United States of America.

Under the responsibility of a coordinator for each major aspect of the guidelines, these draft evaluation documents were reviewed by several scientific institutions and selected experts, and comments were incorporated by the coordinator.
and author prior to submission for final evaluation by a review group. The review group then took a decision as to the health risk assessment and proposed a guideline value.

During the preparation of draft evaluation documents and at the review group meetings, careful consideration was always given to previous risk assessments carried out by IPCS, in its Environmental Health Criteria monographs, the International Agency for Research on Cancer, the Joint FAO/WHO Meetings on Pesticide Residues, and the Joint FAO/WHO Expert Committee on Food Additives, which evaluates contaminants such as lead and cadmium in addition to food additives.

It is clear that not all the chemicals that may be found in drinking-water were evaluated in developing these guidelines. Chemicals of importance to Member States which have not been evaluated should be brought to the attention of WHO for inclusion in any future revision.

It is planned to establish a continuing process of revision of the Guidelines for drinking-water quality with a number of substances or agents subject to evaluation each year. Where appropriate, addenda will be issued, containing evaluations of new substances or substances already evaluated for which new scientific information has become available. Substances for which provisional guideline values have been established will receive high priority for re-evaluation.
Acknowledgements

The work of the following coordinators was crucial in the development of Volumes 1 and 2 of the Guidelines:

J. K. Fawell, Water Research Centre, England (inorganic constituents)
J. R. Hickman, Department of National Health and Welfare, Canada (radioactive materials)
U. Lund, Water Quality Institute, Denmark (organic constituents and pesticides)
B. Mintz, Environmental Protection Agency, United States of America (disinfectants and disinfectant by-products)
E. B. Pike, Water Research Centre, England (microbiology)

The coordinator for Volume 3 of the Guidelines was J. Bartram of the Robens Institute of Health and Safety, England.

The WHO coordinators were as follows:

Headquarters: H. Galal-Gorchev, International Programme on Chemical Safety; R. Helmer, Division of Environmental Health.
Regional Office for Europe: X. Bonnefoy, Environment and Health; O. Espinoza, Environment and Health.

Ms Marla Sheffer of Ottawa, Canada, was responsible for the scientific editing of the guidelines.

The convening of the coordination and review group meetings was made possible by the financial support afforded to WHO by the Danish International Development Agency (DANIDA) and the following sponsoring countries: Belgium, Canada, France, Italy, Netherlands, United Kingdom of Great Britain and Northern Ireland and United States of America.

In addition, financial contributions for the convening of the final task group meeting were received from the Norwegian Agency for Development Cooperation (NORAD), the United Kingdom Overseas Development Administration (ODA) and the Water Services Association in the United Kingdom, the Swedish International Development Authority (SIDA), and the Government of Japan.

The efforts of all who helped in the preparations and finalization of the Guidelines for drinking-water quality are gratefully acknowledged.
**Acronyms and abbreviations used in the text**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAS</td>
<td>atomic absorption spectrometry</td>
</tr>
<tr>
<td>A/C</td>
<td>asbestos-cement</td>
</tr>
<tr>
<td>ADA</td>
<td>ampicillin-dextrin agar</td>
</tr>
<tr>
<td>ADI</td>
<td>acceptable daily intake</td>
</tr>
<tr>
<td>a.i.</td>
<td>active ingredient</td>
</tr>
<tr>
<td>AIDS</td>
<td>acquired immunodeficiency syndrome</td>
</tr>
<tr>
<td>ALAD</td>
<td>aminolaevulinic acid dehydratase</td>
</tr>
<tr>
<td>ALAT</td>
<td>alanine aminotransferase</td>
</tr>
<tr>
<td>AOC</td>
<td>assimilable organic carbon</td>
</tr>
<tr>
<td>APHA</td>
<td>American Public Health Association</td>
</tr>
<tr>
<td>BOD</td>
<td>biochemical oxygen demand</td>
</tr>
<tr>
<td>Bq</td>
<td>Becquerel</td>
</tr>
<tr>
<td>BSP</td>
<td>bromosulfophthalein</td>
</tr>
<tr>
<td>BUN</td>
<td>blood urea nitrogen</td>
</tr>
<tr>
<td>bw</td>
<td>body weight</td>
</tr>
<tr>
<td>CAS</td>
<td>Chemical Abstracts Service</td>
</tr>
<tr>
<td>cfu</td>
<td>colony-forming units</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese hamster ovary</td>
</tr>
<tr>
<td>CMC</td>
<td>carboxymethyl cellulose</td>
</tr>
<tr>
<td>DENA</td>
<td>diethylnitrosamine</td>
</tr>
<tr>
<td>DMAA</td>
<td>dimethylarsinic acid</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOPA</td>
<td>3,4-dihydroxyphenylalanine</td>
</tr>
<tr>
<td>ECG</td>
<td>electrocardiogram</td>
</tr>
<tr>
<td>EDTA</td>
<td>edetic acid</td>
</tr>
<tr>
<td>EEG</td>
<td>electroencephalogram</td>
</tr>
<tr>
<td>EIEC</td>
<td>enteroinvasive <em>E. coli</em></td>
</tr>
<tr>
<td>EP</td>
<td>erythrocyte protoporphyrin</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency (USA)</td>
</tr>
<tr>
<td>ETEC</td>
<td>enterotoxigenic <em>E. coli</em></td>
</tr>
</tbody>
</table>
ACRONYMS AND ABBREVIATIONS

FAO  Food and Agriculture Organization of the United Nations
FPD  flame photometric detection

GC  gas chromatography
GCI  general cognitive index
GEMS Global Environment Monitoring System
GOT glutamic-oxaloacetic transaminase
GPT glutamic-pyruvic transaminase

h  hour
HD  Hodgkin disease
HDL high-density lipoprotein
HPLC high-performance liquid chromatography

IARC International Agency for Research on Cancer
ICRP International Commission on Radiological Protection
ID infective dose
Ig immunoglobulin
IgG immunoglobulin G
IgM immunoglobulin M
ILO International Labour Organisation
IPCS International Programme on Chemical Safety
IQ intelligence quotient
ISO International Organization for Standardization

JECFA Joint FAO/WHO Expert Committee on Food Additives
JMPR Joint FAO/WHO Meeting on Pesticide Residues

LC50 lethal concentration, median
LD50 lethal dose, median
LH luteinizing hormone
LOAEL lowest-observed-adverse-effect level
LT heat-labile enterotoxin

MAC  Mycobacterium avium complex
MAIS  Mycobacterium avium, M. intracellulare, M. scrofulaceum complex
MDI mental development index
MFL million fibres per litre
MIB 2-methylisoborneol
MMAA monomethylarsonic acid
MNCV motor nerve conduction velocity
MS mass spectrometry
MSCA McCarthy Scales of Children's Abilities
MTD maximum tolerated dose
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADPH</td>
<td>nicotinamide adenine dinucleotide phosphate (reduced)</td>
</tr>
<tr>
<td>NAG</td>
<td>non-agglutinable</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute (USA)</td>
</tr>
<tr>
<td>NCV</td>
<td>non-cholera vibrios</td>
</tr>
<tr>
<td>NEU</td>
<td>nitrosoethylurea</td>
</tr>
<tr>
<td>NHANES</td>
<td>US National Health and Nutrition Examination Survey</td>
</tr>
<tr>
<td>NHL</td>
<td>non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>NOAEL</td>
<td>no-observed-adverse-effect level</td>
</tr>
<tr>
<td>NTA</td>
<td>nitrilotriacetic acid</td>
</tr>
<tr>
<td>NTP</td>
<td>National Toxicology Program (USA)</td>
</tr>
<tr>
<td>NTU</td>
<td>nephelometric turbidity unit</td>
</tr>
<tr>
<td>Pa</td>
<td>Pascal</td>
</tr>
<tr>
<td>PDI</td>
<td>psychomotor development index</td>
</tr>
<tr>
<td>PKa</td>
<td>log acid dissociation constant</td>
</tr>
<tr>
<td>PMTDI</td>
<td>provisional maximum tolerable daily intake</td>
</tr>
<tr>
<td>PTWI</td>
<td>provisional tolerable weekly intake</td>
</tr>
<tr>
<td>PVC</td>
<td>polyvinyl chloride</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>SAED</td>
<td>selected-area electron diffraction</td>
</tr>
<tr>
<td>SAP</td>
<td>serum alkaline phosphatase</td>
</tr>
<tr>
<td>SGOT</td>
<td>serum glutamic-oxaloacetic transaminase</td>
</tr>
<tr>
<td>SGPT</td>
<td>serum glutamic-pyruvic transaminase</td>
</tr>
<tr>
<td>SMR</td>
<td>standardized mortality ratio</td>
</tr>
<tr>
<td>ST</td>
<td>heat-stable enterotoxin</td>
</tr>
<tr>
<td>STS</td>
<td>soft tissue sarcoma</td>
</tr>
<tr>
<td>T3</td>
<td>triiodothyronine</td>
</tr>
<tr>
<td>T4</td>
<td>thyroxine</td>
</tr>
<tr>
<td>TCU</td>
<td>true colour unit</td>
</tr>
<tr>
<td>TDI</td>
<td>tolerable daily intake</td>
</tr>
<tr>
<td>TDS</td>
<td>total dissolved solids</td>
</tr>
<tr>
<td>TEM</td>
<td>transmission electron microscopy</td>
</tr>
<tr>
<td>TOC</td>
<td>total organic carbon</td>
</tr>
<tr>
<td>TPA</td>
<td>tetradecanoyl-phorbol-acetate</td>
</tr>
<tr>
<td>UNEP</td>
<td>United Nations Environment Programme</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>WHA</td>
<td>World Health Assembly</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
1. Introduction

This volume of the *Guidelines for drinking-water quality* explains how guideline values for drinking-water contaminants are to be used, defines the criteria used to select the various chemical, physical, microbiological, and radiological contaminants included in the report, describes the approaches used in deriving guideline values, and presents, in the form of brief monographs, critical reviews and evaluations of the effects on human health of the substances or contaminants examined.

This edition of the *Guidelines* considers many drinking-water contaminants not included in the first edition. It also contains revised guideline values for many of the contaminants included in the first edition, which have been changed as a result of new scientific information. The guideline values given here supersede those in the 1984 edition.

Although the number of chemical contaminants for which guideline values are recommended is greater than in the first edition, it is unlikely that all of these chemical contaminants will occur in all water supplies or even in all countries. Care should therefore be taken in selecting substances for which national standards will be developed. A number of factors should be considered, including the geology of the region and the types of human activities that take place there. For example, if a particular pesticide is not used in the region, it is unlikely to occur in the drinking-water.

In other cases, such as the disinfection by-products, it may not be necessary to set standards for all of the substances for which guideline values have been proposed. If chlorination is practised, the trihalomethanes, of which chloroform is the major component, are likely to be the main disinfection by-products, together with the chlorinated acetic acids in some instances. In many cases, control of chloroform levels and, where appropriate, trichloroacetic acid will also provide an adequate measure of control over other chlorination by-products.

In developing national standards, care should also be taken to ensure that scarce resources are not unnecessarily diverted to the development of standards and the monitoring of substances of relatively minor importance.

Several of the inorganic elements for which guideline values have been recommended are recognized to be essential elements in human nutrition. No attempt has been made here to define a minimum desirable concentration of such substances in drinking-water.
1.1 General considerations

The primary aim of the Guidelines for drinking-water quality is the protection of public health. The guidelines are intended to be used as a basis for the development of national standards that, if properly implemented, will ensure the safety of drinking-water supplies through the elimination, or reduction to a minimum concentration, of constituents of water that are known to be hazardous to health. It must be emphasized that the guideline values recommended are not mandatory limits. In order to define such limits, it is necessary to consider the guideline values in the context of local or national environmental, social, economic, and cultural conditions.

The main reason for not promoting the adoption of international standards for drinking-water quality is the advantage provided by the use of a risk-benefit approach (qualitative or quantitative) to the establishment of national standards and regulations. This approach should lead to standards and regulations that can be readily implemented and enforced. For example, the adoption of drinking-water standards that are too stringent could limit the availability of water supplies that meet those standards—a significant consideration in regions of water shortage. The standards that individual countries will develop can thus be influenced by national priorities and economic factors. However, considerations of policy and convenience must never be allowed to endanger public health, and the implementation of standards and regulations will require suitable facilities and expertise as well as the appropriate legislative framework.

The judgement of safety—or what is an acceptable level of risk in particular circumstances—is a matter in which society as a whole has a role to play. The final judgement as to whether the benefit resulting from the adoption of any of the guideline values given here as standards justifies the cost is for each country to decide. What must be emphasized is that the guideline values have a degree of flexibility and enable a judgement to be made regarding the provision of drinking-water of acceptable quality.

Water is essential to sustain life, and a satisfactory supply must be made available to consumers. Every effort should be made to achieve a drinking-water quality as high as practicable. Protection of water supplies from contamination is the first line of defence. Source protection is almost invariably the best method of ensuring safe drinking-water and is to be preferred to treating a contaminated water supply to render it suitable for consumption. Once a potentially hazardous situation has been recognized, however, the risk to health, the availability of alternative sources, and the availability of suitable remedial measures must be considered so that a decision can be made about the acceptability of the supply.

As far as possible, water sources must be protected from contamination by human and animal waste, which can contain a variety of bacterial, viral, and protozoan pathogens and helminth parasites. Failure to provide adequate protection and effective treatment will expose the community to the risk of outbreaks of intestinal and other infectious diseases. Those at greatest risk of waterborne dis-
INTRODUCTION

Infants and young children, people who are debilitated or living under unsanitary conditions, the sick, and the elderly. For these people, infective doses are significantly lower than for the general adult population.

The potential consequences of microbial contamination are such that its control must always be of paramount importance and must never be compromised.

The assessment of the risks associated with variations in microbial quality is difficult and controversial because of insufficient epidemiological evidence, the number of factors involved, and the changing interrelationships between these factors. In general terms, the greatest microbial risks are associated with ingestion of water that is contaminated with human and animal excreta. Microbial risk can never be entirely eliminated, because the diseases that are waterborne may also be transmitted by person-to-person contact, aerosols, and food intake; thus, a reservoir of cases and carriers is maintained. Provision of a safe water supply in these circumstances will reduce the chances of spread by these other routes. Waterborne outbreaks are particularly to be avoided because of their capacity to result in the simultaneous infection of a high proportion of the community.

The health risk due to toxic chemicals in drinking-water differs from that caused by microbiological contaminants. There are few chemical constituents of water that can lead to acute health problems except through massive accidental contamination of supply. Moreover, experience shows that, in such incidents the water usually becomes undrinkable owing to unacceptable taste, odour, and appearance.

The fact that chemical contaminants are not normally associated with acute effects places them in a lower priority category than microbial contaminants, the effects of which are usually acute and widespread. Indeed, it can be argued that chemical standards for drinking-water are of secondary consideration in a supply subject to severe bacterial contamination.

The problems associated with chemical constituents of drinking-water arise primarily from their ability to cause adverse health effects after prolonged periods of exposure; of particular concern are contaminants that have cumulative toxic properties, such as heavy metals, and substances that are carcinogenic.

It should be noted that the use of chemical disinfectants in water treatment usually results in the formation of chemical by-products, some of which are potentially hazardous. However, the risks to health from these by-products are extremely small in comparison with the risks associated with inadequate disinfection, and it is important that disinfection should not be compromised in attempting to control such by-products.

The radiological health risk associated with the presence of naturally occurring radionuclides in drinking-water should also be taken into consideration, although the contribution of drinking-water to total ambient exposure to these radionuclides is very small under normal circumstances. The guideline values recommended in this volume do not apply to water supplies contaminated during emergencies arising from accidental releases of radioactive substances to the environment.
In assessing the quality of drinking-water, the consumer relies principally upon his or her senses. Water constituents may affect the appearance, odour, or taste of the water, and the consumer will evaluate the quality and acceptability of the water on the basis of these criteria. Water that is highly turbid, is highly coloured, or has an objectionable taste or odour may be regarded by consumers as unsafe and may be rejected for drinking purposes. It is therefore vital to maintain a quality of water that is acceptable to the consumer, although the absence of any adverse sensory effects does not guarantee the safety of the water.

Countries developing national drinking-water limits or standards should carefully evaluate the costs and benefits associated with the control of aesthetic and organoleptic quality. Enforceable standards are sometimes set for contaminants directly related to health, whereas recommendations only are made for aesthetic and organoleptic characteristics. For countries with severely limited resources, it is even more important to establish priorities, and this should be done by considering the impact on health in each case. This approach does not underestimate the importance of the aesthetic quality of drinking-water. Source water that is aesthetically unsatisfactory may discourage the consumer from using an otherwise safe supply. Furthermore, taste, odour, and colour may be the first indication of potential health hazards.

Many parameters must be taken into consideration in the assessment of water quality, such as source protection, treatment efficiency and reliability, and protection of the distribution network (e.g., corrosion control). The costs associated with water quality surveillance and control must also be carefully evaluated before developing national standards.

### 1.2 The nature of the guideline values

Guideline values have been set for potentially hazardous water constituents and provide a basis for assessing drinking-water quality.

(a) A guideline value represents the concentration of a constituent that does not result in any significant risk to the health of the consumer over a lifetime of consumption.

(b) The quality of water defined by the *Guidelines for drinking-water quality* is such that it is suitable for human consumption and for all domestic purposes, including personal hygiene. However, water of a higher quality may be required for some special purposes, such as renal dialysis.

(c) When a guideline value is exceeded, this should be a signal: (i) to investigate the cause with a view to taking remedial action; (ii) to consult with, and seek advice from, the authority responsible for public health.

(d) Although the guideline values describe a quality of water that is acceptable for lifelong consumption, the establishment of these guideline values should not be regarded as implying that the quality of drinking-water may be
degraded to the recommended level. Indeed, a continuous effort should be made to maintain drinking-water quality at the highest possible level.

(e) Short-term deviations above the guideline values do not necessarily mean that the water is unsuitable for consumption. The amount by which, and the period for which, any guideline value can be exceeded without affecting public health depends upon the specific substance involved. It is recommended that when a guideline value is exceeded, the surveillance agency (usually the authority responsible for public health) should be consulted for advice on suitable action, taking into account the intake of the substance from sources other than drinking-water (for chemical constituents), the toxicity of the substance, the likelihood and nature of any adverse effects, the practicability of remedial measures, and similar factors.

(f) In developing national drinking-water standards based on these guideline values, it will be necessary to take account of a variety of geographical, socio-economic, dietary, and other conditions affecting potential exposure. This may lead to national standards that differ appreciably from the guideline values.

(g) In the case of radioactive substances, screening values for gross alpha and gross beta activity are given, based on a reference level of dose.

It is important that recommended guideline values are both practical and feasible to implement as well as protective of public health. Guideline values are not set at concentrations lower than the detection limits achievable under routine laboratory operating conditions. Moreover, guideline values are recommended only when control techniques are available to remove or reduce the concentration of the contaminant to the desired level.

In some instances, provisional guideline values have been set for constituents for which there is some evidence of a potential hazard but where the available information on health effects is limited. Provisional guideline values have also been set for substances for which the calculated guideline value would be (i) below the practical quantification level, or (ii) below the level that can be achieved through practical treatment methods. Finally, provisional guideline values have been set for certain substances when it is likely that guideline values will be exceeded as a result of disinfection procedures.

Aesthetic and organoleptic characteristics are subject to individual preference as well as social, economic, and cultural considerations. For this reason, although guidance can be given on the levels of substances that may be aesthetically unacceptable, no guideline values have been set for such substances where they do not represent a potential hazard to health.

The recommended guideline values are set at a level to protect human health; they may not be suitable for the protection of aquatic life. The guidelines apply to bottled water and ice intended for human consumption but do not apply to natural mineral waters, which should be regarded as beverages rather than
drinking-water in the usual sense of the word. The Codex Alimentarius Commission has developed Codex standards for such mineral waters.1

1.3 Criteria for the selection of health-related drinking-water contaminants

The recognition that faecally polluted water can lead to the spread of microbial infections has led to the development of sensitive methods for routine examination to ensure that water intended for human consumption is free from faecal contamination. Although it is now possible to detect the presence of many pathogens in water, the methods of isolation and enumeration are often complex and time-consuming. It is therefore impracticable to monitor drinking-water for every possible microbial pathogen. A more logical approach is the detection of organisms normally present in the faeces of humans and other warm-blooded animals as indicators of faecal pollution, as well as of the efficacy of water treatment and disinfection. The various bacterial indicators used for this purpose are described in Chapter 9. The presence of such organisms indicates the presence of faecal material and, hence, that intestinal pathogens could be present. Conversely, their absence indicates that pathogens are probably also absent.

Thousands of organic and inorganic chemicals have been identified in drinking-water supplies around the world, many in extremely low concentrations. The chemicals selected for the development of guideline values include those considered potentially hazardous to human health, those detected relatively frequently in drinking-water, and those detected in relatively high concentrations.

Some potentially hazardous chemicals in drinking-water are derived directly from treatment chemicals or construction materials used in water supply systems. Such chemicals are best controlled by appropriate specifications for the chemicals and materials used. For example, a wide range of polyelectrolytes are now used as coagulant aids in water treatment, and the presence of residues of the unreacted monomer may cause concern. Many polyelectrolytes are based on acrylamide polymers and co-polymers, in both of which the acrylamide monomer is present as a trace impurity. Chlorine used for disinfection has sometimes been found to contain carbon tetrachloride. This type of drinking-water contamination is best controlled by the application of regulations governing the quality of the products themselves rather than the quality of the water. Similarly, strict national regulations on the quality of pipe material should avoid the possible contamination of drinking-water by trace constituents of plastic pipes. The control of contamination of water supplies by in situ polymerized coatings and coatings applied in a solvent requires the development of suitable codes of practice, in addition to controls on the quality of the materials used.

PART 1

Microbiological aspects
2. Microbiological aspects: introduction

The most common and widespread health risk associated with drinking-water is contamination, either directly or indirectly, by human or animal excreta, particularly faeces. If such contamination is recent, and if those responsible for it include carriers of communicable enteric diseases, some of the pathogenic microorganisms that cause these diseases may be present in the water. Drinking the water, or using it in food preparation, may then result in new cases of infection.

The pathogenic agents involved include bacteria, viruses, and protozoa, which may cause diseases that vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhoea, dysentery, hepatitis, or typhoid fever. Most of them are widely distributed throughout the world. Faecal contamination of drinking-water is only one of several faeco-oral mechanisms by which they can be transmitted from one person to another or, in some cases, from animals to people.

One ingested waterborne pathogen, namely guinea worm (*Dracunculus medinensis*), is not faecal in origin and deserves special mention. Although it is of limited geographical distribution, this helminth is of major public health importance in endemic areas. It is the only human infection that is solely transmitted by the waterborne route, and the World Health Assembly has committed itself to the eradication of dracunculiasis by the end of 1995 (resolution WHA 44.5, 1991).

Other pathogens cause infection when water containing them is used for bathing or for recreation involving water contact, rather than by the oral route. Some may also cause infection by inhalation when they are present in large numbers in water droplets, such as those produced by showers and some air-conditioning systems or in the irrigation of agricultural land.

It is not only by causing infection that microorganisms in drinking-water can affect human health. In some circumstances, cyanobacteria can produce toxins that may remain in the water even when the cyanobacteria themselves have been removed. Finally, there are some organisms whose presence in water is a nuisance, but which are of no significance for public health.

2.1 Agents of significance

The human pathogens potentially transmitted in drinking-water are listed in Table 2.1. Some general information on those included in the table is given below.
Table 2.1 Waterborne pathogens and their significance in water supplies

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Health significance</th>
<th>Main route of exposure</th>
<th>Persistence in water supplies</th>
<th>Resistance to chlorine</th>
<th>Relative infective dose</th>
<th>Important animal reservoir</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Campylobacter jejuni, C. coli</td>
<td>High</td>
<td>O</td>
<td>Moderate</td>
<td>Low</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>Pathogenic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>High</td>
<td>O</td>
<td>Moderate</td>
<td>Low</td>
<td>High</td>
<td>Yes</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>High</td>
<td>O</td>
<td>Moderate</td>
<td>Low</td>
<td>High</td>
<td>No</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigella spp</td>
<td>High</td>
<td>O</td>
<td>Short</td>
<td>Low</td>
<td>Moderate</td>
<td>No</td>
</tr>
<tr>
<td>Vibrio cholerae</td>
<td>High</td>
<td>O</td>
<td>Short</td>
<td>Low</td>
<td>High</td>
<td>No</td>
</tr>
<tr>
<td>Yersinia</td>
<td>High</td>
<td>O</td>
<td>Long</td>
<td>Low</td>
<td>High(?)</td>
<td>Yes</td>
</tr>
<tr>
<td><em>enterococci</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legionella</td>
<td>Moderate</td>
<td>I</td>
<td>May multiply</td>
<td>Moderate</td>
<td>High</td>
<td>No</td>
</tr>
<tr>
<td>Pseudomonas anaerugnosa</td>
<td>Moderate</td>
<td>C,IN</td>
<td>May multiply</td>
<td>Moderate</td>
<td>High(?)</td>
<td>No</td>
</tr>
<tr>
<td><em>Aeromonas</em></td>
<td>Moderate</td>
<td>O, C</td>
<td>May multiply</td>
<td>Low</td>
<td>High(?)</td>
<td>No</td>
</tr>
<tr>
<td>Mycobacterium, atypical</td>
<td>Moderate</td>
<td>I, C</td>
<td>May multiply</td>
<td>High</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoviruses</td>
<td>High</td>
<td>O, C</td>
<td>?</td>
<td>Moderate</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Enteroviruses</td>
<td>High</td>
<td>O</td>
<td>Long</td>
<td>Moderate</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>High</td>
<td>O</td>
<td>Long</td>
<td>Moderate</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>High</td>
<td>O</td>
<td>?</td>
<td>?</td>
<td>Low</td>
<td>Probable</td>
</tr>
<tr>
<td>Norwalk virus</td>
<td>High</td>
<td>O</td>
<td>?</td>
<td>?</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>High</td>
<td>O</td>
<td>?</td>
<td>?</td>
<td>Moderate</td>
<td>No(?</td>
</tr>
<tr>
<td>Small round viruses (other than Norwalk virus)</td>
<td>Moderate</td>
<td>O</td>
<td>?</td>
<td>?</td>
<td>Low(?)</td>
<td>No</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endamoeba bistolytica</td>
<td>High</td>
<td>O</td>
<td>Moderate</td>
<td>High</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Giardia</td>
<td>High</td>
<td>O</td>
<td>Moderate</td>
<td>High</td>
<td>Low</td>
<td>Yes</td>
</tr>
<tr>
<td>Isospora</td>
<td>High</td>
<td>O</td>
<td>Moderate</td>
<td>High</td>
<td>Low</td>
<td>Yes</td>
</tr>
<tr>
<td>Cryptosporidum parvum</td>
<td>High</td>
<td>O</td>
<td>Long</td>
<td>High</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>Acanthamoeba spp</td>
<td>Moderate</td>
<td>C, I</td>
<td>May multiply</td>
<td>High</td>
<td>?</td>
<td>No</td>
</tr>
<tr>
<td>Naegleri fowleri</td>
<td>Moderate</td>
<td>C</td>
<td>May multiply</td>
<td>Moderate</td>
<td>Low</td>
<td>No</td>
</tr>
<tr>
<td>Blastocystis coli</td>
<td>Moderate</td>
<td>O</td>
<td>?</td>
<td>Moderate</td>
<td>Low</td>
<td>Yes</td>
</tr>
</tbody>
</table>
### Table 2.1 (continued)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Health significance</th>
<th>Main route of exposure</th>
<th>Persistence in water supplies</th>
<th>Resistance to chlorine</th>
<th>Relative infective dose</th>
<th>Important animal reservoir</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Helminths</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dracunculus medinensis</td>
<td>High</td>
<td>O</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Low</td>
<td>Yes</td>
</tr>
<tr>
<td>Schistosoma spp</td>
<td>Moderate</td>
<td>O</td>
<td>Short</td>
<td>Low</td>
<td>Low</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* = Not known or uncertain

a O = oral (ingestion), I = inhalation in aerosol, C = contact with skin; IN = ingestion in immunosuppressed patients

b Detection period for infective stage in water at 20°C: short = up to 1 week, moderate = 1 week to 1 month, long = over 1 month

c When the infective stage is freely suspended in water treated at conventional doses and contact times: resistance moderate, agent may not be completely destroyed, resistance low, agent completely destroyed
d Dose required to cause infection in 50% of healthy adult volunteers

#### 2.1.1 Agents of high health significance

Not all potentially waterborne human pathogens are of equal public health significance (Table 2.1). Some of them, including most of the ingested pathogens, present a serious risk of disease whenever they are present in drinking-water, and their elimination from it should be given high priority. Examples include strains of *Escherichia coli*, *Salmonella*, *Shigella*, *Vibrio cholerae*, *Yersinia enterocolitica*, and *Campylobacter jejuni*, the viruses described in Chapter 4, and the parasites *Giardia*, *Cryptosporidium*, *Entamoeba histolytica*, and *Dracunculus*.

While most of the pathogens of high significance in Table 2.1 are found worldwide, others are a public health problem only in limited regions of the world, e.g. guinea worm is found only in certain countries of Africa and Asia. Historically, pandemics of cholera have spread from well defined regions where the outbreaks first occurred. Although high priority should be given to control of these pathogens in drinking-water, this is of regional significance only.

#### 2.1.2 Opportunistic pathogens

Some organisms, naturally present in the environment and not normally regarded as pathogens, may cause disease opportunistically. When such organisms are present in drinking-water, they cause infection predominantly among people whose local or general natural defence mechanisms are impaired. Those most likely to be at risk include the very old, the very young, and patients in hospitals, e.g. those with burns or undergoing immunosuppressive therapy, and those suffering from acquired immunodeficiency syndrome (AIDS). Water used by such patients for drinking or bathing, if it contains excessive numbers of these agents, may produce a variety of infections involving the skin and mucous membranes of the eye, ear, nose, and throat. *Pseudomonas*, *Flavobacterium*, *Acinetoc-
bacter, Klebsiella, and Serratia are examples of such opportunistic pathogens, as is Legionella, which can infect the lungs if inhaled in droplets. Some of these, such as Legionella and Aeromonas, can also cause disease in otherwise healthy individuals when the specific conditions prevailing within a water-supply system have enabled them to multiply to unusually high concentrations. These organisms, while clearly of medical importance, only acquire public health significance under certain conditions. Their removal from drinking-water may therefore be given moderate priority.

2.1.3 Nuisance organisms

Nuisance organisms, by definition, have no public health significance. However, they produce problems of turbidity, taste and odour or appear as visible animal life in the water. As well as being aesthetically objectionable, they indicate that both water treatment and the maintenance and repair of the distribution system are defective.

2.2 Routes of exposure

For the faeco-oral pathogens, drinking-water is only one vehicle of transmission. Contamination of food, hands, utensils, and clothing can also play a role, particularly when domestic hygiene is poor. Because of this multiplicity of transmission routes, improvements in the quality and availability of water, in excreta disposal, and in general hygiene education are all important factors in achieving reductions in diarrhoea morbidity and mortality rates. While many faeco-oral pathogens have been shown to cause waterborne epidemics, none of them causes epidemics exclusively by this means. Neither the identification of a specific pathogen in drinking-water nor the occurrence of a common-source epidemic can therefore be taken as proof of waterborne disease transmission. To obtain confirmatory evidence, an epidemiological investigation is required. Those infections for which there is epidemiological evidence of waterborne transmission are listed in Table 2.1.

The significance of the water route varies considerably both with the disease and with local conditions. Thus, waterborne transmission of poliomyelitis has not been conclusively demonstrated, while waterborne epidemics of giardiasis, typhoid fever, and cholera have frequently been documented. One reason for this is that there are important differences between the pathogens in terms of their persistence in water, their removal by conventional water-treatment processes, and the minimum infective dose, i.e. the number of organisms needed to cause infection when taken by mouth.
2.3 Persistence in water

The persistence of a pathogen in water is a measure of how quickly it dies after leaving the body. In practice, the numbers of a pathogen introduced on a given occasion will tend to decline exponentially with time, reaching insignificant and undetectable levels after a certain period (Table 2.1).

A pathogen that persists outside the body only for a short time must rapidly find a new susceptible host. It is therefore less likely to be transmitted through a water-supply system than within a family or some other group living closely together, where lax personal cleanliness will allow the infection to be transferred from one person to another.

The persistence of most pathogens in water is affected by various factors, of which sunlight and temperature are among the most important. Lifetimes are shorter, sometimes much shorter, at warmer temperatures. For example, whereas enteric viruses may be detected for up to 9 months at around 10 °C, their maximum period of detection at 20 °C is nearer to 2 months (2).

Some pathogens are more resistant than others to conventional water-treatment processes, particularly chlorination at the doses and contact times usually employed. This is also indicated in Table 2.1 and discussed in further detail in Chapter 11.

2.4 Infective dose

For several intestinal pathogens, attempts have been made to determine the number of organisms needed to produce either a clinically apparent infection or intestinal colonization in human subjects (see Table 2.2). The significance of the results of these studies is obscure. While they undoubtedly provide an order of relative infectivity, it is doubtful whether the actual infective doses obtained are relevant to natural infections. The number of subjects exposed to infection in experimental studies is necessarily small and the experiments are designed to ensure that many of them become infected. During an outbreak, the number of subjects exposed may be very large, but only a small proportion become infected. Thus the minimum infective dose in an outbreak, and the attack rate, are probably much lower than in an experimental study. In many outbreaks of typhoid fever, the case rate can be explained only by assuming that the infective dose was low.

The likelihood of ingesting very large numbers of a pathogen on a single occasion from drinking-water is relatively small, both because enteric pathogens cannot normally multiply in water and because the water tends to disperse them. On the other hand, if polluted water is permitted to contaminate food, bacterial pathogens, initially present in small numbers, can multiply to produce very large doses.
Bacteria that cause intestinal infections are able to invade and grow in the intestine. It is convenient, therefore, to develop a model of infection that states that, under the correct circumstances, a single infective organism must be able to initiate a clinically significant infection. The infective dose (ID) required to ensure that infection occurs in a specified proportion of subjects—for example, half the subjects (ID₅₀)—may be considered to represent, for a particular bacterial species, the probability that the single organism will reach the correct portion of intestine under the right circumstances to initiate a clinically apparent infection.

In a natural infection, the variables affecting this probability may be numerous and varied, as shown in Table 2.3. The transfer of the pathogen from one case to the next may appear simple, but numerous factors, including the ability to survive in the environment and the nature of the environment available to the host, play an important role. Socioeconomic factors, such as the practice of food hygiene, the availability of pure water, and the adequacy of excreta disposal, further complicate the picture.

After ingestion of the pathogen, the development of an infection depends on the balance between host factors, such as gastric acidity and intestinal immunity, tending to remove it, and factors aiding the bacteria in their attempt to colonize the intestine, such as the possession of colonization factors and adhesions. Studies in animals suggest that the growth phase and growth rate may be crucial.

After an infection has been initiated, its clinical expression is still not certain. The bacterial virulence factors, such as enterotoxin production and invasiveness, produce pathological changes, but their overt expression is countered by homoeostatic mechanisms in the gut. The multiplication of the bacteria extends the area of pathological changes, while the development of immunity inhibits the expression of bacterial virulence and results in the elimination of the pathogen. The infective dose, as determined in experimental situations, must, in part, represent the number of bacteria needed to produce disease before being overcome by the host defences. The effect of infective dose on incubation period has been demonstrated in a study on typhoid fever, which showed that the larger the adminis-
Table 2.3 Factors determining the probability of developing clinically significant intestinal infections

<table>
<thead>
<tr>
<th>Host</th>
<th>Stage of Infection</th>
<th>Pathogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Socioeconomic factors (food hygiene, availability of potable water, excreta disposal, public health measures)</td>
<td>Ingestion</td>
<td>Environmental survival</td>
</tr>
<tr>
<td>Antimicrobial defences, gastric acidity, etc.</td>
<td>Infection</td>
<td>Growth phase, adhesins and colonization factors</td>
</tr>
<tr>
<td>Immune system, antitoxic immunity</td>
<td>Water loss</td>
<td>Enterotoxin production, mucosal invasion</td>
</tr>
<tr>
<td>Homoeostatic mechanisms of water absorption in the gut</td>
<td>Diarrhoea</td>
<td></td>
</tr>
</tbody>
</table>

tered dose, the shorter the incubation period (4), a finding that may constitute evidence in support of that conclusion.

The physiological state of the host is another important factor. Undoubtedly the response to infection of the healthy north American adults, in whom most studies on infective dose have been performed (Table 2.2), is different from that of malnourished infants in Africa, Asia, or South America. Factors such as gastric acid production and the immune response are both influenced by nutritional status. Caution is therefore necessary in extrapolating from infective dose to epidemiological mechanism.

References


3. Bacteria

3.1 Pathogens excreted

3.1.1 Salmonella

General description

The genus *Salmonella* is a member of the family Enterobacteriaceae. The genus is currently subdivided into the subgenera I–IV, on the basis of biochemical characteristics. Most salmonella strains isolated from humans and warm-blooded vertebrates belong to subgenus I, while subgenera II, III (also called Arizona) and IV are more frequently associated with reptiles, in which they reside commensally. Currently more than 2000 serotypes are named. There are considerable regional variations in the prevalence of serotypes (1).

The virulence of *Salmonella* spp. depends on serotype and strain specificity in host range and infective dose and on host status. *S. typhi* is a specific human pathogen. In particular, *S. typhi*, *S. paratyphi A*, and *S. paratyphi B* are able to invade tissues and cause sepsicaemia with high temperature rather than diarrhoea. This is known as enteric fever. In humans, the majority of the other serotypes give rise to a transient intestinal infection manifesting itself as acute gastroenteritis with diarrhoea. Certain serotypes are highly pathogenic for humans, while others are devoid of any pathogenic action. Many salmonella infections are asymptomatic (2).

Routes of exposure

In the case of *S. typhi* and *S. paratyphi A*, human carriers are the source of infection, whereas milk-borne transmission can also occur with *S. paratyphi B*. The incidence of enteric fever decreases as a country becomes more highly developed in terms of controlled sewerage systems and drinking-water supplies, and the supply of pasteurized milk and dairy products. Most salmonellae are primarily pathogens of animals, which constitute important reservoirs for those infections (2).

---

1 The valuable contribution made by Dr N.F. Pierce, Division of Diarrhoeal and Acute Respiratory Disease Control, WHO, Geneva, in the preparation of this chapter is gratefully acknowledged.
Salmonellae may be present in all kinds of food grown in faecally polluted environments, and are commonly isolated from poultry and livestock and foods prepared from them. Furthermore, animal feedstuffs and fertilizers prepared from animal products may be highly contaminated with salmonellae, and they are also widely distributed in the environment. The contamination of food and animal feedstuffs by water contaminated with salmonellae is considered to be an additional risk factor (3, 4). Dumping of unprocessed slaughterhouse wastes into rivers has been found to be a cause of salmonellae infections. Contamination by salmonellae and conditions favouring their regrowth should be avoided at all stages of the production, transport, storage, and preparation of food, feedstuffs, and drinking-water. Sludge disposal and irrigation must always be accompanied by appropriate hygienic precautions.

The transmission routes of salmonellae are highly complex. Person-to-person transmission may occur, but the relative importance of the human and non-human reservoirs depends on the dietary, agricultural, and sanitary situation in a particular community (2).

**Significance in drinking-water**

Waterborne outbreaks have been chiefly associated with *S. typhi* and much less frequently with *S. paratyphi* B or other *Salmonella* serotypes (2). Epidemiological studies of outbreaks suggest that the ingestion of relatively few cells of *S. typhi* may cause typhoid fever, whereas studies in volunteers (Table 2.2, p. 14) indicate that, for other *Salmonella* serotypes, millions of cells are usually required to cause gastroenteritis.

Salmonellae are excreted in the faeces of infected humans or animals. Faecal contamination of groundwater or surface waters, as well as insufficiently treated and inadequately disinfected drinking-water, are the main causes of epidemic waterborne outbreaks caused by *Salmonella* spp.

Waterborne outbreaks due to heavy contamination are usually characterized by an explosive onset. The majority of cases develop over a period of a few days, and may be followed by a secondary crop of contact cases (2). The geographical distribution of infections in major outbreaks is often strongly correlated with the pattern of a waterworks pipeline network.

Salmonellae can be found in open wells as a result of the drainage or flooding of contaminated surface water into unprotected well shafts. It is uncommon for salmonellae to be isolated from piped water supplies, whether treated or untreated, and their presence suggests a serious fault in the design or management of the system (2).

Penetration of pathogens into water sources must be avoided by the protection of groundwater and surface water catchment areas. A review of the literature has shown that, in general, pathogens will not travel further than the distance that the groundwater flows in 10 days, except in fissured rocks such as limestone and heavily fissured soils (5).
Drinking-water must be of low turbidity after treatment if adequate chlorination is to be achieved. Furthermore, a low load of assimilable organic carbon (AOC) in the treated water is considered to be an important factor in reducing survival time and preventing the regrowth of salmonellae within the distribution system. Reported survival times for salmonellae in drinking-water range from a few days to over 100 days.

Several outbreaks have been caused by the deposition of contaminated sediments in the distribution system for drinking-water, especially in water basins and pipes. Sediments may be shifted to new positions in the system by water pressure oscillations or temporary scarcity of water. Regular flushing of the distribution system is therefore recommended.

3.1.2 Yersinia

**General description**

The genus *Yersinia* is currently placed in the family Enterobacteriaceae and comprises seven species. *Y. pestis, Y. pseudotuberculosis,* and certain serotypes of *Y. enterocolitica* are pathogens for humans. Atypical strains within *Y. enterocolitica,* isolated most frequently from environmental samples, are grouped together as *Y. enterocolitica-like* organisms. They are nonpathogenic for humans and can be subdivided by biochemical means into *Y. intermedia, Y. frederiksenii, Y. kristensenii,* and *Y. aldovae.*

The cells of *Y. enterocolitica* are Gram-negative rods, motile at 25 °C but nonmotile in cultures grown at 37 °C. Certain strains of *Y. enterocolitica* cause acute gastroenteritis with diarrhoea, but other human diseases caused by *Y. enterocolitica* are also known. Biochemical and serological typing of enteric *Y. enterocolitica* strains show that serotypes O:3 and O:9 are commonly found in Africa, Asia, Canada and Europe, whereas serotype O:8 is exclusively isolated in the United States (6-8).

There is some evidence that *Y. enterocolitica* infection may be waterborne. The following discussion is confined to this species.

**Routes of exposure**

The transmission of *Y. enterocolitica* from the natural reservoirs to humans has been the subject of much debate. Many domestic and wild animals are considered to be possible reservoirs of *Y. enterocolitica* because of the high isolation rates of the organism from such sources. It is likely that wild animals, particularly shrews, hares, foxes, and beavers, form a natural reservoir of *Y. enterocolitica.* Swine have been implicated as a major reservoir of *Y. enterocolitica* serotypes involved in human infections.

Available evidence indicates that foods, especially meat and meat products, milk and dairy products, are the major vehicles for the transmission of *Y. enterocolitica.* Furthermore, *Y. enterocolitica* has been isolated from a variety of environ-
mental samples, especially from water, but the serotypes isolated differ from those associated with human disease.

A number of different transmission routes have been suggested for *Y. enterocolitica*, but the ingestion of contaminated food and water is probably the most likely one (8). Direct transmission from person to person and from animals to humans also occurs, but its relative importance has not been clarified. Further research is needed to define the epidemiological importance of “environmental” strains of *Y. enterocolitica*.

**Significance in drinking-water**

The apparent waterborne spread of *Y. enterocolitica* infection has been described in a number of reports. There is some evidence that pathogenic strains of *Y. enterocolitica* enter drinking-water via contaminated surface water or as a result of pollution with sewage (9). Recent studies have shown that human pathogenic serotypes of *Y. enterocolitica* are present in sewage and polluted surface water (10, 11).

In general, pathogenic types of *Y. enterocolitica* are not isolated from untreated or treated drinking-water unless faecal pollution has occurred. Occasionally, nonpathogenic serotypes of *Y. enterocolitica* and nonpathogenic *Y. enterocolitica*-like organisms (*Y. intermedia*, *Y. frederiksenii*, *Y. kristensenii*) may also be isolated from drinking-water. However, none of these isolates exhibit the typical virulence markers. Such isolates are probably of environmental origin without public health importance (12).

Water samples yielding *Y. enterocolitica* often show only slight coliform contamination. One study indicated that 25% of *Y. enterocolitica*-positive samples were negative for both total and faecal coliforms (9). Intensive methods of treatment are not needed in such cases. Other studies have shown a close relationship between faecal pollution and *Y. enterocolitica* isolation rates (13).

Standard chlorination procedures are sufficient to avoid the transmission of *Yersinia* if the treated water is of low turbidity. Free chlorine in the range required for water disinfection (0.2–0.5 mg/litre) for 10 minutes at pH 7.0 completely eradicates the bacteria; 0.05 mg/litre of ozone eradicates the organism after contact for 1 minute regardless of pH (14).

A special feature of *Y. enterocolitica* and *Y. enterocolitica*-like organisms is their ability to grow at low temperatures, even at 4 °C. Accordingly, these organisms can survive for long periods in water habitats. For example, *Y. enterocolitica* was detected in previously sterilized distilled water after over 18 months at 4 °C (15). Such long survival periods make it difficult to determine the origin of contamination when *Yersinia* are detected.
3.1.3 Campylobacter

**General description**

In recent years, considerable attention has been given to *Campylobacter* spp. as important agents of enteritis, gastritis, and other human diseases.

Campylobacters are Gram-negative, slender, comma-shaped rods. They also appear S-shaped and gull-winged when in pairs (7, 16). The organisms show a characteristic corkscrew-like motion, which can be easily seen by phase-contrast microscopy. Campylobacters are microaerophilic organisms, requiring a low oxygen tension (3–6%) for growth. Under unfavourable growth conditions, cells may form coccoid bodies.

A recent review discusses 14 *Campylobacter* species (17). Some are pathogens for humans and animals (e.g. *C. jejuni*, *C. coli*, *C. fetus*), while others are considered to be nonpathogenic (e.g. *C. sputorum*, *C. concisus*) (16, 17). Most of the members of the thermophilic group (growing at 42 °C) of campylobacters cause enteritis in humans. Worldwide, campylobacters are much more important than salmonellae as causes of acute gastroenteritis, but not as important as shigellas. Several major outbreaks of campylobacter enteritis were linked to the ingestion of contaminated food, milk, or water.

From the point of view of water hygiene, the thermophilic campylobacters are of greatest significance; the following discussion is therefore confined to these organisms.

**Routes of exposure**

Thermophilic campylobacters are transmitted by the oral route. The reservoirs for campylobacters include wild birds and poultry which are the most important, and other domesticated animals, such as pigs, cattle, dogs, and cats. Meat, in particular poultry products, and unpasteurized milk are therefore important sources of campylobacter infections (16). Milk may be contaminated with faeces or by the secretion of organisms into milk by cows with mastitis (18). In developing countries, the faeces of infected animals are important reservoirs. The infective dose is low (19).

Recent studies have shown that raw sewage frequently contains $10^5$–$10^6$ thermophilic campylobacters per 100 ml (20, 21). However, *Campylobacter* counts in heavily contaminated sewage can be reduced considerably by wastewater treatment processes. Thermophilic campylobacters were found in crude sludge, but were not detectable in digested conditioned sludge of filter effluent (21). The occurrence of campylobacters in surface waters has proved to be strongly dependent on rainfall, water temperature, and the presence of waterfowl.
**Significance in drinking-water**

Several waterborne outbreaks of campylobacteriosis have been reported in the past decade. The numbers of persons involved ranged from a few to several thousands. In only two of these outbreaks were campylobacters isolated both from patients and from water samples. Unchlorinated surface water and faecal contamination of water storage reservoirs by wild birds were found to be the main causes. The consumption of unchlorinated or inadequately chlorinated surface waters is associated with a considerable risk of outbreaks of campylobacteriosis. Any contamination of drinking-water reservoirs by the excrement of waterfowl must be controlled. Consideration should be given to imposing stricter hygienic requirements for drinking-water, even if obtained from high-quality surface water, since it may be distributed without chlorination.

Campylobacters, like other bacterial pathogens, survive well at low temperatures, suggesting that cold water may be an effective vehicle of transmission. They are able to survive for several weeks (22) in cold groundwater or unchlorinated tapwater. The standard chlorination procedures are sufficient to prevent the spread of campylobacters along water mains if the water is of low turbidity.

3.1.4 *Escherichia coli*

*E. coli* is found in large numbers in the faeces of humans and of nearly all warm-blooded animals; as such it serves as a reliable index of recent faecal contamination of water. This topic is fully covered in Chapters 9–11. Certain strains are pathogenic for humans and it is these that are described below.

**General description**

*E. coli* is a Gram-negative, non-spore-forming, rod-shaped bacterium which can be either motile or nonmotile (motile cells are peritrichous); growth is aerobic or facultatively anaerobic. Metabolism is both respiratory and fermentative; acid is produced by the fermentation of glucose and lactose. Catalase is produced but not oxidase, and nitrates are reduced to nitrites. In the microbiological examination of water, a biochemical description is used (see sections 9.2.1–9.2.3).

Serological typing is based on the somatic O antigens, the capsular K antigens, and the flagellar H antigens. In practice, serogrouping by O antigen is often used alone and, within a particular epidemiological context, may be satisfactory. Biochemical tests do not reliably distinguish pathogenic strains of *E. coli*.

**Health effects**

*E. coli* is a normal inhabitant of the intestine, and most strains are nonpathogenic. However, subtypes able to cause gastrointestinal disease are also known. Such pathogenic *E. coli* strains cause intestinal disease by a variety of mechanisms. Infections may resemble cholera, dysentery, or gastroenteritis due to sal-
monellae. Four classes of pathogenic *E. coli* responsible for diarrhoea are recognized: enteropathogenic, enteroinvasive, enterotoxigenic, and verocytotoxin-producing (23).

Enteropathogenic subtypes of *E. coli* were first recognized as a result of the serological examination of strains isolated from outbreaks of diarrhoea among infants. Associations of particular serotypes with disease were observed, but the corresponding pathogenic mechanisms are not fully understood for most of these organisms. These strains have been particularly associated with outbreaks of infantile gastroenteritis (24).

Enteroinvasive strains of *E. coli* (EIEC) produce dysentery by a mechanism similar to that found with *Shigella* spp. These organisms invade the colonic mucosa and cause bloody diarrhoea. The property seems to be restricted to a few O groups. It must be remembered that *Shigella* and *E. coli* are closely related and that genetic material is readily exchanged between them.

Although enteropathogenic or enteroinvasive strains may cause serious illness, such epidemiological evidence as is available suggests that enterotoxigenic strains are responsible for most episodes of *E. coli* diarrhoea, particularly in developing countries. Enterotoxigenic *E. coli* (ETEC) can cause a cholera-like syndrome in infants, children, and adults.

ETEC produce either a heat-labile enterotoxin (LT), related to cholera enterotoxin, or a heat-stable enterotoxin (ST); some strains produce both toxins. The action of LT is the same as that of cholera toxin. The production of enterotoxin is controlled by plasmids.

The ability of ETEC to cause disease depends not only on the production of enterotoxin but also on their ability to colonize the small intestine. Various colonization factors, or adhesins, have been described, which enable the bacteria to attach themselves to the intestinal mucosa.

The fourth class, verocytotoxic *E. coli*, was first recognized by their production of a cytotoxin active against Vero cells in culture. The organisms commonly belong to the serogroup O157 and cause disease ranging from mild diarrhoea to haemorrhagic colitis characterized by blood-stained diarrhoea, usually without fever, but accompanied by abdominal pain. It is also a cause of the haemolytic uraemic syndrome, commonest in infants and young children, and characterized by acute renal failure and haemolytic anaemia.

**Significance in drinking-water**

Isolation of *E. coli* from a water supply indicates faecal contamination. However, *E. coli* is only one species among many in the family Enterobacteriaceae. Members of the lactose-fermenting species of this group, which may be referred to colloquially as "coliforms", occur in a variety of ecological niches, not all of which are intestinal. Thus, for example, some species are associated with aquatic slimes and vegetation. The picture is further complicated by the fact that other members of the coliform group are also found in the intestine. Thus, the definitive
identification of *E. coli* may be needed to determine the significance of "coli-forms" in a water supply.

Conventionally, thermotolerant coliforms are identified by growth at 44-45 °C, but some isolates of *Klebsiella*, *Citrobacter*, and *Enterobacter* will also grow and ferment lactose under these conditions. The term "faecal coliforms" is often used for this group, but the term "faecal" is to be deprecated, since not all prove to be of faecal origin.

The detection of the pathogenic subtypes of *E. coli* in water supplies has seldom been attempted. Although this may be necessary in epidemiological research, the available methods are not suitable for the routine examination of water samples.

3.1.5 *Vibrio cholerae*

**General description**

*Vibrio* species are motile, non-spore-forming, slightly curved Gram-negative rods with a single polar flagellum; they are both aerobic and facultatively anaerobic. Their metabolism is both respiratory and fermentative without the production of gas. Both catalase and oxidase are formed, and nitrates are reduced to nitrites.

Among the vibrios, special attention has focused on the identity of those causing cholera. It was for a long time believed that *Vibrio cholerae* was a unique and distinct species associated with human disease, and recognized by possession of the O1 antigens. This species was further divided into "classical" and "El Tor" biotypes, the latter distinguished by the ability to produce a dialysable, heat-labile haemolysin, active against sheep and goat red cells (25).

A broader definition of *V. cholerae* has now been adopted. It has been known for many years that vibrios biochemically identical to *Vibrio cholerae* O1, but lacking the O1 antigen, could be found in the aquatic environment. These were termed non-cholera vibrios (NCV) or non-agglutinable (NAG) vibrios. The term "non-O1 *V. cholerae" is now preferred, since some may produce cholera toxin, while investigation of DNA/DNA homology between *Vibrio cholerae*, NCVs, and NAG vibrios has conclusively demonstrated that they are all very closely related. Currently, all are considered to be a single species, *Vibrio cholerae*, divided into more than 80 serological types on the basis of the O or somatic antigens. The H or flagella antigen is common to all the O groups of *V. cholerae*, and H agglutination has been used as a diagnostic test.

Only *V. cholerae* serogroup O1 causes cholera. The O1 group contains two serotypes based on variations in the O antigen factors, namely Ogawa (factors A and B) and Inaba (factors A and C). These serotypes may exist in either the classical or El Tor biotype.
**Routes of exposure**

Cholera has historically been one of the major pandemic diseases. The present pandemic, unlike previous ones, is caused by *V. cholerae* O1 biotype El Tor.

Cholera is usually a waterborne disease, and numerous waterborne outbreaks have been documented. However, foodborne and nosocomial outbreaks are also important, and person-to-person transmission may occur under conditions of extreme crowding and poor hygiene. The problems of the transmission of cholera have been extensively reviewed, and although waterborne transmission is undoubtedly important, many aspects of the epidemiology of cholera are a matter for debate. Evidence has accumulated to suggest that, in some circumstances, *V. cholerae*, including serotype O1, may be part of the autochthonous microbiota of natural waters (25, 26).

**Health effects**

Infection with *V. cholerae* O1 involves the small and large intestine. In the small intestine, the vibrios attach themselves to the mucous layer covering the villous epithelium, chemotactic processes apparently playing a role in their migration to the epithelium. After attachment, vibrios penetrate the layer of mucus and adhere to the surface of the epithelial cells. Motility seems to be essential for mucus penetration to occur. Adherence to the mucosal surface is specific, involving a receptor-adhesion interaction analogous to a lectin-ligand reaction. When present in large numbers, *V. cholerae* O1 produce an enterotoxin (cholera toxin) that causes alterations in the ionic fluxes across the mucosa with the resulting catastrophic loss of water and electrolytes in liquid stools. Cholera toxin is very similar to the heat-labile toxin produced by enterotoxigenic *E. coli*.

It seems likely that other accessory virulence factors, such as mucinase and protease, are also important in the pathogenesis of cholera. Other toxins may also be involved. Enterotoxins similar to the heat-stable toxins of *E. coli* are known to be formed by *V. cholerae* of O groups 2–84, and *V. cholerae* O1 may also produce several toxins. Cholera toxin is not produced by all strains of *V. cholerae* O1, and nontoxigenic strains are considered to be nonpathogenic.

**Significance in drinking-water**

The isolation of *V. cholerae* O1 from water used for drinking is of major public health importance and is evidence of faecal contamination. However, other serogroups of *V. cholerae* may be part of the normal flora of some waters.
3.1.6 Shigella

**General description**

Shigellae are Gram-negative, non-spor-forming, non-motile rods, capable of growth under both aerobic and anaerobic conditions. Metabolism is both respiratory and fermentative; acid, but not usually gas, is produced from glucose. Lactose is seldom fermented. Catalase is usually produced, except by *Shigella dysenteriae* type 1, but oxidase is produced by one serotype only. Nitrates are reduced to nitrates.

Shigellae are serotyped on the basis of their somatic O antigens. Both group and type antigens are distinguished, group antigenic determinants being common to a number of related types. There is evidence that type antigen specificities among *Shigella flexneri* are determined by the presence of lysogenic bacteriophages. It seems likely that biotypic and serological variants are determined by the presence of phage or the carriage of plasmids. Plasmids (transferrable, extrachromosomal genetic elements) were first described in this genus. Phage-typing systems exist for all groups, though they have not been widely applied, serological typing being adequate for all species except *Shigella sonnei*.

**Health effects and routes of infection**

Though shigella infection is not often spread by waterborne transmission, major outbreaks resulting from such transmission have been described. The characteristic bloody diarrhoea results from the invasion of the colonic mucosa by the bacteria. There is good reason to believe that the process is highly species-specific. Shigellae have no natural hosts other than the higher primates, and humans are the only effective source of infection. Of the enteric bacterial pathogens, shigellae seem to be the best adapted to cause human disease. Direct transmission between susceptible individuals is the usual route of infection, and the infective dose is lower than for other bacteria.

**Significance in drinking-water**

The isolation of shigellae from drinking-water indicates recent human faecal contamination and, in view of the extreme pathogenicity of the organisms, is of crucial public health significance. However, this is a rare event, possibly explained in part by the absence of a useful enrichment or selective medium for the isolation of these bacteria. Those generally used have been designed for the isolation of salmonellae and are not optimal for that of shigellae. Furthermore, without confirmatory testing, some anaerogenic strains of *E. coli* may be wrongly identified as shigellae.
3.2 Pathogens that grow in supply systems

All drinking-water contains assimilable organic compounds that will allow a certain degree of bacterial growth. The density of bacteria in drinking-water can and should be controlled for the reasons given in Chapter 8.

Certain bacteria in drinking-water deserve particular attention because they are opportunistic pathogens to humans, i.e. they are able to cause infections in susceptible persons. The most important organisms of this type, namely Legionella and Aeromonas, will be considered here. Other organisms, such as Pseudomonas aeruginosa and opportunistically pathogenic mycobacteria, have been detected in drinking-water supplies.

3.2.1 Legionella

General description

The genus Legionella, a member of the family Legionellaceae, has 22 currently known species, of which L. pneumophila serogroup 1 is most frequently associated with human disease. Other serogroups of L. pneumophila and occasionally other legionellae have also been reported to cause disease. Legionellae are Gram-negative, rod-shaped, non-spore-forming bacteria that require L-cysteine for growth and primary isolation. The cellular fatty acids in legionellae are unique among those found in Gram-negative bacilli in that they consist essentially of branched chains. Preconcentration of legionellae from environmental samples may be required if low levels are to be detected. Immunofluorescence techniques may also be used to detect legionellae in the environment.

Health effects

Legionella infections can lead to two types of disease, namely Legionnaires' disease (legionellosis) and non-pneumonic Legionnaires' disease (Pontiac fever). Legionnaires' disease is a form of pneumonia with an incubation period usually of 3–6 days. Males are more frequently affected than females, and most cases occur in the 40–70 year age group. Risk factors include smoking, alcohol abuse, cancer, diabetes, chronic respiratory or kidney disease, and severe immunosuppression, as in transplant recipients. The fatality rate in untreated cases may be 10% or higher, but the disease can be treated effectively with antibiotics such as erythromycin and rifampicin.

Legionnaires' disease is uncommon, but common-source outbreaks attract much attention. Between 100 and 200 cases are reported each year in England and Wales, and in Germany; in France, the incidence is somewhat higher, with over 400 cases per year (27). For people living in temperate climates, travelling to subtropical areas appears to be a significant risk factor, outbreaks being related to air-conditioning and hot-water systems in hotels. Hospital-associated Legionnaires' disease is the most serious form, because it usually affects debilitated per-
BACTERIA

3.

The non-pneumonic form of the disease is milder, with a high attack rate, an acute onset (5 hours to 3 days) and symptoms similar to those of influenza: fever, headache, nausea, vomiting, aching muscles, and coughing. No fatal cases have been reported and few outbreaks have been recognized, possibly because the non-specific symptoms of the disease hinder its diagnosis.

**Routes of exposure**

Legionellae are widespread in natural sources of water and may also be found in soils. They occur commonly in man-made water systems, particularly in hot-water and cooling-water systems. Infection is the result of the inhalation of aerosols that are small enough to penetrate the lungs and be retained by the alveoli. The degree of risk depends on four key factors: (i) the density of the bacteria in the source; (ii) the extent of aerosol generation; (iii) the number of inhaled bacteria; and (iv) the susceptibility of the exposed individual.

Legionellae multiply in the laboratory at temperatures between 20 and 46 °C. At temperatures higher than 46 °C, the bacteria will survive, but at 60 °C only for a few minutes (28). Temperatures favourable for growth may be found in cooling towers, spas, cold-water systems in buildings, hot-water systems operated below 60 °C or “dead legs” of such systems operated at higher temperatures. Aerosols may be created by the spraying of water in cooling towers or its agitation in spas. Hot-water systems are also likely to create aerosols in showers, through nozzle heads or by splashing in sinks, baths, etc. The number of inhaled bacteria depends on the size of the aerosol generated (<5 μm being most dangerous), the dispersal of the aerosol in the air, and the duration of the exposure. Host defence is an important factor that determines whether exposure to legionellae will lead to clinical disease. It is primarily for this reason that high counts of *L. pneumophila* in water systems have been reported in the absence of disease, whereas similar or lower counts have been associated with epidemics. It is also likely, although not yet adequately proven, that differences in virulence between strains partly account for these observations.

It is now apparent that legionellae can be ingested by the trophozoites of certain amoebae (*Acanthamoeba*, *Hartmanella*, *Valkampfia*, and *Naegleria*) and even grow intracellularly and become incorporated in their cysts (29). This may explain the difficulty of eradicating legionellae from water systems and may be a factor in the etiology of the non-pneumonic disease (30).

The following are generally accepted as requiring disinfection:
- sites implicated in an outbreak of Legionnaires’ disease or Pontiac fever;
- hospital wards housing high-risk patients, such as an organ transplant unit;
- buildings in which the water system has not been used for some time and where large numbers of legionellae are likely to be found.
Nevertheless, it is generally advisable to design and maintain systems in such a way that colonization by *Legionella* is prevented as much as possible. Detailed instructions for achieving this have been given in several publications (31–34) and focus on the following aspects:

- preventing the accumulation of sludge, scale, rust, algae and slime, and removing such deposits regularly;
- maintaining hot-water temperatures permanently above 60 °C or increasing them periodically to above 70 °C, and keeping cold-water supplies below 20 °C;
- selecting materials for contact with water that do not release nutrients that support the growth of *Legionella*.

The use of biocides is generally regarded as a less effective and less desirable means of controlling legionellae in water supplies in buildings. However, their use is essential to prevent the build-up of microbial slimes in air-conditioning systems in which wet, evaporative cooling towers are used. Such systems should be kept clean and well maintained, and should be inspected weekly for fouling, accumulations of slime and scale, and corrosion; they should be thoroughly cleaned and disinfected twice yearly. Biocides are best used intermittently in clean systems (33, 34).

### 3.2.2 Aeromonas

#### General description

*Aeromonas* spp. are Gram-negative, rod-shaped, non-spore-forming bacteria that are currently assigned to the family Vibrionaceae, although they also bear many similarities to the Enterobacteriaceae. MacDonnell et al. (35) have suggested the creation of a new family Aeromonadaceae. The genus *Aeromonas* is divided into two groups of which the first, the group of psychrophilic non-motile aeromonads, consists of only one species, *A. salmonicida*, an obligate fish pathogen that will not be considered further here. The group of mesophilic motile aeromonads has been divided by Popoff into three biochemically distinguishable groups (36), namely *A. hydrophila*, *A. sobria* and *A. caviae*. Each of these three species consists of at least three different DNA-hybridization groups, and later workers have described new species such as *A. veronii*, *A. media*, *A. schubertii*, and *A. eucrenophila*. It may be expected that in the near future the taxonomy of the group of mesophilic aeromonads will be changed still further, but at present the classification of Popoff is that most widely accepted internationally.

#### Health effects

Mesophilic aeromonads have long been known to be pathogenic for cold-blooded animals such as fish and amphibians, but in the last few decades greater attention has been given to their pathogenic significance for humans. Three
major types of infection are described (37): (i) systemic infections, usually in seriously immunocompromised persons; (ii) wound infections (mainly after contact with surface water); and (iii) diarrhoea. In particular, the significance of *Aeromonas* as an enteropathogenic organism is the subject of much discussion. In animal test models, such as the suckling mouse test and the rabbit ileal loop test, pure cultures of *Aeromonas* have been found to cause strong fluid accumulation which can partially be ascribed to the production of extracellular cytotoxins. However, there have been reports that, while the culture filtrate of some *Aeromonas* strains is not enteropathogenic in an animal test model, a suspension of living cells does have this property. Cell-bound factors are apparently also of importance (38). Asao et al. (39) have purified and characterized an *Aeromonas* haemolysin, which was found to be a protein with a relative molecular mass of about 50,000 that was strongly enterotoxic and cytotoxic. It has not yet been possible to purify and characterize other toxins because the toxic activity disappears rapidly when culture filtrates are manipulated. Despite the marked toxin production by *Aeromonas* strains in vitro, diarrhoea has not yet been induced in test animals or human volunteers, and it is assumed that such strains are only poorly able to colonize the gastrointestinal tract (40). Little is known about the adhesion factors of *Aeromonas* or their interaction with receptors in the gastrointestinal tract.

Epidemiological investigations have also resulted in contradictory findings on the significance of *Aeromonas* as an enteropathogenic organism. In some studies, the numbers of *Aeromonas* in the faeces of patients with diarrhoea were greater than those in control groups, whereas in other studies there was no difference, or the bacteria were found in even greater numbers in the latter. In general, it can be said that the significance of *Aeromonas* as an enteropathogenic organism is greater in tropical areas than in the temperate zone. However, infections do occur in the temperate zone as well, albeit less frequently. *Aeromonas*-associated diarrhoea usually causes an acute but self-limiting gastroenteritis but chronic disease with serious complications may also occur. The incidence of *Aeromonas* in human diarrhoeal faeces in the Netherlands was found to vary between 0.5\% in winter and 3\% in summer. Most isolations were made in children under five years of age or in adults above 70 years of age. Young children yielded mainly *A. caviae*, whereas *A. sobria* was usually isolated from the elderly (41).

**Routes of exposure**

*Aeromonas* occurs in water, soil, and food, particularly meat, fish, and milk. The occurrence of *Aeromonas* in drinking-water can be studied by a variety of methods. A membrane filtration method has been described in which a selective ampicillin-dextrin agar (ADA) is used (42). If water samples from house installations are being examined, the addition to the sample of a complexing agent such as the disodium salt of edetic acid (Na₂EDTA) at a concentration of 50 mg/litre is necessary. *Aeromonas* is extremely sensitive to the traces of copper that may be
present in water in domestic installations in which copper piping is used. Complexing of copper was also found to improve the survival of coliform bacteria and heterotrophic bacteria (43).

The number of *Aeromonas* in surface waters can vary between 0.01 and 1000 cfu/ml. Small numbers are found in springs and in seawater that is not contaminated by sewage discharges. If such discharges are present, the number of *Aeromonas* can rise to 100 cfu/ml in seawater, and more than 1000 cfu/ml in fresh water. The species *A. caviae* then being dominant. In fresh waters not subject to sewage pollution, the numbers of *Aeromonas* are usually between 10 and 100 cfu/ml. In these waters, the numbers are higher in summer than in winter, and there is a relation between the eutrophication of the water and the summer density of *Aeromonas*. In stagnant fresh water, *A. sobria* is usually the dominant species. When river water is stored in reservoirs, the number of *Aeromonas* decreases, but there is also a shift in species composition from *A. caviae* to *A. sobria*. *Aeromonas* is not usually found in groundwater or is found only in very small numbers (44).

Irrespective of the contamination level of the raw water, most drinking-water treatment processes appear to be able to reduce the numbers of *Aeromonas* to below 1 cfu/100 ml. However, treated water can contain larger numbers, with maxima of about 1000 cfu/100 ml as a result of regrowth in storage reservoirs with long retention times, polluted filter sand, or sudden changes in the quantities of water to be produced. Regrowth of *Aeromonas* occurs in the distribution network of most drinking-water treatment plants. The size of the *Aeromonas* population will depend on many factors but primarily on the organic content of the water and its temperature, the residence time in the distribution network, and the presence of residual chlorine.

Little is known about the type and concentrations of nutrients for *Aeromonas* in drinking-water. Van der Kooij (45) has suggested that *Aeromonas* prefers to grow on organic matter, e.g. from decaying nitrifying and methane-oxidizing bacteria that develop in drinking-water treatment plants or from biofilm material in distribution networks.

Control of aeromonads in drinking-water requires a multiple approach, which is similar to the general approach to limiting the regrowth of bacteria (see Chapters 8 and 9). The treatment process should effectively remove organic compounds serving as sources of carbon and energy for the growth of bacteria. Furthermore, the amount of biomass produced and subsequently released during the treatment process should be as small as possible. The distribution system should be designed in such a way that residence times are short, and it should be flushed regularly to prevent the accumulation of sediments in stretches with low water velocities. Materials in contact with drinking-water should not be a source of biodegradable compounds. These factors are of greatest importance in supplies that are not disinfected, or where the maintenance of a chlorine residual is not considered desirable for various reasons. Free available chlorine residuals of 0.2–0.5 mg/litre will generally be sufficient to control *Aeromonas* densities in water in the distribution network (46, 47). Chlorine or other disinfectants should not be
used to control occasional increases in *Aeromonas* densities in supplies that are normally not chlorinated because biofilms on pipe walls will be disturbed, and this will result initially in increases rather than decreases in bacterial concentrations. Also, in such systems the chlorine consumption will be rather high and residuals cannot be properly maintained, thus allowing regrowth in remote stretches of the network.

The question whether the presence of *Aeromonas* in drinking-water is a risk to human health cannot be answered with certainty; however, if there is a risk, it must be small because in many countries the bacterium is not important as a causative agent of diarrhoea and is not often able to colonize the gastrointestinal tract of humans. Also, the numbers present in drinking-water are small as compared with those in other sources. In food, for instance, the numbers usually found are of the order of $10^2-10^3$ cfu/g. However, drinking-water is a product that is consumed daily by everyone, including groups with a reduced resistance to infectious diseases. Some of this water is consumed without previous heating, in contrast to most foods contaminated with *Aeromonas*. A cautious approach therefore appears to be justified. Numbers of *Aeromonas* in drinking-water must be controlled as far as possible. Apart from the public health reasons for the control of *Aeromonas* levels in drinking-water, experience has shown that it is a useful and sensitive indicator of general hygiene within the drinking-water production and distribution process. Currently, no guideline value can be given because local conditions (temperature, raw water source) may greatly influence *Aeromonas* counts in drinking-water.

### 3.2.3 Pseudomonas aeruginosa

**General description**

*P. aeruginosa* is a member of the family Pseudomonadaceae and is a monotrichate, Gram-negative rod. It can be recognized by its production of a blue-green fluorescent pigment (pyocyanin), which, in agar cultures, will diffuse into the medium. Pigment may not be produced by strains of *P. aeruginosa* recovered from clinical specimens, and the ability to produce it may be lost on subculture. Like other fluorescent pseudomonads that occur in natural waters, *P. aeruginosa* strains produce catalase and oxidase, and ammonia from arginine, grow with citrate as the sole source of carbon, and are aerobic. *P. aeruginosa*, however, is capable of growth at 41–42 °C, and the blue-green pigment that it produces differs from the fluorescent pale green pigment (fluorescein) produced by other species of fluorescent pseudomonads found in water. It is also capable of growing anaerobically in stab cultures of nitrate agar.

*P. aeruginosa* is commonly found in faeces, soil, water, and sewage but cannot be used as an indicator of faecal contamination, since it is not invariably present in faeces and sewage, and may also multiply in the enriched aquatic environment and on the surface of unsuitable organic materials in contact with water. How-
ever, its presence may be one of the factors taken into account in assessing the
general cleanliness of water distribution systems and the quality of bottled waters (see section 9.3.2).

Routes of exposure

*P. aeruginosa* is an opportunistic pathogen. Most of the illnesses in humans for
which it is responsible are caused, not by drinking water, but by contact with it.
Water containing these bacteria may also contaminate food, drinks, and pharmaceu-
tical products, causing them to deteriorate and to act as secondary vehicles for
transmission. Fixtures in contact with water, such as sinks and sink drains, tap
fittings and showerheads, can also be contaminated by *P. aeruginosa* and can serve
as reservoirs of infection in hospitals.

Health effects and significance in drinking-water

In healthy persons, the illnesses caused by *P. aeruginosa* are usually mild and triv-
ial. Waterborne infections are usually associated with warm, moist environments;
they include the skin rashes and pustules or outer ear canal infections (otitis
externa) reported in users of indoor swimming-pools and whirlpools, where
bacterial counts are high and disinfection is deficient (48, 49). The presence of
this organism in water supplied to hospitals and for the manufacture of pharma-
ceutical preparations and dressings is a matter of concern because *P. aeruginosa* is
a common pathogen in infections of wounds and burns and has caused serious
eye infections after the use of contaminated eye drops (50). Hospital strains of
*P. aeruginosa* can first colonize and then infect patients receiving cancer chemother-
apy (51).

The presence of this organism in potable water also indicates a serious deteri-
oration in bacteriological quality, and is often associated with complaints about
taste, odour, and turbidity linked to low rates of flow in the distribution system
and a rise in water temperature (see section 9.3.2).

3.2.4 Mycobacterium

General description

*Mycobacterium* spp. are rod-shaped bacteria with cell walls having a high lipid
content; this enables them to retain certain dyes in staining procedures that
employ an acid wash, and they are therefore often referred to as acid-fast bacteria.
The characteristics of the cell wall structure also result in a relatively high resis-
tance to disinfectants. All mycobacteria are characterized by slow growth (genera-
tion times under optimal circumstances 2–20 hours), but within this range they
are divided into “slow” and “rapid” growers. Most pathogenic species are found
among the slow growers, which include the strictly pathogenic species *M. tuber-
culosis*, *M. bovis*, *M. africanum*, and *M. leprae*, these are not transmitted by water.
and have only human or animal reservoirs. Other mycobacterial species, often referred to as "atypical", have environmental reservoirs. Although many are considered to be nonpathogenic, several species are opportunistic pathogens for humans, the most important being the slow growers *M. kansasii*, *M. marinum*, *M. avium*, *M. intracellulare*, *M. scrofulaceum*, and *M. xenopi*, and the rapid growers *M. chelonae* and *M. fortuitum*. Some of these species are closely related, and the literature often describes a number of complexes rather than individual species. Examples are the "*M. bovis* complex" (which includes *M. africanum*), the "*M. avium* complex" (or MAC, which includes *M. intracellulare*), or the "*M. avium*, *M. intracellulare*, *M. scrofulaceum* complex" (or MAIS) and the "*M. fortuitum-chelonae* complex."

**Health effects**

The strictly pathogenic mycobacteria are associated with classical infectious diseases such as tuberculosis and leprosy. The environmental mycobacteria may cause a range of diseases including tuberculous lung disease and disseminated infections which may also involve the skeleton (*M. kansasii*, *M. avium* complex), infections of the lymph nodes (MAIS complex), and infections of the skin and soft tissues (*M. marinum*, *M. fortuitum-chelonae* complex) (52, 53). Diseases caused by opportunistic pathogenic mycobacteria are not normally transmitted from person to person but are usually the result of environmental exposure in combination with predisposing factors, such as dust retained in the lungs, surgical wounds, or immunosuppression produced by medication (transplant patients) or by underlying disease (AIDS, malignancies). Mycobacteria are generally resistant to many antimicrobial agents, hence effective treatment may be difficult.

**Routes of exposure**

An extensive review of the occurrence of mycobacteria in environmental sources has been published (54). Tapwater has long been known to harbour saprophytic mycobacteria; in fact, one of the most commonly occurring species, *M. gordonae*, is known as the tapwater bacillus. The occurrence of opportunistic pathogenic species in tapwater has also been demonstrated by various authors (55, 56). These organisms may accidentally contaminate clinical specimens during and after collection, or during processing in the laboratory; this may falsely suggest that the patients concerned are suffering from a mycobacterial infection (57, 58). A link between the occurrence of mycobacteria in drinking-water and disease has sometimes been suggested. Endemic *M. kansasii* infections in Czechoslovakia were studied from 1968 onwards, the peak incidence being found in a small, densely populated district in which workers were engaged in mining, heavy industry, and power generation. *M. kansasii* could also be isolated from shower outlets in collieries, and it was later shown that the drinking-water system in the entire region was widely contaminated. It was suggested that mycobacteria from drinking-
water were spread via aerosols (59). The high isolation frequency of *M. kansasii* from clinical specimens in Rotterdam, the Netherlands, led to an investigation of the water supply system. The organisms were frequently isolated from tapwater, and were of the same phage type and showed the same weak nitratase activity as clinical strains (60). The increase in the isolation frequency of the *M. avium* complex in Massachusetts, USA, has also been attributed to their presence in drinking-water (61). It should be noted that in all these cases there is only circumstantial evidence of a causal relationship between the occurrence of mycobacteria in drinking-water and human disease. Certainly, the low infectivity of environmental mycobacteria does not warrant the setting of standards or the institution of eradication programmes.

The ecology of opportunistic mycobacteria in water supplies is poorly understood. The bacteria have been isolated infrequently from treated water or mains water (52, 57) but appear to multiply within the plumbing systems in buildings as well as in taps. Increased isolation frequencies have been associated with higher temperatures (hot-water systems or cold-water pipes in the vicinity of central heating). Older buildings appear to be more frequently colonized than new ones (61), and transport of drinking-water over long distances also seems to increase the content of mycobacteria (58). Haas et al. (62) attempted to correlate total microscopic counts of acid-fast bacteria (hence including both pathogenic and saprophytic species) with a range of physicochemical parameters. A negative correlation with total chlorine residual and a positive correlation with turbidity and total organic carbon (TOC) was established, but these variables only accounted for a small proportion of the overall variance of counts. It might also be expected that materials used for plumbing would have an effect on mycobacterial densities, but no experimental evidence of such an effect has yet been presented.

References


3. BACTERIA


5. Lewis WJ, Foster SSD, Dragar BS. The risk of groundwater pollution by on-site sanitation in developing countries: a literature review. Dubendorf, Switzerland, International Centre for Wastes Disposal, 1982 (Report No. 01/82).


4. Viruses

4.1 General description

The viruses of greatest significance in the waterborne transmission of infectious disease are essentially those that multiply in the intestine of humans and are excreted in large numbers in the faeces of infected individuals. Although viruses cannot multiply outside the tissues of infected hosts, some enteric viruses appear to have a considerable ability to survive in the environment and remain infective. Discharges of sewage and human excreta constitute the main source of human enteric viruses in the aquatic environment. With the various analytical methods currently available, wide variations are found in the numbers of viruses present in sewage. These belong to the families shown in Table 4.1. The numbers of viruses and the species distribution will reflect the extent to which they are being carried by the population. Sewage treatment may reduce the numbers of viruses 10–1000-fold, depending on the nature and extent of the treatment given. However, it will not eliminate them entirely, and the sludge produced during sewage treatment will often contain large numbers. As sewage mixes with receiving water, viruses are carried downstream, remaining detectable for varying periods of time, depending on the temperature, the degree to which they are adsorbed on to sediments, the depth to which sunlight penetrates into the water, and other factors. Consequently, enteric viruses can be found in sewage-polluted water at the intakes to water-treatment plants.

The relationship between the occurrence of viruses in water and risks to health is not a simple one; the factors involved are discussed in section 4.3. Table 4.1 lists those viruses, infective for humans, which have been found in sewage-polluted water and the illnesses with which they have been associated.

4.1.1 The nature of viruses

Viruses are replicating infectious agents that are among the smallest of all microorganisms. In essence, they are nucleic acid molecules that can enter cells and replicate in them, and code for proteins capable of forming protective shells around them. The following characteristics are shared by all viruses:

---

1 The valuable contribution made by Dr N.F. Pierce, Division of Diarrhoeal and Acute Respiratory Disease Control, WHO, Geneva, in the preparation of this chapter is gratefully acknowledged.
Table 4.1 Viruses pathogenic to humans which can occur in polluted water and diseases attributed to them

<table>
<thead>
<tr>
<th>Virus family</th>
<th>Members</th>
<th>No. of serotypes</th>
<th>Diseases caused</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picornaviridae</td>
<td>Human polioviruses</td>
<td>3</td>
<td>Paralysis, meningitis, fever</td>
</tr>
<tr>
<td></td>
<td>Human echoviruses</td>
<td>32</td>
<td>Meningitis, respiratory disease, rash, fever, gastroenteritis</td>
</tr>
<tr>
<td></td>
<td>Human coxsackieviruses A1-22,24</td>
<td>23</td>
<td>Enteroviral vesicular pharyngitis, respiratory disease, meningitis, enteroviral vesicular stomatitis with exanthem (hand, foot and mouth disease)</td>
</tr>
<tr>
<td></td>
<td>Human coxsackieviruses B1-6</td>
<td>6</td>
<td>Myocarditis, congenital heart anomalies, rash, fever, meningitis, respiratory disease, meningitis, epidemic myalgia (pleurodynia)</td>
</tr>
<tr>
<td></td>
<td>Human enteroviruses B8-71</td>
<td>4</td>
<td>Meningitis, encephalitis, respiratory disease, rash, acute enteroviral haemorrhagic conjunctivitis, fever</td>
</tr>
<tr>
<td></td>
<td>Hepatitis A virus</td>
<td>1</td>
<td>Hepatitis A</td>
</tr>
<tr>
<td>Reoviridae</td>
<td>Human reoviruses</td>
<td>3</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>Human rotaviruses</td>
<td>5</td>
<td>Gastroenteritis, diarrhoea</td>
</tr>
<tr>
<td>Adenoviridae</td>
<td>Human adenoviruses</td>
<td>41</td>
<td>Respiratory disease, conjunctivitis, gastroenteritis</td>
</tr>
<tr>
<td>Parvoviridae</td>
<td>Adeno-associated viruses</td>
<td>4</td>
<td>Latent infection following integration of DNA into the cellular genome</td>
</tr>
<tr>
<td>Caliciviridae</td>
<td>Human caliciviruses</td>
<td>5</td>
<td>Gastroenteritis in infants and young children</td>
</tr>
<tr>
<td></td>
<td>Small round structured viruses (including Norwalk virus)</td>
<td>14</td>
<td>Gastroenteritis, acute viral gastroenteropathy (Winter vomiting disease)</td>
</tr>
<tr>
<td>Caliciviridae (?)</td>
<td>Hepatitis E virus</td>
<td>?</td>
<td>Hepatitis E</td>
</tr>
<tr>
<td>Unknown</td>
<td>Astroviruses</td>
<td>1</td>
<td>Gastroenteritis, neonatal necrotizing enterocolitis</td>
</tr>
<tr>
<td>Papovaviridae</td>
<td>Papillomaviruses</td>
<td>2</td>
<td>Plantar warts</td>
</tr>
</tbody>
</table>
1. The virus particle or virion consists of a genome, either RNA or DNA, that is surrounded by a protective protein shell called the capsid. This shell is itself often enclosed within an envelope that contains both protein and lipid.

2. Viruses replicate only inside specific host cells. They are totally dependent on the host cell's synthetic apparatus and energy sources, and are thus parasites at the genetic level.

4.1.2 Classification of animal viruses

The present universal system for virus taxonomy is set arbitrarily at the hierarchical levels of family, genus, and species by the International Committee on Taxonomy of Viruses (1). The fundamental criteria used for classification purposes are the type and strandedness of the nucleic acid of the viral genome and the presence or absence of a lipoprotein envelope.

Virus families, designated by terms ending in -viridae, represent clusters of genera of apparently common evolutionary origin. Virus genera are designated by terms ending in -virus and are based on common evolutionary origin and biophysical or serological properties (see Table 4.1). Virus species have not been designated formally except for the family Adenoviridae, where the term is now defined on the basis of immunological distinctiveness.

4.1.3 Virus families occurring in water

Picornaviruses are 27-28 nm particles consisting of positive-sense single-stranded RNA enclosed in a protein coat of icosahedral symmetry, which are stable at pH 3; one member of this family, hepatitis A virus, is particularly stable, e.g. it can survive for some hours at pH 1. They resist inactivation by various environmental factors for a number of weeks, particularly when associated with sediments in natural waters. The genus Enterovirus, which is one of the three genera of the Picornaviridae family pathogenic to humans, contains six major groups: human polioviruses, human echoviruses, human coxsackievirus groups A and B, the new enterovirus serotypes 68-71, and, as mentioned above, hepatitis A virus.

The family Reoviridae contains six genera, two of which—human reoviruses (orthoreoviruses) and human rotaviruses—have been detected in polluted water. The virus particles are approximately 70 nm in diameter and have both an inner capsid 50-65 nm in size, of icosahedral symmetry, enclosing a double-stranded, segmented genome, and an outer one, in which striking differences are apparent in the different genera. The orthoreoviruses have a well-defined outer capsid, composed of hexagonal and pentagonal subunits. The rotavirus outer capsid lacks visible subunit structures. Both genera lose infectivity relatively slowly even at ambient temperatures and are stable over a wide range of pH values.

The family Adenoviridae contains two genera. The mammalian adenoviruses include 41 human species, subdivided on the basis of their biophysical, biochem-
4. VIRUSES

The virion is a non-enveloped regular icosahedron (20 triangular surfaces and 12 vertices), which is 65–80 nm in diameter. A fibre-like structure projects from each of the vertices. The genome is a single linear molecule of double-stranded DNA.

Parvoviridae are among the smallest of the DNA animal viruses. The virion is 18–26 nm in diameter, is of icosahedral symmetry and has a single-stranded DNA genome. The family Parvoviridae contains three genera, for two of which, Parvovirus and Dependovirus, waterborne transmission is a possibility. The virion is extremely resistant to inactivation; it is stable between pH 3 and 9, and at 56 °C for 60 minutes. The genus Dependovirus (adeno-associated virus, AAV), has a relatively wide host range; infection is common in the general population. AAV only infects human cells cryptically; no overt disease has been observed.

The so-called “small round structured viruses”, which include Norwalk virus, contain RNA and a single capsid polypeptide typical of caliciviruses; they are therefore currently included in the Caliciviridae family (see also p. 47).

Hepatitis E virus is an important cause of acute hepatitis in tropical and subtropical countries. Classification of this virus is difficult, but many have placed it among the Caliciviridae (2).

The Papovaviridae consist of several genera, among them the papilloma viruses. Papovaviridae are non-enveloped, icosahedral particles, 45–55 nm in diameter, which contain one molecule of double-stranded DNA. They are highly resistant to inactivating environmental factors. Natural transmission is presumed to be through contact, and the diseases that they cause have been associated with swimming-pools.

4.2 Routes of exposure

4.2.1 General considerations

Acute gastrointestinal and diarrhoeal illnesses continue to be the major waterborne diseases throughout the world. Rapid methodological advances have recently been made in the study of their etiology that have revolutionized the diagnosis of viral diarrhoeal diseases. Waterborne outbreaks due to viruses have now been recorded from developed and developing countries all over the world (2–5). Many different strains of viruses have been isolated from raw and treated drinking-water (6). Isolation from water does not prove beyond all possible doubt that water is a vehicle for the transmission of disease, although it does indicate that a hazard exists. Proper treatment and disinfection should result in drinking-water that is essentially virus-free. Epidemiological proof of the waterborne transmission of viral diseases is very difficult to obtain for a variety of reasons (7), including the following:

- the symptoms may not resemble those of typical waterborne diseases;
- asymptomatic carriage and excretion occur in a large proportion of those infected;
some infections have long incubation periods, e.g. hepatitis caused by hepatitis A virus,
waterborne transmission may be at a low level, and secondary spread may occur by other routes;
suitably sensitive methods for detecting the infectious agent in water may be lacking.

Waterborne transmission has been unequivocally demonstrated for hepatitis A and hepatitis E viruses, rotaviruses and Norwalk virus, and the explosive epidemics that they cause have been well documented. For the other viruses included in Table 4.1, waterborne transmission is a probability but has not been definitely established.

Low-level transmission may occur in which small numbers of viruses present in drinking-water, either sporadically or continuously, produce asymptomatic infections that remain unrecognized. The person-to-person spread of such infections in the community could lead to disease outbreaks apparently unconnected with water. However, the existence of such a mechanism has not been confirmed.

In a prospective epidemiological study among city dwellers receiving bacteriologically satisfactory drinking-water, it was found that the group receiving water not treated by reverse osmosis at the point of use had 25% more gastrointestinal symptoms than those receiving water treated by this process (8). The symptoms observed were compatible with infection caused by the Norwalk virus or astroviruses, which were probably incompletely removed from the sewage-contaminated river water used as the source.

In some areas, water sources may be heavily polluted, and the water-treatment processes used may not be reliable. For this reason, and because of the large number of persons at risk, drinking-water must be regarded as having a very significant potential as a vehicle for the environmental transmission of enteric viruses. As with other microbial infections, enteric viruses may also be transmitted by contaminated food and aerosols, as well as by direct contact, the usual mode of transmission.

Schemes for the recycling of wastewater for domestic use are being considered in some cities, while in many others, water for potable supplies is obtained from contaminated surface sources containing a significant proportion of wastewater. The risk of viruses penetrating the water-treatment processes—including pretreatment storage and disinfection—must be carefully evaluated whenever wastewater is to be reused in this way.

4.2.2 Specific families of viruses

Enteroviruses have a worldwide distribution, their prevalence increasing during the warm months of the year in temperate climates. The epidemiology of these infections suggests that faecal-to-oral transmission is the major means of spread and that various types of enterovirus can give rise to large outbreaks when they are transmitted by the water route.
Rotaviruses and orthoreoviruses have been detected in sewage, rivers, and lakes and in treated drinking-water in some countries (9–12). Transmission occurs via the faecal-to-oral route. The infection is usually associated with sporadic cases, but several large waterborne outbreaks have been well documented (13, 14). The rotaviruses are of considerable public health importance as a common cause of acute diarrhoea, particularly in young children. They infect and multiply in mature or differentiated enterocytes located on the villi of the duodenum and small intestine, and are excreted in large numbers; as many as 1000 virus particles may be present per gram of faeces for approximately 8 days after the onset of symptoms.

Adenoviruses generally infect conjunctival, respiratory, and intestinal epithelium in addition to regional lymphoid tissue. Prolonged excretion of viruses both from the pharynx and from the intestinal tract has been described. Several species, particularly subgroups B, C, D and E, and serotypes 1, 2, 3, 4, 5, 6, 7 and 15, have been isolated from sewage, rivers, lakes, groundwater, and water used for drinking and swimming. Waterborne transmission occurs by the faecal-to-oral route, by inhalation of adenovirus aerosols into the lower respiratory tract, and by eye contact when the conjunctival surface is mildly irritated. Several large outbreaks of pharyngoconjunctival fever have been associated with swimming-pools (15, 16).

The use of electron microscopy for the examination of faecal specimens from persons with nonbacterial gastroenteritis resulted in many observations of small viruses ranging in size from 20 to 40 nm, the “small round structured viruses” already mentioned on p. 45. The first of these viruses to be described was the Norwalk agent which was detected in volunteers fed filtered faecal suspension obtained from patients in an outbreak of winter vomiting disease. Morphologically similar viruses known as the Hawaii, Wollan, Ditching, Parramatta, Snow Mountain and Montgomery County agents were subsequently found. Failure to culture any of these agents satisfactorily delayed definitive classification but, as previously noted, they are now assigned to the Caliciviridae family.

Norwalk virus infects the villi of the jejunum. Virus shedding in stools occurs during the first 72 hours after the onset of illness. The virus is transmitted by the faecal-to-oral route. Of all Norwalk-related outbreaks, water seems to be responsible for about 40%, the type of water involved including drinking-water supplies, recreational bathing water, and shellfish-harvesting water (17).

4.3 Health effects

Enteric viruses are capable of producing a wide variety of syndromes, including rashes, fever, gastroenteritis, myocarditis, meningitis, respiratory disease, and hepatitis (Table 4.1). In general, asymptomatic infections are common and the more serious manifestations rare. However, when drinking-water is contaminated with sewage, gastroenteritis and hepatitis may occur in epidemic proportions. Apart from these infections, there is little, if any, epidemiological evidence to

47
show that adequately treated drinking-water is involved in the transmission of virus infections.

Gastroenteritis of viral origin may be associated with a variety of agents (Table 4.1). It is usually of 24–72 hours’ duration with nausea, vomiting and diarrhoea; it occurs in susceptible individuals of all ages, but is most serious in the very young and very old, where dehydration and electrolyte imbalance can occur rapidly and threaten life if not corrected without delay.

Dependoviruses (adeno-associated virus), together with adenoviruses, have been recovered from surface water (18); it is therefore suspected that waterborne transmission of these viruses can occur.

Hepatitis A virus (human enterovirus 72) and enterically transmitted hepatitis E virus cause infections of the liver typically accompanied by lassitude, anorexia, weakness, nausea, vomiting, headache, abdominal discomfort, fever, dark urine, and jaundice. Hepatitis, if mild, may require only rest and restricted activities for a week or two, but when severe may cause death from liver failure, or may result in chronic disease of the liver. Severe hepatitis is tolerated less well with increasing age, and the fatality rate increases sharply beyond middle age. The mortality rate is higher among those with pre-existing malignancy and cirrhosis (19). A fulminant form leading to death within days occurs in 0.1–0.6% of cases. Hepatitis E infection in pregnant women has a high mortality rate. Local epidemics are usually traceable to contaminated food or water. The virus has been detected in polluted rivers (20) and in drinking-water (21). Several very large outbreaks of drinking-water-transmitted hepatitis have occurred in India (2), China (Mendong, personal communication), Algeria (22) and the former Soviet Union (23).

Adenoviruses are among the viral agents associated with acute nonbacterial infectious gastroenteritis. Of the various species, two (types 40 and 41) cannot routinely replicate in cell cultures and are called fastidious variants. Such fastidious adenoviruses have been found in many parts of the world and are probably second only to rotaviruses as a cause of gastroenteritis in young children. They tend to be endemic rather than epidemic although outbreaks have occurred. Cytotoxic adenoviruses can easily be detected in all kinds of water, so that waterborne transmission of the fastidious variants has also been suspected (6).

Rotaviruses are responsible for a large proportion of severe episodes of diarrhoea in small children and infants, and may also cause gastroenteritis in the elderly (24). They are responsible for as much as 50% of the gastroenteritis in infants and children admitted to hospital during the cooler months of the year in temperate climates. Rotaviruses have occasionally been isolated from drinking-water in some countries, but more often from sewage (9, 25). Acute infection is characterized by the abrupt onset of severe watery diarrhoea with fever and vomiting. Dehydration and metabolic acidosis may develop, resulting in death if untreated. Those most severely infected and affected are between 6 and 24 months old.
The Norwalk virus usually causes self-limiting explosive epidemics of gastroenteritis that last for 24–48 hours, are community-wide, and involve school-age children, family contacts, and adults. Roughly one-third of such outbreaks of gastroenteritis can be attributed to the Norwalk virus. Infections result in delayed gastric emptying, nausea, vomiting, and abdominal cramps. About 50% of infected persons have associated diarrhoea; some have fever and chills. A transient lymphopenia has been observed. Norwalk and Norwalk-like viruses (small round structured viruses) primarily infect and cause disease in older children and adults, and have been responsible for a large number of outbreaks of acute infectious nonbacterial gastroenteritis. Infection may be spread by municipal water systems, semi-public water supplies, recreational swimming, and stored water (4, 26, 27) although other modes of transmission, including person-to-person spread, are usually more important.

References


4. VIRUSES


5. Protozoa

Drinking-water plays a major role in the spread of three of the intestinal protozoa pathogenic for humans, namely *Giardia intestinalis* (syn. *G. lamblia*, the etiological agent of human giardiasis), *Cryptosporidium parvum* (human cryptosporidiosis), and *Entamoeba histolytica* (amoebic dysentery). *Balantidium coli* infection (balantidiasis) is uncommon, although the parasite has a worldwide distribution. These pathogenic intestinal protozoa can be transmitted to humans by any mechanism whereby material contaminated with faeces containing viable organisms from infected individuals can reach the mouth. However, infections with pathogenic *Naegleria fowleri* (naegleriasis or primary amoebic meningoencephalitis) and *Acanthamoeba* spp. (meningitis, keratitis) are associated primarily with recreation and the inhalation of warm soil-contaminated water, and are comparatively rare.

5.1 Giardia

5.1.1 General description

**Life cycle**

Organisms in the genus *Giardia* (also called *Lamblia*) are flagellated protozoa that parasitize the intestines of humans and animals. These flagellates have a simple two-stage life cycle consisting of the reproductive trophozoite stage and the environmentally resistant cyst stage. When ingested by a susceptible host, the cysts are induced to excyst by exposure to acid in the stomach and perhaps also by contact with enzymes or other as yet undefined digestants (1). After excysting, the trophozoite leaves the cyst wall behind and rapidly undergoes cytokinesis, splitting by binary fission into two daughter trophozoites (2) which are bilaterally symmetrical and vary in shape from ellipsoidal to pyriform (3). The anterior end is rounded and contains two nuclei, while the posterior end tends to be pointed. The dorsal side is convex, and the ventral side contains an adhesive or sucking disc by which the organism attaches itself to intestinal surfaces. Each trophozoite has two slender median rods or axostyles, four pairs of flagella, and a pair of median bodies. The trophozoites may be 9–21 μm long, 5–15 μm wide, and 2–4 μm thick.
Perhaps in response to population pressures, the trophozoites release their hold on the intestinal epithelium and enter the lumen. As they travel down the intestines, they are apparently induced to encyst by exposure to bile, alkaline pH, and possibly bacterial metabolites. The cysts are ovoid, 8–12 μm long by 7–10 μm wide, and contain the same structures (nuclei, axostyles, median bodies) as the trophozoites; however, up to four nuclei may be visible within each cyst. The cysts are discharged with the faeces and thereby returned to the environment.

The length of time that cysts can survive depends on the temperature. G. intestinalis cysts have survived for at least 77 days and G. muris cysts for at least 84 days when suspended in water at less than 10 °C. Above 20 °C, cyst inactivation is relatively rapid. Sharp decreases in cyst viability have been noted after 3 days' storage in water at 20 °C or after only 1 day at 37 °C. The thermal death point for G. muris cysts has been reported to be 54 °C, and G. intestinalis have been inactivated by exposure to 55 °C for 5 minutes. Cysts may be inactivated in water by bringing the temperature to boiling point.

Host range

Giardia organisms are widely distributed in nature and have been reported as occurring in more than 40 species of animals including amphibians, birds, and mammals. However, whether or not giardiasis is, or can be, a zoonosis is debatable. Some investigators have reported infecting a variety of animals—including dog, beaver, muskrat, gerbil, and rat—with cysts from human sources, but others have been unable to infect mice, hamsters, rats, cats, and dogs with such cysts. However, all of them have been able to infect some species of animals with cysts derived from different ones. While there are anecdotal reports to suggest that humans may become infected with cysts from deer, beavers, and muskrats, no controlled studies on human volunteers inoculated with organisms from animal sources have yet been reported. It appears that some species of Giardia may be host-specific while others may not be. In addition, since at least some animals that inhabit watersheds can become infected with cysts from humans, they may act as intermediaries for human Giardia infection rather than as primary reservoirs. Methods are needed capable of differentiating between the cysts causing human infections and those found in environmental samples. Until such methods are developed, it would seem prudent, as has been suggested, to assume that humans may be susceptible to many of the Giardia infecting lower animals.

The North American literature strongly supports the concept that animal vectors have been the source of the contamination of watersheds and of waters all but inaccessible to humans.
5.1.2 Routes of exposure

As with other pathogenic intestinal protozoa, *Giardia* can be transmitted by any mechanism whereby material contaminated with faeces containing viable organisms from infected individuals can reach the mouth. Documented routes of exposure include drinking-water, recreational water, food, and person-to-person contact.

**Water**

Epidemic giardiasis associated with contaminated drinking-water has been reported in the United States of America (16), Canada (17), England (18), Scotland (19), and Sweden (20). Drinking-water has also been implicated as the vehicle of transmission in outbreaks occurring among travellers in the former Soviet Union (21). The USA has experienced a great number of reported waterborne outbreaks, over 25 occurring between 1986 and 1988 (22). In some of the outbreaks, water supplies had been contaminated with human sewage; in others, faecal discharges from watershed animals were the suspected sources of the contamination. Surveys of such animals have shown very high *Giardia* prevalence in aquatic voles (23) and muskrats (24). Most of the outbreaks in the USA have been attributed to contaminated surface water treated only by disinfection (16). *Giardia* cysts can be inactivated by disinfection, but are among the most resistant waterborne pathogens; effective disinfection calls for consideration of the water pH, turbidity, and temperature, as well as controlling the disinfectant dose and contact time (25). The wide distribution of *Giardia* in humans and animals, the uncertainty concerning cross-species infectivity, the resistance of the cysts to inactivation by disinfection, and experience with the outbreaks led the USA to develop regulations on the disinfection of all surface water supplies in the country (26). Risk analysis, using a probabilistic model, suggests that an annual risk of infection of less than one per 10 000 population can be achieved for source waters with 0.7–70 cysts per 100 litres, when treatment to achieve a $10^5$-fold reduction is applied (27).

Endemic giardiasis has also been associated with the consumption of contaminated drinking-water in such diverse locations as the USA (16) and South Africa (28). In addition to endemic and epidemic giardiasis from drinking-water supplies, there have been reported outbreaks in the USA (29) and in Canada (30), affecting children and adults, caused by the ingestion of swimming-pool water. The source of contamination in these outbreaks was apparently related to defecation in the water by infected children.

**Relative significance of routes of exposure**

Quantifying the degree of significance of the various routes of transmission of giardiasis is difficult because of a lack of information on the total prevalence or incidence of infection or disease. Bennett and co-workers (31), using published
Material and survey data from the National Center for Health Statistics, estimated that 60% of the cases of giardiasis occurring in the USA were waterborne. Kappus & Juranek (32) suggested that 45–50% of giardiasis cases in the USA were associated with drinking unfiltered municipal water. They also suggested that 40–45% of cases were associated directly or indirectly with person-to-person transmission at day-care centres, and that the remaining cases (about 10%) involved exposure while travelling, engaging in sexual practices that involve faecal exposure, or ingesting untreated surface water while hiking or camping. The contribution of waterborne as opposed to person-to-person transmission may be expected to vary from country to country depending on a number of factors including the extent of water treatment, the sanitation facilities, and local customs. However, apart from Cryptosporidium (see p. 56), Giardia probably has the greatest potential for transmission through drinking-water of all the waterborne parasitic protozoa since:

- cysts from humans are infective for a wide variety of domestic and wild animals and are widely distributed in the environment;
- some waterborne outbreaks have been attributed to the contamination of drinking-water by cysts of nonhuman origin;
- the cysts are highly resistant to disinfection.

### 5.1.3 Health effects

Although the pathogenicity of the organisms was for long controversial, it is now widely accepted that *Giardia* can cause disease, and Koch's postulates have been satisfied by experimental human infections (33). Much of the controversy apparently arose from the highly variable illness-to-infection ratio observed. Asymptomatic infections with *Giardia* have been reported to account for up to 76% of the total under epidemic conditions (34). The time between ingestion of the organism and the appearance of the parasite in the stool is about 9–14 days, while the incubation period may range from 1 to 75 days with a median value of 8–15 days (35). Symptomatic infections may be acute, subacute, or chronic, and the condition may last for months if not diagnosed and treated. Symptoms that have been commonly reported include diarrhoea, flatulence, foul-smelling stools, cramps, distension, fatigue, anorexia, nausea, weight loss, and vomiting. Intolerance to lactose may develop during the infection and persist even after the organism has been eradicated (35). Infection in children may interfere with growth and normal development (36), but mortality has rarely been reported in patients of any age.

The pathophysiological mechanisms in giardiasis remain to be clarified. As with the clinical effects, histopathological changes in the intestinal mucosa can cover a wide spectrum ranging from minimal to significant enteropathy with enterocyte damage, villus atrophy, and crypt hyperplasia (37).

No explanation can be given for the broad range of clinical and pathological effects observed but both parasite and host factors are probably involved. Strain variation in pathogenicity has been demonstrated in humans (33), while strain
and host variations have been observed in animals (38). In addition to local effects that can be produced directly by the parasites, their metabolic activity (39), and secretion products, host factors that could contribute to the degree of tissue damage include nutritional status, systemic immune responses, and mucosal immunity (37, 40). *Giardia* isolated from humans and animals have been found to be associated with bacteria, virus-like particles and mycoplasma-like organisms (41). It has been suggested that these apparent symbionts may be transmitted via *Giardia* cysts. In addition, a double-stranded RNA virus has been found in *Giardia* (42). Some isolates of *G. intestinalis* are susceptible to infection with this virus while others are not (43). What effect, if any, these associated organisms might have on the virulence of *Giardia* or on the pathogenesis of the disease is not known.

5.2 *Cryptosporidium* spp.

5.2.1 General description

*Cryptosporidium* spp. are intracellular coccidian parasites of the gastrointestinal and respiratory tracts of numerous animals, including mammals, birds, and fish, and have a worldwide distribution. At present, six species are known, namely *C. parvum* and *C. muris*, which infect mammals, *C. baileyi* and *C. meleagris*, which infect birds, and *C. serpentis* and *C. nasorum*, which infect reptiles and fish, respectively. *C. parvum* is the major species responsible for clinical disease in humans and domestic animals (44). As with both *E. histolytica* and *G. intestinalis*, infection occurs by ingestion of the transmissive phase which, for *Cryptosporidium* spp., is the oocyst. Person-to-person transmission occurs (45), and oocysts from humans are infective for numerous mammals, including cattle and sheep (46), while both domestic and feral animals may be reservoirs of human infection (47). Infected humans can excrete $10^9$ oocysts a day, and calves and lambs can excrete up to $10^{10}$ oocysts daily for up to 14 days (48). The average density of oocysts in raw sewage has been estimated at 5000 per litre (49). The broad host range together with the high output of oocysts ensures a high level of contamination in the environment. *Cryptosporidium* is an obligate parasite that develops only within a living host cell; unlike the other protozoa transmitted by drinking-water, but in common with other coccidia, it has several characteristic developmental stages (44). Infection is initiated following ingestion of the oocyst, which contains four naked, motile sporozoites. These are released through the suture in the oocyst wall following exposure to trypsin and bile salts, and attach themselves intimately to the surface of adjacent epithelial cells. They develop within a parasitophagous vacuole which is intracellular but extracytoplasmic, initially as a fixed trophozoite, then through asexual and sexual stages to finally become oocysts.

*Cryptosporidium* completes its life cycle within a single host; however, unlike *E. histolytica* and *G. lamblia*, endogenous reinfection (autoinfection) occurs
which, together with recycling of the asexual stage, allows parasite numbers to build up to a high level. In addition, external maturation of oocysts is not required, and the thin-walled oocysts, which account for up to 20% of the total, excyst during passage through the intestine, releasing sporozoites which further increase the infection. The majority of the oocysts become detached and sporulate during passage through the gut to become thick-walled oocysts which are infective when excreted. *C. parvum* oocysts are spherical; their modal size is $4.5 \times 5.0 \mu m$ (range 4–6 μm).

In various surveys conducted throughout the world, *Cryptosporidium* infection in immunocompetent persons has been found in 26 countries, with a reported prevalence of 0.6-20% in developed countries and 4-20% in developing ones. The infection is more common in children than in adults (50). Among AIDS patients, cryptosporidiosis has a prevalence of 3-4% in the USA and over 50% in some African countries and Haiti. An asymptomatic carrier state exists, but the ratio of cases to carriers has not been determined. At present, no effective drug is available for the treatment of cryptosporidiosis.

### 5.2.2 Routes of exposure

As with other pathogenic intestinal protozoa, *Cryptosporidium* can be transmitted by any mechanism whereby material contaminated with faeces containing viable organisms from infected humans or animals can reach the mouth.

**Drinking-water**

Humans and other mammals are reservoirs for infection, and the contamination of water supplies with either human or animal sewage can lead to the transmission of *Cryptosporidium* through drinking-water. Outbreaks have been traced to the contamination of drinking-water by both human and animal wastewaters (51–54). Oocysts can survive several months in water at 4 °C and are among the most chlorine-resistant pathogens known (55). Waterborne outbreaks of cryptosporidiosis have been reported from both the USA and the United Kingdom and, in most of the recently documented outbreaks, oocysts have been identified in drinking-water. Outbreaks have been associated with untreated drinking-water, water treated by chlorination only, and water subjected to conventional treatment (coagulation, sedimentation, sand filtration and chlorination). Because oocysts are only 4–6 μm in size, the extent to which those present in raw water are removed by various water-treatment processes is still unclear. As with other intestinal protozoa pathogenic to humans, the infective dose is thought to be small. When two primates were given a dose of 10 oocysts, disease was produced in both (56). Information both on oocyst survival in the environment and on resistance to disinfection is incomplete at present; however, oocysts lose their infectivity at temperatures below 0 °C or when kept at above 45 °C for 5–20 minutes (55–57).
Apart from *Giardia* (see p. 52), *Cryptosporidium* probably has the greatest potential for transmission through drinking-water of all the waterborne parasitic protozoa since:

- oocysts from humans are infective for a wide variety of domestic and wild animals, and are widely distributed in the environment;
- some waterborne outbreaks have been attributed to the contamination of drinking-water by oocysts of nonhuman origin;
- among the protozoa under consideration, *Cryptosporidium* spp. have the smallest and most chlorine-resistant oocysts.

**Other routes of exposure**

Swimming-pools have been incriminated in the transmission of cryptosporidiosis (54), but the evidence for the outdoor recreational water route for the transmission of infection is circumstantial (52, 53). However, as oocysts can be detected in recreational waters, and such waters are being increasingly used for immersion sports, it is likely that the importance of this route of infection will increase in the future.

Since both animals and humans are reservoirs of infection and both *E. histolytica* and *G. intestinalis* can be transmitted by food, it seems likely that this may also be true for *Cryptosporidium* spp.

The transmission of *Cryptosporidium* infection between children and adults appears to be rare where good personal hygiene is practised. However, the transmission of infection among preschool children in day-care centres (46, 58) and similar institutions is probably common.

### 5.2.3 Health effects

**Immunocompetent patients**

While infection may be asymptomatic, it is usually associated with diarrhoea (80–90% of cases). Gastrointestinal symptoms, which may be accompanied by an influenza-like illness (20–40% of cases), include vomiting, anorexia, and flatulence. Symptoms typically last 7–14 days, and prolonged excretion of oocysts is unusual.

**Immunocompromised patients**

In patients with AIDS, other acquired abnormalities of T-lymphocytes, congenital hypogammaglobulinaemia, severe combined immunodeficiency syndrome, those receiving immunosuppressive drugs, and those with severe malnutrition, a severe cholera-like illness is produced, resulting in intractable nausea, weight loss, and severe dehydration (as much as 20 litres of liquid stool may be lost per day).

Except in those patients in whom the suppression of the immune system can be relieved by stopping immunosuppressant drugs, symptoms persist unabated until the patient dies (59).
5.3 Entamoeba histolytica

5.3.1 General description

*E. histolytica* is distributed worldwide and exists in trophozoite and cyst stages. Infection occurs by ingestion of cysts; these range in size from 10 to 20 μm (average 12 μm). Since *E. histolytica* is primarily a parasite of primates, humans are the reservoir of infection. Dysenteric individuals pass only trophozoites, which are adversely affected by environmental factors such as drying and changes in temperature and salt concentration, while most or all of the parasites in this active amoeboid stage are destroyed by gastric juice (60). Consequently, chronic cases and carriers who excrete cysts are more important sources of infection. Various surveys throughout the world have indicated a prevalence of 10–45% for *E. histolytica* infections and carriers can discharge up to $1.5 \times 10^7$ cysts daily (61).

5.3.2 Routes of exposure

Since humans are the primary reservoir for infection with *E. histolytica*, the contamination of water supplies with domestic sewage can lead to the transmission of this organism through drinking-water. Outbreaks have been traced to sewage contamination of drinking-water (61). The potential for waterborne transmission may be greater in the tropics, where the carrier rate sometimes exceeds 50%, as compared with more temperate regions where the prevalence in the general population is generally less than 10%. The cysts can survive for several months in water at 0 °C, 3 days at 30 °C, 30 minutes at 45 °C, and 5 minutes at 50 °C (55), and are extremely resistant to chlorination (62).

*E. histolytica* may also be transmitted by food, including raw vegetables, and food handlers may be important in transmission (61). Although swimming-pools have not been definitely incriminated, they are a potential source.

Of the intestinal protozoan pathogens, *E. histolytica* is the most prevalent worldwide. Person-to-person spread and contamination of food by infected food handlers appear to be the most significant means of transmission, although contaminated drinking-water also plays a role.

5.3.3 Health effects

Though most infections with *E. histolytica* are asymptomatic or cause only minor symptoms, deaths can occur. The usual clinical manifestations are gastroenteritis with symptoms ranging from mild diarrhoea to fulminating bloody dysentery. Liver abscess is the most common metastatic complication. Pathogenicity appears to depend both on strain virulence and on host factors, including the nutritional status of the individual and the associated bacterial flora (61).
5.4 *Balantidium coli*

5.4.1 General description

*Balantidium coli* is a ciliated organism of worldwide distribution; both the trophozoite and cyst stages can be infective for humans. The spherical to ovoid cysts are 40–60 μm in diameter, yellowish to greenish in colour, and have a twomembrane wall. Human infections usually occur as a result of the ingestion of food or water contaminated with faecal material from infected swine. Other hosts include lesser primates and, rarely, dogs and rats. *B. coli* is very common in swine but is considerably less prevalent in humans. Asymptomatic carrier infections can occur in humans and the world incidence is estimated at less than 0.7% (60).

5.4.2 Route of exposure

The only reported waterborne outbreak of balantidiasis occurred in the Truk District of Micronesia in 1971. It was concluded that the epidemic probably resulted from the contamination of water supplies by pig faeces when a devastating typhoon destroyed pig pens and precarious water-catchment facilities (63).

5.4.3 Health effects

The incidence of balantidiasis in humans is low, and direct contact with pigs appears to be the main route of transmission of the causative organism. The potential exists for the transmission of the organism in food and water contaminated with pig faeces.

Balantidiasis can present as an acute bloody dysentery, but an asymptomatic carrier state also occurs in humans (60).

5.5 *Naegleria* and *Acanthamoeba*

5.5.1 General description

Free-living amoebae cause severe human disease of waterborne origin. *Naegleria fowleri* is the etiological agent of primary amoebic meningoencephalitis (64). Although another species of *Naegleria, N. australiensis*, is known to produce fatal brain infection in experimental animals, no human cases due to this species have been reported (65). Various species of the genus *Acanthamoeba* cause keratitis, skin and pulmonary infections, and granulomatous amoebic meningitis (66). Infections by *Hartmannella* reported in the older literature were all due to *Acanthamoeba*. Infections with *N. fowleri* are almost always associated with recreational contact rather than with the drinking of water. *Acanthamoeba* eye infections are mostly related to inadequate cleaning or disinfection of contact lenses.
Naegleria spp. exist in three forms, namely as a trophozoite, a flagellate, and a cyst stage (67). The trophozoites (10–20 μm) move by eruptive pseudopod formation. They have a single nucleus with a central nucleolus, although binucleated and multinucleated forms do occur. A sexual stage is unknown, and reproduction is by simple binary fission. The trophozoite can transform into a flagellate stage with two anterior flagella. The flagellate does not divide but reverts to the trophozoite stage. Under adverse conditions, the trophozoite transforms into a circular cyst, 7–15 μm in diameter. Although the cyst is quite resistant to chlorination, prolonged contact does kill it.

Acanthamoeba spp. have two forms (67). The trophozoites (10–30 μm) are characterized by needle-like projections called filopodia or acanthopodia. Like Naegleria, they usually have a single nucleus with a central nucleolus, and reproduce by binary fission. In most species, the cyst stage (14–25 μm) is typically polygonal or starlike and has two easily distinguished cell walls. In some species, including the most virulent ones, the cyst is more or less rounded, and the two cell walls are difficult to discern. Cysts of Acanthamoeba are extremely resistant to chlorination.

Pathogenic species can be differentiated from nonpathogenic ones by prescreening on cell lines and then by intranasal instillation of the cultured amoebae into mice. Different species of pathogenic Naegleria and Acanthamoeba can be identified by antigen, isoenzyme and/or DNA studies. Naegleria fowleri is typically thermophilic, growing in water at temperatures up to 45 °C. Pathogenic Acanthamoeba rarely thrive at such high temperatures.

5.5.2 Routes of exposure

Because of its thermophilic nature, N. fowleri is distributed worldwide in surface waters that are naturally heated by the sun or in industrial cooling waters and geothermal springs (68). Most infections are reported in industrialized countries. In Australia, many fatal cases occurred through the use of unfiltered, chlorinated water for washing and bathing (69). Cases in developing countries are most probably under-reported.

Some Acanthamoeba infections are related to water, but most, except for keratitis, occur in debilitated persons. Keratitis can occur following a minor trauma to the eye and subsequent washing, or as a result of wearing contact lenses. In particular, inadequate cleaning and disinfection of contact lenses favour the occurrence of Acanthamoeba keratitis. Contact lens cases appear to be breeding places for this organism.

Acanthamoeba can be found in all environments, and particularly frequently in chlorinated swimming-pools and drinking-water. Although airborne transmission of free-living amoebae does occur, the evidence for infection by this route is controversial.
5.5.3 Health effects

*Naegleria fowleri* causes fatal meningoencephalitis particularly in young and healthy individuals after swimming or activities causing infected water to be inhaled. The amoeba enters the brain by penetrating the olfactory mucosa and cribriform plate (70). The infection is very severe, and patients often die (5–10 days after penetration) before the infectious agent can be diagnosed. In addition, treatment is difficult, as only amphotericin B appears to be effective. Administration of other antibiotics together with amphotericin B might increase success rates. Although the infection remains rare (about 100 cases had been described up to 1980), new cases are encountered every year.

*Acanthamoeba* can cause diseases ranging from meningitis to pulmonary and wound infections, but few cases have been reported. However, the number of cases of keratitis increased considerably in the 1980s. While only 20 cases of keratitis were reported up to 1984, the number of cases in the USA had increased to over 200 by 1989 (71). Very few treatments are effective against *Acanthamoeba* infections, although keratitis cases can now be treated effectively; corneal transplants were usually necessary in the past.

*Legionella* bacteria can grow inside the cells of *Naegleria, Acanthamoeba* (72) and other free-living amoebae, and are protected against disinfection when inside the cysts of these amoebae. This is discussed further on p. 29.

**References**


64. Carter R. Description of a *Naegleria* sp. isolated from two cases of primary amoebic meningo-encephalitis, and of the experimental pathological changes induced by it. *Journal of pathology*, 1970, 100:217-244.


6. Helminths

The helminths or parasitic worms comprise two unrelated groups of organisms, namely flatworms belonging to the phylum Platyhelmintha, and roundworms belonging to the phylum Nematoda. Apart from the guinea worm, *Dracunculus medinensis*, which is transmitted solely by drinking-water, it is rare for any of those listed in Table 6.1 to be so transmitted. On the other hand, the continued use of poor-quality borehole or piped water is a major factor in the risk of acquiring the other helminth infections.

**Table 6.1 Helminths potentially transmitted by drinking-water**

<table>
<thead>
<tr>
<th>Zoological classification</th>
<th>Species</th>
<th>Infective stage and usual mode of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum Nematoda (roundworms)</td>
<td><em>Dracunculus medinensis</em></td>
<td>Larvae in cyclops ingested in water</td>
</tr>
<tr>
<td></td>
<td><em>Ascaris lumbricoides</em></td>
<td>Eggs ingested from soil</td>
</tr>
<tr>
<td></td>
<td><em>Toxocara canis</em></td>
<td>Eggs ingested from soil</td>
</tr>
<tr>
<td></td>
<td><em>Trichuris trichiura</em></td>
<td>Penetrative larvae in soil</td>
</tr>
<tr>
<td></td>
<td><em>Necator americanus</em></td>
<td>Penetrative larvae in soil</td>
</tr>
<tr>
<td></td>
<td><em>Ancylostoma duodenale</em></td>
<td>Penetrative larvae in water</td>
</tr>
<tr>
<td></td>
<td><em>Strongyloides stercoralis</em></td>
<td>Penetrative larvae in water</td>
</tr>
<tr>
<td>Phylum Platyhelmintha, class Trematoda (flukes)</td>
<td><em>Schistosoma spp</em></td>
<td>Free-swimming cercarial larvae penetrate skin</td>
</tr>
<tr>
<td></td>
<td><em>Fasciola spp</em></td>
<td>Cercarial larvae encysted and ingested on vegetation</td>
</tr>
<tr>
<td>Class Cestoidea, subclass Cestode (tapeworms)</td>
<td><em>Taenia solium</em></td>
<td>Cysticerci consumed in raw pork or wild boar</td>
</tr>
<tr>
<td></td>
<td><em>Echinococcus spp</em></td>
<td>Eggs ingested from soil</td>
</tr>
<tr>
<td></td>
<td><em>Spirometra spp.</em></td>
<td>Larvae in cyclops ingested in water or from soil</td>
</tr>
</tbody>
</table>
6.1 Dracunculus medinensis

6.1.1 General description

The guinea worm is the longest nematode parasite of humans, the female worm measuring up to 700 mm in length. When the female is ready to discharge its embryos, its anterior end emerges from a blister, usually on the foot or lower limb, and releases many thousands of embryos when the affected part of the body is immersed in water. The male worms measure only 25 mm in length, remain in the tissues and so are never seen. Embryos can be released on several occasions on contact with water in ponds, or in large open step-wells, used as sources of drinking-water. After a few weeks the entire worm is expelled from the body. Embryos can live in water for about 3 days but, when ingested by certain species of freshwater cyclopoid Copepoda (Crustacea), penetrate into the haemocoelom, moult twice, and are infective to a new host in about 2 weeks. If the cyclops, which measure 0.5–2 mm in length, are swallowed in drinking-water, the larvae are released in the stomach, penetrate the intestinal and peritoneal walls, and inhabit the subcutaneous tissues. Mature gravid female worms emerge about 1 year after infection (1).

Infection with guinea worm is geographically limited to rural areas of India, Pakistan, and 16 countries in sub-Saharan Africa (Benin, Burkina Faso, Cameroon, Chad, Côte d’Ivoire, Ethiopia, Ghana, Kenya, Mali, Mauritania, Niger, Nigeria, Senegal, Sudan, Togo, and Uganda). The annual incidence of dracunculiasis is estimated to be less than 2 million cases; approximately 140 million people are at risk (2).

6.1.2 Routes of exposure

Drinking-water containing infected cyclops is the only source of infection with Dracunculus, which is therefore the only human parasite that can be eradicated solely by the provision of safe drinking-water. The eradication of guinea worm infection from the world by 1995 was a target of the International Drinking Water Supply and Sanitation Decade (1981–1990), and the World Health Assembly formally committed itself to this goal in 1991 (resolution WHA 44.5).

The disease occurs in rural areas where piped water supplies are not always available. Control is based principally on the provision of boreholes and safe wells, but also includes measures aimed at preventing contamination of water sources, filtering of water by consumers, and in some situations chemical treatment of ponds and open wells. There are no effective antihelminthic drugs for the clinical treatment of the infection.

Transmission is usually highly seasonal, depending on changes in water sources. For instance, transmission is highest in the early rainy season in a dry

---

1 The valuable contribution made by Dr P. J. A. Ranque, Dracunculiasis Eradication, WHO, Geneva, in the preparation of this section is gratefully acknowledged.
savanna zone of Mali with under 800 mm annual rainfall, but in the dry season in the humid savanna area of southern Nigeria with over 1300 mm annual rainfall.

6.1.3 Health effects

As previously mentioned, when a female guinea worm emerges, it causes the formation of a blister, which bursts, and a portion of the worm is extruded. In about 50% of all cases, the whole worm is extruded in a few weeks, the lesion then heals rapidly, and disability is of limited duration. However, in the remaining cases, complications ensue, and the track of the worm becomes secondarily infected, leading to morbidity, which lasts for months. Mortality is extremely rare, but permanent disability can result from contractures of tendons and chronic arthritis; in 1988, it was estimated that, in Nigeria, there were 12,000 such cases annually out of more than 600,000 infections each year.

Usually only one worm emerges, but there may be two, three, or occasionally many. Worms do not survive for more than one transmission season, but there does not appear to be any acquired immunity, and the same individuals can be reinfected many times. Incidence rates in infected communities can be very high, 30% of the 14-45-year age group often becoming infected each year. The economic effect on agricultural productivity can be important: for instance, an 11% annual reduction in rice production has been reported from an area of eastern Nigeria, at a cost of US$ 20 million (3).

6.2 Schistosoma

6.2.1 General description

_Schistosoma_ spp. belong to the class of trematodes or flukes, whose infective larvae are able to penetrate the human skin or mucous membranes, causing schistosomiasis. They may be transmitted through drinking-water, but are more of a hazard when water is used for washing or bathing.

Schistosome eggs are excreted in the urine or faeces of an infected person, and break open on reaching fresh water, releasing a tiny parasite (a miracidium). This must penetrate a freshwater snail within 8-12 hours if it is to develop further. Once it has penetrated the snail, the parasite divides many times until, within 4-7 weeks or longer, depending on the type of parasite, thousands of new forms (cercariae) break out of the snail into the water. The cercariae can live for up to 48 hours, and can penetrate human skin within a few seconds.

After penetration, the young parasites migrate through the lymphatic system to the blood vessels of the portal system, affecting the intestine (intestinal schis-
HELMINTHS

tosomiasis) or the blood vessels around the bladder (urinary schistosomiasis), and
develop into male or female adult worms within about 4 weeks. The adult worms
live less than 5 years on average, although they can live for up to 40 years. Of the
eggs produced by the female worm—over 200 per day for some species—only
about half leave the body in the faeces (intestinal schistosomiasis) or in the urine
(urinary schistosomiasis), the rest remaining embedded in the body, where they
damage important organs. Heavy infections with schistosomes, which occur
mainly in children, cause the actual disease.

Intestinal schistosomiasis caused by *Schistosoma mansoni* occurs in 52 countries
in Africa, the Eastern Mediterranean Region, the Caribbean, and South America.
Oriental or Asiatic intestinal schistosomiasis, caused by the *S. japonicum* group of
parasites (including *S. mekongi* in the Mekong river basin), is endemic in seven
countries in the South-East Asia and Western Pacific Regions. (Another form of
intestinal schistosomiasis caused by *S. intercalatum* has been reported from ten cen-
tral African countries.) Urinary schistosomiasis, caused by *S. haematobium*, is
endemic in 54 countries in the African and Eastern Mediterranean Regions.

6.2.2 Routes of exposure

Schistosome infections are acquired when infected water is used for domestic
activities, bathing or washing, or while working in contact with water. Ingested
cercariae can penetrate the buccal mucous membranes, but this is a relatively
unimportant route of entry. If safe drinking-water is readily available it will be
used for washing, thus reducing the need to use contaminated surface water.

While there is a real possibility of piped untreated surface water transmitting
schistosomiasis, most transmission is from unpiped sources such as pools, wells,
and cisterns. In regions where schistosomiasis is endemic, the construction of
dams and large reservoirs often leads to an increase in the population of the
aquatic snail host and thus favours the spread of the disease. There are also many
examples of increased transmission of schistosomiasis as a result of irrigation, the
most dramatic being found along the Nile valley in Egypt and Sudan (4).

Schistosome infections are a hazard of recreational and irrigational water use
rather than of drinking-water. However, improvements in community water sup-
plies will reduce the incidence of schistosomiasis, particularly in communities
where incidence is high (4, 5).

6.2.3 Health effects

The human schistosomes are a cause of severe morbidity and sometimes death in
the 200 million people infected worldwide. In terms of socioeconomic and
public health importance in tropical and subtropical areas, the disease now ranks
second to malaria (4).

In communities where it is endemic, the prevalence of infection is greatest in
10–14-year-old children; in many African communities over 70% of village chil-
GUIDELINES FOR DRINKING-WATER QUALITY

dren may be infected. Pathology is due mainly to the host's reaction to eggs that fail to escape. Primary lesions are mainly in the liver, intestine, and around the bladder, but the most severe pathological effects are the consequence of secondary damage to the upper urinary tract, bladder cancer, and liver fibrosis and its haemodynamic consequences.

Schistosomiasis has a significant effect on health (6). In infected people without clinical evidence of disease, it is estimated that 30 work days are lost per year as a result of *S. japonicum* infection and 4 work days per year as a result of *S. haematobium* infection. After a latency period of 5–15 years, approximately 10% of infected people will develop severe disease. An 18% reduction in the work output of persons with severe *S. mansoni* infection can be expected. A reduction of 12% or more in exercise capacity was found in children with *S. haematobium* infection in Zimbabwe, but this was recovered by 1 month after treatment. Similarly, a 7–10% improvement in exercise capacity was found in children with *S. haematobium* infection 1 month after treatment in Kenya (KE Mott, personal communication).

A specific type of bladder cancer occurs in countries where urinary schistosomiasis is endemic, and is the leading cause of death due to cancer in Egypt among men aged 20–44 years. Diseases of the central nervous system, affecting the spinal cord, are more frequent and cause more debility than is widely recognized, especially among migrants into endemic areas of *S. mansoni* transmission.

In persons with schistosomiasis and intercurrent hepatitis B or typhoid fever, the severity and duration of both increase markedly, with an increased risk of chronic liver disease.

Since a single cercaria is infective, there is no safe level and cercariae should be absent from drinking-water. In the absence of routine monitoring assays, reliance must be placed on preventive measures if a significant risk from drinking-water is suspected in an area. The cercariae have a free-living life of under 48 hours, and storage for this period renders water safe (7). It is likely that storage for 24 hours will greatly reduce infectivity. Slow sand filters, provided that they are properly operated, will remove the majority of cercariae, and disinfection at a residual level of 0.5 mg of free chlorine per litre for 1 hour will kill cercariae of the human schistosomes (8). A sounder approach is to use a source that does not contain the host snails and is not subject to excretal contamination.

### 6.3 Other helminths

A great variety of helminth eggs and larvae have been detected in drinking-water, and it is clear that none of those infective to humans should be present if the drinking-water is to be safe. However, the vast majority of such helminths are not primarily waterborne, and it is neither feasible nor necessary to monitor water for them on a routine basis (9).

Helminths that could conceivably be transmitted through drinking-water are listed in Table 6.1. *Fasciola* spp., which belong to the same class as the schisto-
HELMINTHS

6.

Parasites that regularly parasitize farm and domestic animals, such as trematodes (Trematoda), principal parasites of farm and domestic animals. The cercariae which emerge from freshwater snails encyst on water plants and infect humans if these are ingested. Some tapeworms (Cestoda) have very resistant eggs, and those of *Taenia solium* (the pork tapeworm) and *Echinococcus* spp. can develop in humans. Eggs are liberated from the gravid proglottids (segments), and are passed out in faeces. They can then be ingested from soil or on salad vegetables, although the normal route of infection with *T. solium* is the ingestion of raw pork containing the larval cysticercus stage. Eggs of *Echinococcus* have been recovered from wells in an area of East Africa and might be transmitted in drinking-water. Another tapeworm, *Spirometra*, has its tapeworm stage in carnivores, and two intermediate hosts, the first being a cyclopoid copepod and the second an amphibian, reptile, rodent or herbivore, depending on species. Humans occasionally act as intermediate hosts for the larvae (spargana) by ingesting first-stage larvae inside cyclops when drinking water from ponds.

The resistant eggs of various common, ubiquitous, intestinal nematode parasites of humans, such as *Ascaris* and *Trichuris* (and the common dog ascarid, *Toxocara*), the eggs of which can hatch in humans and the larvae cause visceral damage, are passed in faeces and normally ingested in soil or on salad vegetables. The eggs occasionally enter water but have a high relative density and settle quickly; drinking-water does not play an important part in their transmission.

Other intestinal nematode parasites which infect many millions of people in the tropics and subtropics are *Necator* and *Ancylostoma* (the hookworms) and *Strongyloides*. Eggs (or, in the case of *Strongyloides*, larvae) are passed in the faeces, and the larvae develop in the soil into an infective stage which can penetrate the skin of a new host. While the larvae of *Ancylostoma* are sometimes ingested on salad vegetables, there is little evidence that drinking-water is ever a source of infection for these soil-transmitted nematodes.

References


7.
Toxins from cyanobacteria

Blooms of cyanobacteria (commonly called blue-green algae) are very common in lakes and reservoirs used for potable water supply. These bacteria are capable of producing various toxins which fall into the following three categories: (i) hepatotoxins produced in fresh water by *Microcystis*, *Oscillatoria* and *Anabaena*, and by *Nodularia* in brackish water; (ii) neurotoxins produced by species of *Anabaena*, *Oscillatoria*, *Nostoc*, *Cylindrospermum* and *Aphanizomenon*; (iii) lipopolysaccharides from a number of species (1).

The most commonly encountered are the hepatotoxins, which induce death by circulatory shock as a result of massive liver haemorrhage within 2–24 hours of oral intake of a sufficiently large quantity (2–7). At present, there are thought to be more than 13 variants of the toxin, which is a cyclic structure containing seven amino acids of relative molecular mass varying from about 800 to 1050. The best studied of these hepatotoxins is microcystin LR:R, which has a relative molecular mass of 994.

The LD₅₀ of microcystin LR:R has been shown to be about 30 μg/kg of body weight in mice by intraperitoneal injection (8). A lethal dose is about 1–2 μg of pure toxin per mouse; however, the toxicity by the oral route appears to be about an order of magnitude less. There appear to be no other toxicity data available on the pure toxin, although studies with diluted extracts of a toxic bloom, reported to contain 56.6 μg/ml of an unknown variant of microcystin, showed that liver damage could be induced in mice given a one-quarter dilution of the extract in their drinking-water for 1 year (9). Microcystin was not mutagenic in the Ames test (10), but purified microcystin LR inhibited protein phosphatase *in vitro* with the same potency and specificity as the tumour promoter okadaic acid (11).

The hepatotoxic cyclic peptide from *Nodularia*, termed nodularin, has a structure similar to that of microcystin, but contains only five amino acids. The oral LC₅₀ for mice has been determined as 67 μg/ml for females and 73 μg/ml for males receiving 4.5–7 ml of drinking-water per day containing crude extracts (7).

There are a number of unconfirmed reports of algal toxins in drinking-water supplies causing health problems, including an outbreak of hepatenteritis in Palm Island, Australia (12). However, the most convincing evidence comes from an epidemiological study by Falconer et al. (13) of an Australian community in which raised serum enzymes indicative of mild, reversible liver damage were observed in hospital patients who drank water from a local reservoir with a very
large toxic bloom of *Microcystis aeruginosa*. Recent surveys of large numbers of fresh waters worldwide, which produce heavy growths of cyanobacteria, have shown the presence of cyanobacterial toxins at over 60% of sites (14). Algal blooms may change from being nontoxic to toxic in a very short time, but there is at present no well established method for analysis of the toxin in drinking-water.

It has been reported that activated carbon removes microcystin to a significant extent (15–17) and that ozone at a dose of 1.0–1.5 mg/litre destroys toxicity (17) by converting microcystin into a less toxic substance (18). The use of algicides such as copper sulfate at the height of the bloom is not recommended, since this leads to a massive release of toxin into the water, and may have been responsible for the unusual problems seen on Palm Island (12).

At present, it is not clear how great a hazard algal toxins pose in drinking-water, and the data are insufficient to enable any guidelines to be drawn up. However, problems resulting from the progressive eutrophication of inland waters appear to be increasing and with them the likelihood of cyanobacterial blooms. This emphasizes the need for the protection of sources, and particularly of lakes and reservoirs, from discharges of nutrient-rich effluent.

**References**


18. Dahlem AM. Structure-toxicity relationships and fate of low molecular weight peptide toxins of cyanobacteria. Department of Veterinary Medical Science, Graduate College, University of Illinois at Urbana-Champaign, 1989 (PhD thesis).
8. Nuisance organisms

Nuisance organisms constitute a morphologically and physiologically diverse group, including planktonic and benthic cyanobacteria (blue-green algae), actinomycetes, iron, manganese, and sulfur bacteria, crustacea, and protozoa. These organisms cause problems when the conditions in reservoirs or distribution systems are such as to support their growth. Thus organic matter in drinking-water supports the growth of bacteria and fungi, which in turn will help to maintain populations of protozoa and crustacea. Many invertebrate animals can feed on bacteria, fungi, and protozoa. The content of organic compounds in treated water should therefore ideally be so low as to inhibit the growth of bacteria and to prevent that of other organisms during distribution.

8.1 Microbiological problems

Although the raw water itself does not usually contain large numbers of nuisance organisms, problems may develop during the water-treatment process. Nuisance organisms become concentrated on the surfaces and inside the beds of filters, where they autolyse and release cellular compounds that cause colour, turbidity, tastes, and odours. Activated carbon filters will, after a while, contain large amounts of organic matter, thus providing an excellent substrate for bacteria, which can create problems in the water supply, either by causing taste, odour, and turbidity, or microbiologically by increasing the colony counts of aerobic heterotrophic bacteria. Significant amounts of organic carbon can cause the growth of *Aeromonas* spp. in the distribution system during the warmer months of the year (see section 3.2.2). Large numbers of aerobic, heterotrophic bacteria in treated water can interfere with the interpretation of the tests for the coliform group by masking their presence or giving false positive reactions. A particular problem exists with some strains of *Aeromonas* spp., which produce acid and gas with coliform media, even at 44 °C.

Most of these nuisance organisms can be controlled relatively easily by care in operating water-treatment processes. Nutrient-rich raw water should be avoided if proper water treatment cannot be applied.

The compounds produced by nuisance organisms have low taste and odour thresholds, e.g. the earthy taints of geosmin (trans-1,10-dimethyl-trans-9-deca-dole) and MIB (2-methylisoborneol) produced by actinomycetes and cyanobac-
8. NUISANCE ORGANISMS

teria. These compounds cause problems in drinking-water at threshold values of 10 and 25 ng/litre respectively, and are therefore often the cause of complaints by consumers before they are detected by analytical methods. It is therefore advisable to use panels of trained judges of taste and odour so that the compounds can be detected and the necessary measures taken before they become a problem in the drinking-water supply. Another way to prevent nuisance organisms from causing taste and odour problems is by means of regular microscopic examination of the organisms present in the water. As soon as a group of organisms known to cause these problems becomes dominant, appropriate measures should be taken to deal with them.

Some of these organisms can also produce colour in drinking-water. Pigmented organisms, such as cyanobacteria and algae, can be crushed on filters, resulting in the release of pigments, while microalgae can pass through the filters and cause both coloration and turbidity.

If water contains ferrous or manganous salts, these can be oxidized by iron or manganese bacteria, resulting in rust-coloured or black deposits in storage tanks and on the walls of pipes in parts of the distribution system where the flow rate is low. If the flow rate is subsequently increased, however, these deposits can be loosened and transported to consumers. Rust-coloured deposits can stain laundry. The slurry will also contain organic deposits which can decompose to produce tastes and odours. Manganese-oxidizing microorganisms (bacteria, fungi, and, very rarely, protozoa) produce deposits in aquifers, wells, and water conduits, the problems caused by such deposits including reduced yield, clogging of slots in well pipes, increased turbulence in pipes resulting in reduced flow velocity, damage to equipment for measuring water flow, black-coloured water, stains on laundry, and problems with food-handling establishments. The deposits can contain heavy metals such as arsenic, lead, zinc, and copper. Bacteria can become attached to them, so that, if they are disturbed, the colony count of the water will be increased. Prevention is based on the removal of Mn(II) from raw water; if a value of about 0.1 mg/litre is exceeded.

Iron and sulfur bacteria may contribute to the corrosion of iron and steel well pipes and drinking-water mains. Such microbially mediated corrosion can occur as a consequence of:

- the adsorption of nutrients and the depletion of dissolved oxygen by the colonies of microorganisms that have accumulated at the metal surface;
- the liberation of corrosive metabolites, such as organic acids and other complex-forming compounds;
- the production of sulfuric acid from sulfides or elemental sulfur; and
- the inclusion of sulfate-reducing bacteria in the cathodic process under anaerobic conditions.

The presence of certain organisms in water may be an indication either of the corrosion of cast iron or of the biodeterioration of construction materials to form substances that support the growth of microorganisms. The latter include nonmetallic materials, such as plastics, rubber-jointing compounds, and pipe-lining
materials, which can provide organic nutrients and thus encourage the growth of microorganisms, sometimes including coliform organisms other than *Escherichia coli* and *Pseudomonas aeruginosa*. Deterioration can occur in pipelines carrying groundwater or surface water. Unchlorinated waters, or water in which the chlorine residual has disappeared, appear to support higher rates of attack than those in which a residual can be detected.

Nuisance organisms may also cause problems in groundwater sources by encrusting well screens, thus reducing yield and impairing the aesthetic quality of the supply. Their presence may also indicate organic pollution of the aquifer.

Routine monitoring of such nuisance organisms cannot be recommended because of their diverse nature and unpredictable occurrence, although bacteriologists should be aware that they can impair water quality. It is not practicable to specify any quantitative guideline values for nuisance microorganisms.

### 8.2 Problems caused by invertebrate animals

Invertebrate animals often infest shallow, open wells, from which supplies are drawn by bucket, but problems are not uncommon in large, public supplies. The animals derive their food from the bacteria, algae, and protozoa in the water or present on slimes on pipe and tank surfaces.

The types of animal concerned can be considered, for control purposes, as belonging to two groups. Firstly, there are free-swimming organisms in the water itself or on water surfaces, such as the crustacea *Gammarus pulex* (freshwater shrimp), *Crangonyx pseudogracilis*, *Cyclops* spp. and *Chydorus sphaericus*. Secondly, there are other animals that either move along surfaces or are anchored to them (such as *Asellus aquaticus* (water louse), snails, *Dreissena polymorpha* (the zebra mussel) and other bivalve mollusces, and the bryozoan *Plumatella* sp.), or inhabit slimes (such as *Nais* spp., nematodes and the larvae of chironomids) ([I]). In warm weather, slow sand filters can sometimes discharge the larvae of gnats (*Chironomus* and *Culex* spp.) into the water, if the top layer of the bed collapses, causing a draw-down of unfiltered water.

The only health hazard positively identified arises in tropical countries where water fleas (*Cyclops*) are the intermediate host of the guinea worm (*Dracunculus medinensis*) (see section 6.1).

Penetration of waterworks and mains is more likely to be a problem when low-quality raw waters are abstracted and high-rate filtration processes used. Prechlorination assists in destroying animal life and in its removal by filtration but, if excessive, may produce chlorinated organic compounds and convert total organic carbon into a biodegradable form. Maintenance of chlorine residuals in the distribution system, the production of high-quality water, and the regular cleaning of water mains by flushing or swabbing will usually prevent infestation.

Bryozoan infestation can be treated with a shock dose of chlorine, maintained at 10 mg/litre for about 24 hours, followed by flushing. Permethrin treatment of water at an average dose of 0.01–0.02 mg/litre for 24–48 hours has been
used to destroy *Asellus* and other crustacea, but treated water must not be discharged into watercourses, as it is rapidly toxic to fish and other aquatic life at this concentration (2, 3). The most effective procedure is to draw treated water into the main by opening hydrants downstream of the injection point. These are then closed, allowing sufficient contact time (ideally 24 hours) to paralyse the crustacea, after which the mains are cleared by flushing and swabbing. Persons using renal dialysis should not be supplied with permethrin-treated water, and those rearing fish should be warned not to replenish the culture tanks with mains water while it is being treated. The treated water can be safely discharged into sewers for treatment at sewage works (2).

**References**


9. Microbial indicators of water quality

9.1 Rationale

The recognition that faecally polluted water is responsible for spreading enteric diseases led to the development of sensitive methods of verifying that drinking-water is free from faecal contamination. Even though many waterborne pathogens can now be detected, the methods are often difficult, relatively expensive, and time-consuming. Furthermore, pathogens are shed into water only from infected people and animals, and it is not possible to examine water for every possible pathogen that might be present. It is prudent to regard as unsafe all water that contains bacteria indicating faecal pollution, because of the risk that enteric pathogens may be present. The bacteria selected as indicators of faecal pollution should be universally present in the faeces of humans and warm-blooded animals in large numbers. Other desirable properties of faecal indicators are that they should be readily detected by simple methods and that they do not grow in natural waters. Furthermore, it is essential that their persistence in water and the extent to which they are removed by water treatment are similar to those of waterborne pathogens.

Examination for faecal indicator bacteria in drinking-water provides a very sensitive method of quality assessment. It is also important to determine the quality of the raw water, not only to assess the degree of pollution but also to enable the best local source to be selected and the best form of treatment chosen. Microbiological examination for faecal indicators is the most sensitive and specific method for detecting recent faecal pollution, i.e. pollution that is potentially dangerous, since simple chemical analysis is not adequate for this purpose. Water must be examined regularly and frequently because pollution is often intermittent and may not be detected if examination is limited to only one or a small number of samples. For this reason it is better to examine drinking-water frequently by means of a simple test rather than less often by several tests or a more complicated test. When personnel and facilities are limited, routine microbiological examination for evidence of faecal contamination must always be given first priority (1).

Microbiological examinations can also be carried out with other objectives than assessing the degree of faecal contamination. They may give information on the effectiveness with which specific groups of microorganisms have been removed by treatment processes; thus, if bacteriophages are present this may
indicate that viruses have not been removed, and the presence of spores of sulfite-reducing clostridia also shows highly persistent microorganisms may have survived. Colony counts of aerobic, heterotrophic bacteria, or microscopic or indirect chemical methods (e.g. the assay of adenosine triphosphate by luminescence) may provide information on the availability of nutrients in the water that support bacterial growth, which may result in aesthetic problems or in the presence of opportunistic pathogens. For some of these latter organisms, specific culture methods are also being used, namely for *Pseudomonas aeruginosa*, *Legionella* and *Aeromonas* (see section 3.2); however, these should not be used routinely, but only when necessary to solve problems related to the occurrence of the organisms concerned.

9.2 Indicators of faecal contamination

The use of normal intestinal organisms as indicators of faecal pollution rather than the pathogens themselves is universally accepted for monitoring and assessing the microbial safety of water supplies. In practice, the criteria to be satisfied by an ideal indicator (see section 9.1) cannot all be met by any one organism. However, many of them are best fulfilled by *Escherichia coli*, and to a lesser extent by the thermotolerant coliform bacteria; *E. coli* is thus the indicator of choice when resources for supplementary microbiological examination are limited. Other microorganisms that satisfy some of these criteria, though not to the same extent as *E. coli* and the thermotolerant coliform organisms, can also be used as supplementary indicators of faecal pollution in certain circumstances.

Because enteroviruses and the cysts of some parasites are known to be more resistant than *E. coli* and coliform organisms to disinfection, the absence of these organisms in surface water that has only been disinfected will not necessarily indicate freedom from enteric viruses and the resting stages of *Cryptosporidium*, *Giardia*, amoebae, and other parasites.

The significance that can be attached to the presence or absence of particular faecal indicators varies with each organism and particularly with the degree to which that organism can be specifically associated with faeces. For example, some of the genera detected by the methods for enumeration of thermotolerant and total coliform bacteria have nonfaecal sources in the environment, e.g. in soil or decaying vegetation, or can even grow in the aquatic environment, thus limiting their usefulness as indicators of faecal contamination. Other bacterial indicators have useful properties which enable them to be used for particular purposes. For example, although the faecal streptococci and enterococci and the spores of sulfite-reducing clostridia, typified by *Clostridium perfringens*, are less numerous than coliforms in faecally polluted water, they have greater powers of survival and so may be used to confirm the presence of faecal contamination when *E. coli* is not found or to assess the efficiency of treatment processes. Anaerobic bacteria, such as bifidobacteria and the *Bacteroides fragilis* group, are more abundant than coliform organisms in faeces, but decay rapidly in water, and accepted standard
methods for their detection and enumeration are not yet available. Full identification of these indicator organisms would require such an extensive series of tests as to be impracticable in routine monitoring.

9.2.1 *Escherichia coli*

*Escherichia coli* is abundant in human and animal faeces, where numbers may attain $10^9$ per gram of fresh faeces. It is found in sewage, treated effluents, and all natural waters and soils subject to recent faecal contamination, whether from humans, farm animals, or wild animals and birds. The presence of *E. coli* in water always indicates potentially dangerous contamination requiring immediate attention. Complete identification of *E. coli* is too complicated for routine use, hence certain tests have been evolved for identifying this organism rapidly with a high degree of certainty. Some of them are the subject of international and national standards and have been accepted for routine use, whereas others are still being developed or evaluated. Detection of *E. coli* on complex media entails incubation at the restrictive temperature of 44-45 °C in combination with demonstration of the production of acid and gas from lactose and of specific biochemical reactions such as indole production and β-glucuronidase activity, and the absence of urease activity. In other tests, chemically defined media with specific substrates for the growth and detection of enzymatic activities of *E. coli*, such as β-galactosidase and β-glucuronidase, are used. Confirmation of the presence of *E. coli*, as indicated by these methods, requires extensive biochemical identification or the use of alternative, commercially available test systems. Such confirmation is not recommended as a routine, but may be necessary to validate the use of routine tests under specific conditions.

9.2.2 Thermotolerant (faecal) coliform organisms

These are defined as the group of coliform organisms that are able to ferment lactose at 44-45 °C. They comprise the genus *Escherichia* and, to a lesser extent, species of *Klebsiella*, *Enterobacter*, and *Citrobacter*. Of these organisms, only *E. coli* is specifically of faecal origin, being always present in the faeces of humans, other mammals, and birds in large numbers, and rarely found in water or soil that has not been subject to faecal pollution. Thermotolerant coliforms other than *E. coli* may also originate from organically enriched water such as industrial effluents or from decaying plant materials and soils. In tropical and subtropical waters, thermotolerant coliform bacteria may occur without any obvious relation to human pollution and have been found on vegetation in a tropical rainforest (2). This means that the occurrence of the thermotolerant coliform group in subtropical or tropical waters or those enriched with organic wastes does not necessarily indicate faecal contamination by humans since they can originate from wild animals, including birds. However, their presence in waters in warm climates should not be ignored, as the basic assumption that pathogens may be present and that treatment has been inadequate still holds good.
Regrowth of thermotolerant coliform organisms in the distribution system is unlikely unless sufficient bacterial nutrients are present (biochemical oxygen demand (BOD) greater than 10 mg/litre) or unsuitable materials are in contact with the treated water, the water temperature is above 15 °C, and there is no free chlorine residual.

Thermotolerant coliforms are less reliable indicators of faecal contamination than *E. coli*, although under most circumstances their concentrations are directly related to *E. coli* concentrations in water. Their use for water-quality examination is therefore considered acceptable. Internationally standardized methods and media for their detection have been validated, and are relatively simple and widely available. When necessary, thermotolerant coliform isolates can be subjected to further confirmatory tests to detect those that are presumptive *E. coli*. Normally, a test for the ability to produce indole from tryptophan at 44 ± 0.5 °C is sufficient. The detection and identification of these organisms as faecal organisms or presumptive *E. coli* provide strong evidence of recent faecal contamination and of the need for immediate investigation.

Because thermotolerant coliform bacteria are readily detected by single-step methods, they have an important secondary role as indicators of the efficiency of individual water-treatment processes in removing faecal bacteria. They may therefore be used in assessing the degree of treatment necessary for waters of different quality and for defining performance targets for bacterial removal (see section 11.3).

9.2.3 Coliform organisms (total coliforms).

Coliform organisms have long been recognized as a suitable microbial indicator of drinking-water quality, largely because they are easy to detect and enumerate in water. The term “coliform organisms (total coliforms)” refers to Gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties, and able to ferment lactose at 35–37 °C with the production of acid, gas, and aldehyde within 24–48 hours. They are also oxidase-negative and non-spore-forming. These definitions have recently been extended by the development of rapid and direct enzymatic methods for enumerating and confirming members of the coliform group. By definition, coliform bacteria display β-galactosidase activity. Traditionally, coliform bacteria were regarded as belonging to the genera *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella*. However, the group of coliform bacteria, as defined by modern taxonomical methods, is heterogeneous and includes lactose-fermenting bacteria which can be found in both faeces and the environment, namely in nutrient-rich waters, soil, decaying vegetation and drinking-water containing relatively high levels of nutrients. Examples of such species are *Enterobacter cloacae* and *Citrobacter freundii*.

The coliform group also contains species that are rarely, if ever, found in faeces and which can multiply in relatively good-quality drinking-water, e.g.
Serratia fonticola, Rahnella aquatilis, and Buttiauxella agrestis. Several lactose-fermenting species of Serratia and Yersinia can be isolated from uncontaminated water or soil. There are also many reports of the existence of non-lactose-fermenting but otherwise characteristic coliform bacteria. Lactose-negative strains which otherwise resemble the traditional coliform genera lack the lactose permease enzyme. They do, however, possess the \( \beta \)-galactosidase enzyme and will appear as coliform bacteria if a \( \beta \)-galactosidase test is applied. The existence of nonfaecal bacteria that fit the definition of coliform bacteria and of lactose-negative coliform bacteria limits the applicability of this group of bacteria as indicators of faecal pollution.

Coliform bacteria should not be detectable in treated water supplies and, if found, suggest inadequate treatment, post-treatment contamination, or excessive nutrients. In this sense, the coliform test can be used to assess treatment efficiency and the integrity of the distribution system. Although coliform organisms may not always be directly related to the presence of faecal contamination or pathogens in drinking-water, the coliform test is still useful for monitoring the microbial quality of public water supplies. If there is any doubt, especially when coliform organisms are found in the absence of faecal coliforms and \( E. \) coli, secondary indicator organisms may be used to determine whether faecal contamination is present; these include the faecal streptococci and sulfate-reducing clostridia, especially Clostridium perfringens.

9.2.4 Faecal streptococci

The term “faecal streptococci” refers to those streptococci generally present in the faeces of humans and animals. All possess the Lancefield group D antigen. Taxonomically, they belong to the genera Enterococcus and Streptococcus. The genus Enterococcus has recently been defined to include all streptococci sharing certain biochemical properties and having a wide tolerance of adverse growth conditions. It includes the species \( E. \) avium, \( E. \) casseliflavus, \( E. \) cecorum, \( E. \) durans, \( E. \) faecalis, \( E. \) faecium, \( E. \) gallinarum, \( E. \) hirae, \( E. \) malodoratus, \( E. \) mundtii, and \( E. \) solitarius.

Most of these species are of faecal origin and can generally be regarded as specific indicators of human faecal pollution under many practical circumstances. They may, however, be isolated from the faeces of animals, whereas certain species and subspecies, such as \( E. \) casseliflavus, \( E. \) faecalis var. liquefaciens, \( E. \) malodoratus, and \( E. \) solitarius occur primarily on plant material. The taxonomy of enterococci has recently undergone important changes, and detailed knowledge of the ecology of many of the new species is lacking.

In the genus Streptococcus, only \( S. \) bovis and \( S. \) equinus possess the group D antigen and are members of the faecal streptococcus group. They occur mainly in animal faeces. Conventional media for the isolation and identification of faecal streptococci, such as m-enterococcus agar, KF-streptococcus agar, and azide-glucose broth, generally support the growth of all faecal streptococci. However, particularly in warm climates, other cocci may also develop on these media, so
that confirmatory tests are needed. More restrictive media that support the growth of the enterococci in particular have also been proposed and have been widely used in the USA (3). The applicability and specificity of these media need to be further tested under a wide range of conditions. Faecal streptococci rarely multiply in polluted water and are more persistent than \textit{E. coli} and coliform bacteria. Their main value in assessing water quality is therefore as an additional indicator of treatment efficiency. Furthermore, streptococci are highly resistant to drying and may be valuable for purposes of routine control after new mains have been laid or distribution systems repaired, or for detecting pollution by surface run-off to groundwater or surface waters.

9.2.5 Sulfite-reducing clostridia

These are anaerobic, spore-forming organisms, of which the most characteristic, \textit{Clostridium perfringens} (\textit{C. welchii}), is normally present in faeces, though in much smaller numbers than \textit{E. coli}. However, they are not exclusively of faecal origin and can be derived from other environmental sources. Clostridial spores can survive in water much longer than organisms of the coliform group and will resist disinfection. Their presence in disinfected waters may thus indicate deficiencies in treatment (4). In particular, the presence of \textit{C. perfringens} in filtered supplies may be a sign of deficiencies in filtration practice, while \textit{C. perfringens} spores may indicate the presence of protozoan cysts; because of their longevity, they are best regarded as indicating intermittent or remote contamination and thus are of special value. However, they are not recommended for the routine monitoring of distribution systems. Because they tend to survive and accumulate, they may be detected long after pollution has occurred and far from the source, and thus give rise to false alarms.

9.2.6 Bacteriophages

Bacteriophages are viruses that infect bacterial host cells. They usually consist of a nucleic acid molecule (genome) surrounded by a protein coat (capsid). Bacteriophages may contain either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) as the genome and may have a very simple, cubic structure or a more complex one with heads, tails, tail fibres, or other attachments. They are in the size range 25–100 nm. Bacteriophages have been proposed as indicators of water quality, particularly with respect to human enteric viruses, both because of their similar nature and because they are relatively easy to detect in water samples (5). Furthermore, data are accumulating showing the similarities between certain groups of bacteriophages and human enteric viruses in terms of survival in the aquatic environment and responses to water- and wastewater-treatment processes. Two groups of bacteriophages have been studied extensively in the context of viral indicators in water, namely the somatic coliphages, which infect standard \textit{E. coli} host strains via cell wall (somatic) receptors, and the F-specific RNA bac-
teriophages which infect *E. coli* and related bacteria through the F- or sex-pili. Neither of these groups of organisms occurs in high concentration in faeces, but they are invariably found in sewage. They are used, therefore, primarily as an index of sewage contamination and, because of their high persistence as compared with bacterial indicators, as an additional indicator of treatment efficiency or groundwater protection.

9.2.7 Miscellaneous indicators

The bifidobacteria and the *Bacteroides fragilis* group are anaerobes which are specific to faeces, where they outnumber the coliform group. They do not survive or multiply in natural waters, and have been seen as an alternative to the coliform group in tropical and semitropical regions, where the latter can multiply in warm and organically enriched water (6). However, their numbers decline more rapidly than those of thermotolerant coliforms and *E. coli* in passing from faeces through sewage and into polluted waters, indicating that their rate of decay is greater than that of other bacterial indicators (6). This is a disadvantage, since bacteria in the coliform group are themselves more sensitive to decay than viral and protozoal pathogens. In addition, the methods of detecting them in water are not very reliable and have not been standardized.

9.3 Indicators of water quality and treatment efficacy

9.3.1 Heterotrophic plate counts (colony counts)

Heterotrophic plate counts may be used to assess the general bacterial content of water. They do not represent all the bacteria present in the water but only those able to grow and produce visible colonies on the media used and under the prescribed conditions of temperature and time of incubation. Colony counts are often determined following incubation at 22 °C and 37 °C to assess the relative proportions of naturally occurring water bacteria unrelated to faecal pollution and of bacteria derived from humans and warm-blooded animals, respectively. The count at 22 °C is of little sanitary value, but is useful in assessing the efficiency of water treatment, specifically the processes of coagulation, filtration, and disinfection, where the objective is to keep counts as low as possible. The 22 °C count may also be used to assess the cleanliness and integrity of the distribution system and the suitability of the water for use in the manufacture of food and drink, where high counts may lead to spoilage. Any increase in counts in the test at 37 °C as compared with those normally found may be an early sign of pollution.

9.3.2 *Aeromonas* spp. and *Pseudomonas aeruginosa*

Apart from heterotrophic plate counts, the use of other microorganisms, including *Aeromonas* and *Pseudomonas aeruginosa*, has been advocated as a means of
9 MICROBIAL INDICATORS OF WATER QUALITY

assessing the hygienic quality of drinking-water \((7, 8)\). However, neither examination for these organisms nor heterotrophic plate counts are essential for the routine monitoring of hygienic quality. They are of value in certain circumstances in giving an indication of the general cleanliness of the distribution system and in assessing the quality of bottled water. However, high heterotrophic plate counts and counts of these bacteria may interfere with the detection of \(E.\ coli\), coliforms, and other bacterial indicators of faecal pollution.

9.4 Methods

9.4.1 Standard methods

Microbiological examination provides the most sensitive, although not the most rapid, indication of the pollution of drinking-water supplies. Because the growth medium and the conditions of incubation, as well as the nature and age of the water sample, can influence the species isolated and the count, the accuracy of microbiological examinations may vary. This means that the standardization of methods and of laboratory procedures is of great importance if uniform criteria for the microbiological quality of water are to be used in different laboratories and countries. International standard methods should be evaluated in local circumstances before being adopted in national surveillance programmes. Information is given here on established standard methods, particularly those of the International Organization for Standardization (ISO), Geneva (Table 9.1), to encourage their use. There are also other well established national standards, such as those of the American Public Health Association (APHA) \((3)\) and of the United Kingdom \((9)\). Established standard methods should be used for routine examinations.

Whatever method is chosen for the detection of \(E.\ coli\) and the coliform group, some means of "resuscitating" or recovering environmentally or disinfectant-damaged strains must be used, such as preincubation for a short time at a lower temperature \((3, 9)\)

9.4.2 Methods for pathogenic bacteria, protozoa, and cytopathic enteroviruses

Although the direct search for specific pathogenic bacteria has no place in the routine microbiological examination of water, there are occasions when examination for intestinal pathogens may be necessary as, for example, during an epidemic, when pollution is suspected, or in the evaluation of a new source. The chances of detecting pathogens will be greater if large samples of water are examined, and if media selective for certain intestinal pathogens are used. Examination for bacterial pathogens will include some, if not all, of the following steps: concentration of the organisms in the sample, inoculation into enrichment broth, subculture onto selective agar media, and biochemical and serological
Table 9.1 ISO standards for water quality

<table>
<thead>
<tr>
<th>Standard no.</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>5667-3:1985</td>
<td>Sampling — Part 3. Guidance on the preservation and handling of samples</td>
</tr>
<tr>
<td>5667-5:1991</td>
<td>Sampling — Part 5. Guidance on sampling of drinking-water and water used for food and beverage processing</td>
</tr>
<tr>
<td>6222:1988</td>
<td>Enumeration of viable micro-organisms — colony count by inoculation in or on a nutrient agar culture medium</td>
</tr>
<tr>
<td>7704:1985</td>
<td>Evaluation of membrane filters used for microbiological analyses</td>
</tr>
<tr>
<td>8199:1988</td>
<td>General guide to the enumeration of micro-organisms by culture</td>
</tr>
<tr>
<td>9308-2:1990</td>
<td>Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive Escherichia coli — Part 2. Multiple tube (most probable number) method</td>
</tr>
<tr>
<td>6579:1990</td>
<td>General guidance on methods for the detection of Salmonella</td>
</tr>
</tbody>
</table>

Examination of suspect colonies. Rather than rely on a single method, it is better to use as many methods as possible so that no opportunity to detect a pathogen is missed (3, 9). This is especially true for the detection of Salmonella, since no single method is suitable for all serotypes.

Isolation methods are available for Salmonella spp. (3, 9), Shigella spp. (10), Vibrio cholerae (11), Yersinia enterocolitica (12), Campylobacter spp. (13–15), Legionella spp. (16, 17), P. aeruginosa (3, 9), Aeromonas spp. (18), Giardia spp. (19–22), Cryptosporidium spp. (21–23), and Naegleria spp. (24).

Standard methods are now available for concentrating and recovering cytopathic enteroviruses from large volumes of water (i.e., in the range 10–1000 litres) (3, 25–27). A method for enumerating male-specific bacteriophages in water has been described (28), and some of the factors influencing their recovery have been reviewed (5).
References


10. Microbiological criteria

10.1 Rationale

10.1.1 Overall strategy

A supply of drinking-water should be sufficient in quantity, wholesome, and not injurious to health. These requirements are all inter-related. The history of water-supply engineering has repeatedly shown that the provision of safe drinking-water is the most important step which can be taken to improve the health of a community by preventing the spread of waterborne disease.

Microbiological monitoring provides a sensitive indication of the extent to which source protection, treatment, and distribution are effective barriers to the transmission of infectious agents of waterborne disease at the time that the samples were taken. It is important to realize at the outset that microbiological integrity is provided by source protection and treatment, and that sudden loss of this integrity or steady deterioration may be missed if monitoring is not frequent enough. Proper design of sampling schemes is important.

The task of monitoring is properly that of the water-supply agency whereas surveillance—keeping public health and the safety and acceptability of water supplies under continuous review—is the duty of the local and national health authorities. Good communications between bodies responsible for monitoring and surveillance are essential. The user has an important role in preserving the quality of the water delivered to the premises through the proper design, construction, and maintenance of storage tanks, taps, and associated plumbing so as to prevent deterioration.

10.1.2 Treatment objectives and microbiological criteria

It is difficult with the epidemiological knowledge currently available to assess the risk to health presented by any particular level of pathogens in water, since this will depend equally on the infectivity and invasiveness of the pathogen and on the innate and acquired immunity of the individuals consuming the water. It is only prudent to assume, therefore, that no water in which pathogenic microorganisms can be detected can be regarded as safe, however low the concentration.

Furthermore, only certain waterborne pathogens can be detected reliably and easily in water, and some cannot be detected at all. This has led over many years
to the adoption of the concept of faecal indicator species (see section 9.2) and to universal agreement that the most specific and suitable bacteriological indicator of faecal pollution is *Escherichia coli*. Any water that contains *E. coli* must be regarded as faecally contaminated and unsafe, and requiring immediate remedial action.

Only strict attention to source protection and to the design and operation of efficient treatment and distribution will guarantee the exclusion of pathogens from drinking-water delivered to the consumer. For each water supply, the quality of the source water must guide the selection of the treatment processes, and due attention must be given to the ability of these processes to eliminate different pathogens (see Chapter 11). The microbiological water criteria presented here provide the means for demonstrating that these measures have been satisfactory at the time of sampling. The selection and design of water-treatment processes capable of achieving the necessary reductions in faecal and pathogenic agents will ensure that, if properly operated, these systems will always be able to produce water of the desired quality. This strategy is the only one that can be adopted in the case of pathogens, such as *Giardia*, *Cryptosporidium*, and viruses, that are more resistant than *E. coli* to terminal disinfection.

10.1.3 Water supplies for small remote communities

The provision of water supplies to small remote communities encounters particular problems worldwide, in that location, available facilities, and financial constraints often mean that only untreated water can be supplied, treatment is limited in extent because only local resources are available, or monitoring is infrequent or impossible. In such circumstances, sanitary assessment of the supply is all-important, and it is recommended that such assessments should be carried out periodically and at least yearly. In addition, the guideline values recommended here should be regarded as a goal for the future, not an immediate requirement. The guideline values recommended for the elimination of hazards may be very difficult to achieve under some conditions, and must then be applied, together with adequate methods of excreta disposal, in an appropriate manner depending on those conditions and on the availability of resources. Unless other sources of risk are adequately controlled, the effect of providing pure water on the transmission of diarrhoeal diseases may not be achieved.

The particular problems of supplies for small remote communities and their management are the subject of Volume 3 of *Guidelines for drinking-water quality*.

10.2 Bacteriological quality

Guideline values for bacteriological quality are summarized in Table 10.1, but they should not be used uncritically without reference to the information given in the text. It is most important that the reasons for adopting them are properly understood.
Table 10.1 Bacteriological quality of drinking-water

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Guideline value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All water intended for drinking</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> or thermotolerant coliform bacteria <em>b, c</em></td>
<td>Must not be detectable in any 100-ml sample</td>
</tr>
<tr>
<td>Treated water entering the distribution system</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> or thermotolerant coliform bacteria <em>b</em></td>
<td>Must not be detectable in any 100-ml sample</td>
</tr>
<tr>
<td>Total coliform bacteria</td>
<td>Must not be detectable in any 100-ml sample</td>
</tr>
<tr>
<td>Treated water in the distribution system</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> or thermotolerant coliform bacteria <em>b</em></td>
<td>Must not be detectable in any 100-ml sample</td>
</tr>
<tr>
<td>Total coliform bacteria</td>
<td>Must not be detectable in any 100-ml sample</td>
</tr>
<tr>
<td></td>
<td>Must not be detectable in any 100-ml sample. In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12-month period.</td>
</tr>
</tbody>
</table>

*a* Immediate investigative action must be taken if either *E. coli* or total coliform bacteria are detected. The minimum action in the case of total coliform bacteria is repeat sampling. If these bacteria are detected in the repeat sample, the cause must be determined by immediate further investigation.

*b* Although *E. coli* is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.

*c* It is recognized that, in the great majority of rural water supplies in developing countries, faecal contamination is widespread. Under these conditions, the national surveillance agency should set medium-term targets for the progressive improvement of water supplies, as recommended in Volume 3 of *Guidelines for drinking-water quality*.

It is self-evident and unquestionable that water intended for drinking must not contain agents of waterborne disease. However, many pathogens, including bacteria, viruses, and parasites, are difficult or even impossible to detect. For this reason, as already explained in Chapter 9, microbial indicators of water quality, i.e. bacteria indicating either the potential for faecal pollution or that such pollution has occurred, are used, since their presence shows that pathogens could also be present. The most numerous of the faecal indicator bacteria is the coliform group, and the most suitable member of this group is *Escherichia coli*, since it alone is derived exclusively from the faeces of humans and warm-blooded animals. In practice, thermotolerant coliform organisms or *E. coli* should not be detectable in any 100-ml sample of any water intended for drinking.

A further reason for adopting this criterion is that it is readily achievable by water treatment. Efficient treatment, together with terminal disinfection, should yield water free from coliform organisms, no matter how polluted the original water may have been. Furthermore, in nearly all epidemics of waterborne disease,
it can be shown that the bacteriological quality of the water was unsatisfactory and that there was evidence of contamination or a failure of terminal disinfection (1, 2).

In practice, the fact that *E. coli* can be found in wild and domestic animals and birds is not important because they can also carry pathogens infectious for humans.

During the passage of water from the treatment works to the consumer, its bacteriological quality may deteriorate. Members of the coliform group may be present in inadequately treated supplies or those contaminated after leaving the treatment plant, as a result either of growth on unsuitable materials in contact with the water (those used for washers, packing materials, lubricants, plastics and plasticizers, for example) or of entry from soil or natural water through leaky valves and glands, repaired mains, or back-siphonage. This type of post-contamination will most probably be found when the water is untreated or undisinfected, or where there is limited or no residual disinfectant. The occasional occurrence of coliform organisms in water in the distribution system or untreated supplies in up to 5% of samples taken over any 12-month period, in the absence of *E. coli*, can be regarded as acceptable. It should be stressed that the regular occurrence of these organisms, as opposed to their occasional and sporadic detection, in such samples must be a cause of concern.

Bottled natural mineral waters constitute a special case; their quality is the subject of Codex Alimentarius standards. The water must be collected and bottled under conditions such that it will retain its original quality. When such water is marketed, the Codex standard specifies that it shall not contain *E. coli*, coliform bacteria, group D streptococci, or *Pseudomonas aeruginosa* in 250-ml samples, provision being made for re-examination if not more than two coliforms (but not *E. coli*) or group D streptococci are found in 250 ml (3).

10.3 Virological quality

10.3.1 Rationale

It is essential that drinking-water supplies should be essentially free of human enteric viruses so that the risk of transmission of waterborne viral disease is negligible. It must be assumed that any drinking-water supply subject to faecal contamination exposes consumers to the risk of viral disease. There are thus two approaches to preventing viral contamination of drinking-water, namely: (i) providing drinking-water from a source that is free of faecal contamination; and (ii) producing drinking-water from a faecally contaminated source by treating it in a manner capable of reducing enteric viruses to a negligible level.

Although methods of concentrating and detecting low levels of viruses in water are available, they are too complex, expensive, and time-consuming for routine monitoring. Furthermore, not all relevant viruses can be detected by the methods currently available. As a result, failure to detect viruses in even very large
volumes of water does not prove that the water is virus-free and that consumers are not at risk of viral disease. In fact, a recent epidemiological–virological study indicated that drinking-water produced by conventional treatment from a faecally contaminated surface source might have been responsible for 25% of all gastrointestinal illness of probable viral etiology, even though the treated water was of acceptable microbiological quality (4).

Progress has recently been made in modelling, assessing, and predicting the risks of waterborne disease associated with drinking-water containing different concentrations of viruses and protozoan cysts (5). Although this has provided estimates of the health risks linked to the consumption of contaminated drinking-water, the modelling and risk analyses are based on limited dose–response data and require further refinement and verification; they are therefore not sufficiently developed to provide quantitative criteria for virus concentrations in drinking-water. Even if such health risk assessments for waterborne viruses were possible, the inability to monitor viruses in drinking-water reliably would preclude their practical application.

In the light of the foregoing, the guidelines for viruses in drinking-water presented in Table 10.2 are based on the probable virological quality of the source water and the required degree of treatment for source waters containing different levels of faecal contamination and hence different levels of viruses. The aim of these source-protection and water-treatment guidelines is to ensure that no viruses are present even in very large volumes of drinking-water.

10.3.2 Guidelines for groundwaters

If groundwater is obtained from a protected source and found to be free of faecal contamination, it can be assumed that the water is of acceptable virological quality for drinking if other essential criteria are met. The water source and its delivery system (casing, pump, pipes, and other appurtenances) must be free of faecal contamination from either surface (e.g. waste infiltration) or subsurface (e.g. septic tanks) sources. Specifically, the water must meet the guideline criteria for turbidity and pH (Table 10.2), bacteriological quality (Table 10.1), and parasitological quality (section 10.4)

Groundwater obtained from a protected source showing evidence of faecal contamination (1–20 E. coli per 100 ml) or exposed indirectly to obvious surface or subsurface sources of faecal contamination (e.g. wastewater infiltration of septic tanks) must be adequately disinfected to reduce enteric viruses to negligible levels. Adequate disinfection is defined (see Table 10.2) as the application of chlorine to achieve a free residual of at least 0.5 mg/litre after a minimum contact time of 30 minutes in water having a median turbidity not exceeding 1 NTU and a pH of <8.0, or an equivalent disinfection process in terms of virus inactivation. All such disinfection processes must produce at least 99.99% reduction of enteric viruses.

Groundwater sources and their delivery systems not adequately protected from either surface or subsurface faecal contamination, such as shallow dug wells
**Table 10.2 Recommended treatments for different water sources to produce water with negligible virus risk**

<table>
<thead>
<tr>
<th>Type of source</th>
<th>Recommended treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ground water</strong></td>
<td></td>
</tr>
<tr>
<td>Protected, deep wells; essentially free of faecal contamination</td>
<td>Disinfection</td>
</tr>
<tr>
<td>Unprotected, shallow wells, faecally contaminated</td>
<td>Filtration and disinfection</td>
</tr>
<tr>
<td><strong>Surface water</strong></td>
<td></td>
</tr>
<tr>
<td>Protected, impounded upland water; essentially free of faecal contamination</td>
<td>Disinfection</td>
</tr>
<tr>
<td>Unprotected impounded water or upland river; faecal contamination</td>
<td>Filtration and disinfection</td>
</tr>
<tr>
<td>Unprotected lowland rivers, faecal contamination</td>
<td>Pre-disinfection or storage, filtration, disinfection</td>
</tr>
<tr>
<td>Unprotected watershed, heavy faecal contamination</td>
<td>Pre-disinfection or storage, filtration, disinfection, additional treatment and disinfection</td>
</tr>
<tr>
<td>Unprotected watershed; gross faecal contamination</td>
<td>Not recommended for drinking-water supply</td>
</tr>
</tbody>
</table>

---

*a* For all sources, the median value of turbidity before terminal disinfection must not exceed 1 nephelometric turbidity unit (NTU) and must not exceed 5 NTU in single samples.

Terminal disinfection must produce a residual concentration of free chlorine of $\geq 0.5$ mg/litre after at least 30 minutes of contact in water at pH $\leq 8.0$, or must be shown to be an equivalent disinfection process in terms of the degree of enterovirus inactivation ($\geq 99.99\%$).

Filtration must be either slow sand filtration or rapid filtration (sand, dual, or mixed media) preceded by adequate coagulation-flocculation (with sedimentation or flotation). Diatomaceous earth filtration or a filtration process demonstrated to be equivalent for virus reduction can also be used. The degree of virus reduction must be $\geq 90\%$.

Additional treatment may consist of slow sand filtration, ozonation with granular activated carbon absorption, or any other process demonstrated to achieve $\geq 99\%$ enterovirus reduction.

*b* Disinfection should be used if monitoring has shown the presence of E. coli or thermotolerant coliform bacteria.

---

or other unsealed or uncased wells, may be used as drinking-water sources if their E. coli count does not exceed 2000 per 100 ml and the water is treated by filtration and disinfection. Unprotected groundwaters containing more than 2000 E. coli per 100 ml are considered grossly faecally contaminated and are not recommended as water-supply sources regardless of treatment, unless no higher-quality water sources are available. In this situation, the water must be treated by at least three unit processes each of which is individually capable of reducing viruses, as prescribed for contaminated surface water (see Table 10.2).

### 10.3.3 Guidelines for surface water sources

In general, surface waters are never completely pure and will always be subject to some degree of faecal contamination, so that treatment to reduce viruses to negligible levels will always be required. The quality of the source water in terms of the degree of faecal contamination (as defined by E. coli counts in the raw source water) will determine the degree of treatment required (see Table 10.2).
For source waters derived from protected watersheds essentially free of human faecal contamination, possibly subject only to low levels of faecal contamination from indigenous animals, and containing fewer than 20 \( E. coli \) per 100 ml, the required degree of treatment is adequate disinfection. However, it is essential that the source water should have a median turbidity not exceeding 1 NTU and a maximum turbidity not exceeding 5 NTU in single samples in order to ensure adequate virus inactivation by disinfection. Surface water sources that meet these virological criteria may nevertheless be contaminated with unacceptable levels of \textit{Giardia} cysts and \textit{Cryptosporidium} oocysts, neither of which can be adequately controlled by disinfection treatment to control viruses. If there is a risk of protozoal contamination, the source water may have to be treated by means of strictly controlled coagulation and filtration to ensure that these agents are removed. Where necessary, special investigations can be conducted to determine whether source water contamination by protozoan cysts or oocysts is probable or demonstrable.

Surface waters from inadequately protected watersheds contaminated by both human and animal faeces and containing 20–2000 \( E. coli \) per 100 ml must be treated by both filtration and disinfection in order to reduce enteric viruses to negligible levels. Because such waters are likely to contain protozoal cysts or oocysts as well as enteric viruses, filtration and disinfection must be adequate to control both of these classes of pathogens.

Surface waters from inadequately protected watersheds heavily contaminated by human and animal faeces and having \( E. coli \) counts of over 2000 per 100 ml but not more than 20 000 per 100 ml, will require extensive treatment consisting of filtration, disinfection, and at least one other process (e.g. long-term storage or an additional filtration or disinfection process) capable of producing additional reduction of viruses of >99%. Such surface waters are clearly inferior as sources of drinking-water and should be used only when no other source of higher quality is available. If such a source is used, the local authorities will have to bear the considerable burden of ensuring that the treatment is properly designed, operated, and maintained and that there is adequate monitoring and surveillance of the water system and its water quality to ensure a continuous supply of acceptable virological quality.

Surface waters from inadequately protected sources containing more than 20 000 \( E. coli \) per 100 ml are considered to be grossly faecally contaminated and hence unsuitable for drinking-water supply regardless of the extent and type of treatment. Production of drinking-water from such a source carries a great risk of inadequate virological quality and would be undertaken only under the most extraordinary circumstances.

10.4 Parasitological quality

It is not possible to set guideline values for pathogenic protozoa, helminths, and free-living organisms, other than that these agents should not be present in
drinking-water, because only one or very few organisms are required for humans to become infected. The analytical methods for protozoan pathogens are expensive and time-consuming and cannot be recommended for routine use. Methods for concentrating the resting stages of *Giardia* and *Cryptosporidium* from large volumes of water are being standardized. When facilities are available for studying the incidence of these parasites in surface water, these methods could be used for measuring the efficiency of different water-treatment processes in removing them and the incidence of carriage of these parasites by animal vectors in the watershed. A better understanding of information on the epidemiology and zoonotic relationships of these parasites from the point of view of human health will then be possible.

The control of parasitic disease and of invertebrate animal life in water mains is best accomplished by means of appropriate treatment.

### 10.5 Monitoring

#### 10.5.1 Approaches and strategies

The monitoring of drinking-water quality ideally consists of the following components:

- the control of quality on a routine basis to verify that treatment and distribution comply with the prescribed objectives and regulations;
- the surveillance, usually at specified intervals, of the entire water-supply system from source to consumer from the point of view of microbiological safety.

Continuous control is an integral part of the responsibilities of the water-supply agency, through which the waterworks management ensures the satisfactory performance of the treatment processes, the quality of the water produced and the absence of secondary contamination within the distribution network. In principle, an independent body should verify that the waterworks correctly performs its duties. This surveillance is usually the responsibility of the local, regional and national health authorities.

#### 10.5.2 Sampling frequencies and procedures

The frequency of sampling will be determined by the resources available. The more frequently the water is examined, the more likely it is that chance contamination will be detected. Two main points should be noted. Firstly, the chance of detecting pollution which occurs periodically, rather than randomly, is increased if samples are taken at different times of the day and on different days of the week. Secondly, frequent examination by a simple method is more valuable than frequent examination by a complex test or series of tests. Sampling frequencies for raw water sources will depend on their overall quality, their size, the likelihood of contamination, and the season of the year. They should be established by local control agencies and are often specified in national regulations and guide-
lines. The results, together with information from the sanitary inspection of the gathering grounds, will often indicate whether increased vigilance is needed.

Sampling frequencies for treated water leaving the waterworks depend on the quality of the water source and the type of treatment. Minimum frequencies are one sample every 2 weeks for waterworks with a groundwater source, and one sample every week for waterworks with a surface water source.

The frequency of sampling should be greater where a large number of people are supplied because of the larger number of people at risk. Advice on the design of sampling programmes and on the frequency of sampling is given in ISO standards (see Table 9.1) and in many national regulations. The minimum number of samples to be taken each month for water in the distribution system is given for different population sizes in Table 10.3.

**Table 10.3 Minimum sampling frequencies for drinking-water in the distribution system**

<table>
<thead>
<tr>
<th>Population served</th>
<th>Samples to be taken monthly</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 5000</td>
<td>1 sample</td>
</tr>
<tr>
<td>5000–100 000</td>
<td>1 sample per 5000 population</td>
</tr>
<tr>
<td>More than 100 000</td>
<td>1 sample per 10 000 population, plus 10 additional samples</td>
</tr>
</tbody>
</table>

Samples should be taken at random intervals in each month from fixed points, such as pumping stations and tanks, from random locations throughout the distribution system and from taps connected directly to the mains in houses and large multi-occupancy buildings, where there is a greater risk of contamination through cross-connections and back-siphonage. The frequency of sampling should be increased at times of epidemics, flooding, and emergency operations, or following interruptions of supply or repair work. With systems serving small communities, periodic sanitary surveys are likely to yield more information than infrequent sampling.

No general recommendation can be made for unpiped supplies and untreated water because the quality and likelihood of contamination will vary seasonally and with local conditions. The frequency should be established by the local control agency and reflect local conditions, including the results of sanitary surveys.

Detailed advice on the procedures to be used for sampling different sources of water or treatment plants and distribution systems and at the tap are given in Volume 3 of *Guidelines for drinking-water quality* and in standard methods (6, 7) (see Table 9.1). However, the following general points should be noted.

Care must be taken to ensure that samples are representative of the water to be examined and that no accidental contamination occurs during sampling. Sample collectors should therefore be trained and made aware of the responsible
nature of their work. Samples should be clearly labelled with the site, date, time and other relevant information, and sent to the laboratory for analysis without delay.

If the water to be examined is likely to contain chlorine, chloramine, chlorine dioxide, or ozone, sodium thiosulfate should be added to neutralize any residual disinfectant. Properly controlled, the concentration of thiosulfate has no significant effect on the coliform organisms, including *E. coli*, either in chlorinated or in unchlorinated water samples during storage (6). If heavy metals, particularly copper, are present, chelating agents (e.g. edetic acid (EDTA) or nitrilotriacetic acid (NTA)) should also be added.

When samples of disinfected water are taken, the concentration of residual disinfectant at the sampling point and the pH should be determined at the time of collection.

When a number of samples are to be taken for various purposes from the same location, the sample for bacteriological examination should be collected first to avoid the risk of contamination of the sampling point.

Samples must be taken from different parts of the distribution system to ensure that all parts of it are tested. When streams, lakes, or cisterns are being sampled, the water must be taken from below the surface, away from banks, sides of tanks, and stagnant zones, and without stirring up sediments. Taps, sampling ports, and the orifices of pumps should, if possible, be disinfected and a quantity of water run to waste to flush out the stagnant water in the pipe before the sample is taken. Sampling ports in treatment processes and on water mains must be carefully sited to ensure that samples are representative. The length of pipework to the tap should be as short as possible.

The changes that may occur in the bacterial content of water on storage can be reduced to a minimum by ensuring that samples are not exposed to light and are kept cool, preferably between 4 and 10 °C, but not frozen. Examination should begin as soon as possible after sampling and certainly within 24 hours. If samples cannot be cooled, they must be examined within 2 hours of sampling. If neither condition can be met, the sample should not be analysed.

10.5.3 Surveillance programme requirements

Surveillance is the continuous and vigilant public health assessment and review of the safety and acceptability of drinking-water supplies. Each component of the drinking-water system—the source, treatment, storage, and distribution—must function without risk of failure. A failure in one part will jeopardize and nullify the effects of other parts that function perfectly, as well as the care that has been taken to ensure that they do so. Water is liable to contamination at all stages in the process of supply, hence the need for constant vigilance. At the same time, careful and intelligent assessment of likely sources of risk and breakdown is necessary before a supply system is planned and installed and, indeed, continuously thereafter, because of possible changes in conditions and potential sources of con-
tamination. Contingency plans must be made to deal with any emergencies that may arise through natural or man-made disasters, such as accidents, wars, and civil commotions, or the cessation of supplies of essential chemicals used in treatment.

An essential part of surveillance is the establishment of a proper network for regulation and command. At government level, this means the establishment and enforcement of national standards, and the promulgation of national guidelines for achieving compliance with the laws and standards; for the water-supply agency, it means promotion of local codes of good waterworks practice together with formal instruction and training. A national inspectorate should be established to ensure that the legal requirements are met and standards complied with. This body should be separate from that representing the interests of the water provider.

Both the water provider and the inspectorate should have properly equipped laboratory facilities staffed by trained and properly qualified personnel, adequate facilities for sustaining at all times the level of monitoring required, and sufficient capacity to carry out additional examinations as required to meet special needs. Operational staff at the waterworks, should also be appropriately trained and qualified.

Lines of communication and command must be established at the outset and must be properly understood by all staff up to the highest levels. This will ensure the effective functioning of day-to-day operations and also that immediate remedial action is taken in emergencies and when contamination is discovered. Bacteriological failures must be acted on as soon as discovered, so that the microbiologist must have the authority to instruct the engineer and the operational staff to take the necessary action. The lines of communication needed in an emergency will be complex, involving not only different public bodies but also authorities responsible for different geographical regions. Appropriate instructions must be laid down and understood at each site.

The scope of surveillance, together with examples covering the points made in this section, has been considered in a separate WHO publication, which should be consulted (8). The importance of surveillance is highlighted repeatedly in official reports of serious outbreaks of waterborne disease, which usually reveal deficiencies in more than one area (1, 2, 9). Surveillance procedures are described further in Volume 3 of Guidelines for drinking-water quality.

The levels of surveillance of drinking-water quality differ widely in developing countries in line with the differences in economic development and the provision of community water supplies in those countries. Surveillance should be progressively developed and expanded by adapting the level to the local situation and economic resources, with gradual implementation, consolidation, and development to the level ultimately desired.
10.6 Action to be taken when contamination is detected

No surface water can be assumed to be free from enteric pathogens, including viruses and parasites, since they can be derived from wild or farm animals living in the catchment area as well as from human faecal contamination. The geographical and seasonal distribution of specific pathogens in natural waters can provide valuable information on the epidemiology of disease in animal populations and the routes of transmission to the human population. Such information also indicates the precautions needed to safeguard the sources of water and the degree of treatment needed.

The occurrence of any pathogenic agent, bacterial, viral, or animal, in a drinking-water supply is always a matter for the gravest concern, demanding immediate attention to treatment and to determining the cause. The examination of drinking-water for a particular pathogen will most probably be required when the water is suspected to be the cause of an outbreak of disease in the community supplied with the water. The finding of the causal agent of the disease in the water, together with the distribution of primary cases among those using it, prove that the supply is implicated, particularly if the disease is not found among those not using the water and not acquiring infection secondarily.

10.6.1 Bacterial indicators of faecal contamination

The finding of *Escherichia coli* in any sample of water intended for drinking is a matter for concern, since it indicates that the water has been recently contaminated by faecal material from humans or animals, and that there is a likelihood that pathogens will also be present. If the water is a treated piped supply, there is also the strongest reason for suspecting that there has been a breakdown in disinfection, treatment before disinfection has failed, or contaminated water has entered the system. Immediate action must be taken to discover the source of contamination and to increase the dosage of disinfectant so as to ensure that an adequate residual is present in the water delivered to the consumer, until the problem is overcome. Consideration should be given to telling consumers to boil water intended for drinking. If the quality of water leaving the works is satisfactory, ingress of contaminated water will most usually arise as a result either of damage to the distribution system or repairs to it, or through infiltration into underground service reservoirs or directly into the mains as a result of low pressure and back-siphonage or cross-connections. Where treatment is minimal, because source water is normally of high quality, sudden deterioration resulting from storms, flooding, or massive pollution incidents will mean that the disinfection applied is inadequate. Prior assessment of hazards by sanitary survey or by the establishment of “early-warning” monitoring systems in the catchment area will help avoid such events.

A finding of thermotolerant or total coliform bacteria in the presumptive test demands instant attention. The positively reacting tubes or colonies must be
examined further using confirmatory tests, and for the presence of *E. coli*. The water must immediately be resampled from the same source. The waterworks engineer must be informed at once, so that investigations can be made to discover the source of the contamination. Such action must be regarded as the minimum. Where the failure concerns water leaving the treatment works, investigations and corrective action as outlined in the previous paragraph are necessary immediately and before the results of the confirmatory test are known.

The finding of total coliform organisms, in the absence of thermotolerant coliforms or *E. coli*, in a treated piped supply usually indicates post-treatment contamination, or growth on pipes or fittings, when the treated water entering the supply system is satisfactory. It suggests either that materials coming into contact with water, such as those used for pipes, washers, pipe sealants, and packings, or rubber and plastics used for other purposes, are supporting growth or that untreated water is entering the distribution network. Because total coliforms of nonfaecal origin can exist in natural waters, their presence can occasionally be tolerated in unpiped or untreated water, if thermotolerant coliform bacteria and *E. coli* are absent. If they are present repeatedly or in consecutive samples, as indicated in Table 10.1, action must be taken to improve the sanitary protection of the source.

In temperate or cold climates, the finding of total or thermotolerant coliform bacteria in the presumptive test leads in a high proportion of cases to confirmation of the presence of *E. coli* and therefore of evidence of faecal contamination. This may be less common in tropical and semitropical regions, particularly where the water is untreated. Nevertheless, the indication must not be ignored for the reasons given in section 9.2. If desired, the faecal origin of such coliform organisms can be confirmed using the faecal streptococcus and sulfite-reducing clostridia tests.

10.6.2 Miscellaneous indicators

Occasionally, and particularly where the source water is derived from lowland rivers and where the water temperatures in the distribution system are 20 °C or higher, *Aeromonas* spp. can occur and will interfere with the interpretation of the total coliform tests. At these temperatures and where the free chlorine residual is below 0.2 mg/litre, these bacteria are able to grow on assimilable organic carbon in the water. Similar significance attaches to the finding of *Pseudomonas aeruginosa* in supply systems. Both these organisms can occur in the absence of coliform bacteria and will interfere with the interpretation of the coliform tests. Their sanitary significance is unclear, although they can be opportunistically pathogenic and are undesirable where water is used in the manufacture of food and drink, or is supplied to hospitals. Measures to eradicate them must be taken, and may include eliminating unsatisfactory materials in contact with the water, cleaning distribution systems and plumbing in the buildings affected, and maintaining adequate residual disinfectant in the supply.

Bacteria recovered in colony counts at 22 °C are without sanitary significance. However, the occurrence of such bacteria in numbers exceeding 100 per
100 ml in piped water may indicate enrichment of the water with assimilable organic carbon. Large numbers are undesirable in water used for preparing food and drink or for bottling. Any increase in the numbers of colonies above normal levels when counts are made at 37 °C should be regarded with suspicion, since it may be caused by the onset of polluting conditions, particularly if not accompanied by an increase in the count at 22 °C. An increase in the count at 37 °C is often a valuable indication of undesirable changes and should prompt an investigation of the supply or of the gathering grounds, if the water is untreated.

The presence of macroscopic animal life in drinking-water is aesthetically objectionable if nothing else. With piped supplies, it is an indication that flushing and cleaning of the distribution system are needed (see section 8.2). Their occurrence may sometimes reflect unusually high water temperatures as, for example, when chironomid larvae are discharged from slow sand filters into the treated water.

References


The emphasis in this section is on protecting and improving the microbiological quality of drinking-water. It is a basic principle, long established as a result of the lessons learned from serious outbreaks of waterborne disease, that a single barrier to the spread of pathogenic organisms is not sufficient to ensure the purity of drinking-water (I, 2). Purity is not the only requirement, however; the drinking-water supply must also be capable of meeting the anticipated demand. Inadequate supply, together with geographical factors, often means that raw water of poor microbiological quality and possibly containing significant amounts of wastewater has to be used. A second principle is that to ensure that the drinking-water delivered to the consumer is free from pathogens, the level of treatment should be related to the degree of pollution expected in the source water. In the case of contaminated water sources, several treatment processes, designed primarily for such water will be necessary. Together, these processes will progressively remove pathogens and other contaminants from raw water and consistently produce a safe and wholesome supply of drinking-water. Ideally, safety should be achieved before the final treatment step, so that the failure of any one process will not result in waterborne disease, i.e., the system is fail-safe. The protection of the source from pollution and the provision of adequate and properly operated treatment processes constitute the essential barriers to the transmission of disease on which the supply of wholesome water depends (I, 2).

11.1 Water sources

11.1.1 Selection of sources

Before a new source of drinking-water supply is selected, it is important to ensure that: (1) the quality of the water is satisfactory or can be improved by treatment to make it suitable for drinking; (2) the source will yield enough water to meet the needs of the community not only under the normal conditions of the average annual cycle but also under conditions which are unusual but can be expected, say, once in 10 years; (3) under normal abstraction conditions, the change in local water flow patterns will not cause any unacceptable deterioration in the quality of the water abstracted; and (4) the water to be abstracted can be protected against pollution.
A full sanitary and microbiological survey of the catchment area should be carried out to locate all the sources of pollution. It may be that the treatment or diversion of a small polluting discharge could make a large potential source acceptable (3). If remote upland sources of surface waters are being considered, it is important to assess whether access by people or livestock can be prevented and whether wild animals are likely to be vectors of salmonellae, Giardia, and Cryptosporidium.

11.1.2 Source protection

In the past, isolation of the watershed from human activity was an important means of protecting a waterway or aquifer from contamination. The rising cost of land and increases in population have made this procedure more costly and difficult, especially when new sources must be found in an area that is already developed. It is still desirable for water suppliers to own or control the land to the extent that this is feasible (1).

Another line of defence is to prevent polluting activities in the area which may be a source of infection. This means, for instance, defining areas where sewage sludge may not be applied, and exercising strict control over discharges of sewage effluents and agricultural wastes, the location of sites for the dumping of garbage and toxic wastes, and drilling, mining, and quarrying. Control of such activities does not necessarily mean that they should be banned, but that, in the interests of public health, they should be licensed and open to inspection and monitoring whenever water quality could be affected. Where potentially harmful substances are handled or made, steps should be taken to ensure that any effluents are either adequately treated or conveyed safely over the catchment area (3).

Sources of groundwater such as springs and wells should be sited and constructed so as to be protected from surface drainage and flooding. Zones of groundwater abstraction should be fenced off to prevent public access, kept free from rubbish, and sloped to prevent the formation of pools in wet weather. Animal husbandry should be adequately controlled in such zones (1, 3).

Protection of open surface water is difficult. It may be possible to protect a reservoir from major human activity, but the protection of a river, if possible at all, may be feasible only over a limited stretch. Often it is necessary to accept existing and historical uses of a river or lake and to design the treatment accordingly. Adequate sewage treatment is important in preserving water quality at downstream intakes (3).

11.2 Treatment processes

The fundamental purpose of water treatment is to protect the consumer from pathogens and from impurities in the water that may be injurious to human health or offensive. Where appropriate, treatment should also remove impurities which, although not harmful to human health, may make the water unappealing
to drink, damage pipes, plant, or other items with which the water may come into contact, or render operation more difficult or costly.

These purposes are achieved, as previously mentioned, by introducing successive barriers, such as coagulation, sedimentation (or flotation), and filtration, to remove pathogens and impurities. The final barrier is disinfection. The function of the entire system, and indeed of much of water treatment, may with some justification be regarded as that of conditioning the water for effective and reliable disinfection (3).

11.2.1 Storage

The storage of water in reservoirs creates favourable conditions for the self-purification of the stored water, but may also cause undesirable changes in water quality. The benefits of storage include the provision of a continuous supply of water, reduction in turbidity, reduction in pathogens through the action of sunlight and sedimentation, dilution of undesirable substances that may accidentally enter the intake, and oxidation of impurities. It also provides a buffer should pollution occur in the river. Undesirable conditions created by storage include those associated with the production of algae, pollution by birds and animals, evaporation, and the leaching of iron and manganese from soils and rocks (2, 3).

Reservoirs should either be constructed in series or designed to prevent short-circuiting, since this will enhance removal of pathogens and self-purification. The benefits of reservoir storage are greatest in the summer and when residence periods are about 3-4 weeks.

11.2.2 Presedimentation

Highly turbid surface water may require presedimentation before further treatment. Presedimentation basins are constructed in excavated ground or of steel or concrete. Such basins may be preceded by equipment for the addition of chemicals to provide partial coagulation during periods when the water is too turbid to clarify by sedimentation alone.

11.2.3 Prechlorination

Prechlorination to breakpoint has been widely used as an alternative to storage for water derived from lowland rivers and is also used when stored water contains much planktonic life. Its purpose is to reduce counts of fecal bacteria and pathogens, destroy animal life and algae, and oxidize ammoniacal nitrogen, iron, and manganese, thereby assisting in their removal. The combined and free chlorine which remains effectively discourages microbial activities, such as protozoal predation and nitrification, as well as microbial growth during subsequent filtration. When used to disinfect raw water, the oxidative effect of chlorine and even more of ozone will result in the partial conversion of total organic carbon into bio-
degradable organic carbon which, if not removed by biological activity during treatment (e.g. during slow sand or granular activated carbon filtration), can result in the growth of nuisance organisms during distribution. Prechlorination of organically enriched surface waters has been shown to produce a substantial increase in the content of trihalomethanes and is often a wasteful use of chlorine. It is important to balance the maintenance of the microbiological safety of drinking-water against possible hazards associated with the formation of such disinfection by-products.

11.2.4 Coagulation and flocculation

To remove particulate matter, a water-treatment plant will generally include equipment for coagulation and flocculation, followed by sedimentation and filtration.

Coagulation involves the addition of chemicals (e.g. aluminium sulfate, ferric sulfate) to neutralize the charges on particles and facilitate their agglomeration during the slow mixing provided in the flocculation step (1). Flocs thus formed co-precipitate, adsorb and entrap natural colour and mineral particles, and can bring about major reductions in counts of protozoa, faecal bacteria, pathogens, and viruses.

Coagulation and flocculation require a high level of operational skill. Chemical dosages and pH control must be correct, and the plant must be designed to ensure proper floc formation. Before it is decided to use coagulation as part of a treatment process, careful consideration must be given to the likelihood of process stability, the availability of regular supplies of chemicals, and the need for qualified personnel (3).

11.2.5 Sedimentation or flotation

The purpose of sedimentation is to permit settleable floc to be deposited and thus reduce the concentration of suspended solids that must be removed by filters (2). Flotation is an alternative to sedimentation, and has advantages when the amount of floc is small.

The factors that influence sedimentation include the size, shape, and weight of the floc, viscosity and hence the temperature of the water, the detention time, the number, depth, and areas of the basins, the surface overflow rate, the velocity of flow, and the inlet and outlet design (1, 2).

Arrangements must be made for the collection and safe disposal of sludge from sedimentation tanks.

11.2.6 Rapid filtration

Typically, rapid sand filters consist of 0.4–1.2 m of sand, usually of an effective size of 0.5–1.0 mm, supported by gravel and underdrains. In recent years, single-
medium filters have often been replaced by dual-medium or multimedia ones. During filtration, residual particles of floc not removed by sedimentation are trapped in the interstices of the bed, and may induce further flocculation of particles. A limited amount of biological activity may also occur, if it is not suppressed by prechlorination or by high flow rates. Both sand and mixed-media filters are normally cleaned by reversal of the flow though the bed (backwashing). Backwash water is either discharged to the sewer or drying beds or recycled after removal of sludge.

The performance of rapid filters in removing microorganisms and turbidity varies over the duration of the run between backwashings. Immediately after backwashing, performance is poor, until the bed has compacted. In some plants, water is filtered and diverted for recycling for 15-30 min at the start of each filter run. In some waterworks, a 30-min slow start for each filter run is included to prevent the initial breakthrough. Performance will also deteriorate progressively when backwashing is needed, since floc may escape through the bed into the treated water, thereby increasing its turbidity. In view of the foregoing, proper supervision and control of filtration at the waterworks are essential.

11.2.7 Slow sand filtration

Typically, slow sand filters consist of 0.5-1.5 m of silica sand with an effective size of 0.3-0.6 mm. The upper layer of fine sand is supported on gravel and a system of underdrains.

Slow sand filtration is simpler to operate than rapid filtration, as frequent backwashing is not required (4, 5). It is therefore particularly suitable for developing countries and small rural systems, but is applicable only if sufficient land is available. On the other hand, the filters are readily clogged by algal blooms and do not remove heavy metals and many micropollutants efficiently. They effectively remove biodegradable organic carbon and oxidize ammonia.

When the filter is first brought into use, a microbial slime community (schmutzdecke) develops at the surface of the bed. This consists of bacteria, free-living ciliated protozoa and amoebae, crustacea, and invertebrate larvae acting in food chains, resulting in the oxidation of organic substances in the water and of ammoniacal nitrogen to nitrate. Pathogenic bacteria, viruses, and resting stages of parasites are removed, principally by adsorption on to the schmutzdecke and by subsequent predation. When correctly loaded and operated, slow sand filtration is capable of bringing about a great improvement in water quality (6). Slow sand filters, operated at a filtration rate of 1.1-4.2 m/day, were able to remove 97-99% of enteroviruses at water temperatures of 6-11 °C. This was somewhat greater than for E. coli, and removal was greatest when the water was warmest (7). A slow sand filter is more efficient than any other process in removing parasites (helminths and protozoa). Nevertheless, the effluent from such a filter might well contain a few E. coli and viruses, especially during the early phase of a filter run. It is usual to divert or recycle the filtered water produced immediately after
commissioning or cleaning a filter bed until the *schmutzdecke* has been established and become effective.

11.2.8 Infiltration

Surface water can also be treated by infiltration of the raw or partly treated water into river banks or sand dunes, followed by underground passage; this is an effective means of removing undesirable microorganisms and viruses. Infiltration is applicable only in areas where suitable geological conditions exist. Pretreatment is required to prevent clogging of the infiltration area. In addition, water abstracted from the aquifer usually needs some additional treatment, such as aeration and filtration, to remove, e.g., iron and manganese present in anaerobic groundwater. The residence time underground should be as long as possible to obtain a water comparable in quality to groundwater.

11.2.9 Disinfection

The overall objective of disinfection is to ensure that the quality criteria specified in Table 10.1 (see p. 95) are always met.

Terminal disinfection of piped drinking-water supplies is of paramount importance and is almost universal, since it is the final barrier to the transmission of waterborne bacterial and viral diseases. Although chlorine and hypochlorite are most often used, water may also be disinfected with chloramines, chlorine dioxide, ozone, and ultraviolet irradiation (3, 8).

The efficacy of any disinfection process will depend on the degree of purity achieved by prior treatment, as disinfectants are highly active and will be neutralized to a greater or lesser extent by organic matter and readily oxidizable compounds in water. Microorganisms that are aggregated or adsorbed on to particulate matter will also be partly protected from disinfection. It is therefore recommended that the median turbidity of water before disinfection should not exceed 1 NTU; it should not exceed 5 NTU in any individual sample.

Practical experience has shown that the kinetics of the disinfection of drinking-water follow the first-order model of Chick's law, in which the fraction of the original population surviving, \( x_t/x_0 \), after treatment for a time \( t \) is given by

\[
x_t/x_0 = e^{-kt}
\]

where \( k \) is the specific death rate. This law is based on the assumption that all the agents being removed are equally sensitive to the disinfectant and that they are randomly distributed and not clumped together.

The specific death rate with disinfection, \( k \), or the contact time, \( t \), required to kill a given percentage of the original population is usually proportional to the concentration, \( C \), of disinfectant, as in Watson’s empirical dilution law:

\[
C^n t = k
\]

where \( k \) is a constant of proportionality and \( n \) is the dilution exponent. For water disinfection, the value of \( n \) is close to 1, and it is therefore convenient to express
the product of the concentration and the time required to bring about 99% removal of a given agent as a \( Ct \) value. This must be done with caution, because it is assumed that Chick's law is followed and that the conditions of disinfection (temperature, pH, chemical composition of the water, its disinfectant demand, and the physiological state of the agents being disinfected) are constant (9). Table 11.1 lists \( Ct \) values for different agents and disinfectants, and shows that, of the microorganisms listed, *E. coli* is generally the most sensitive, that the three viruses differ in sensitivity not only among themselves but also to the different disinfectants, and that the parasites *Giardia* and *Cryptosporidium* are the most resistant (10-12). Table 11.1 also indicates that normal chlorination conditions (i.e. free chlorine residual of 0.5 mg/litre, a contact time of 30 min, pH less than 8.0, and water turbidity less than 1 NTU) can be expected to bring about reductions greatly in excess of 99% for *E. coli* and the viruses specified but not for the parasitic protozoa.

Table 11.1 \( Ct \) values (mg.min/l) for 99% inactivation of various agents by disinfectants at 5 °C

<table>
<thead>
<tr>
<th>Agent</th>
<th>Free chlorine pH 6-7</th>
<th>Preformed chloramine pH 6-9</th>
<th>Chlorine dioxide pH 6-7</th>
<th>Ozone pH 6-7</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>0.034-0.05</td>
<td>95-180</td>
<td>0.4-0.75</td>
<td>0.02</td>
</tr>
<tr>
<td>Poliovirus type 1</td>
<td>0.1-2.5</td>
<td>76-3720</td>
<td>0.2-6.7</td>
<td>0.1-0.2</td>
</tr>
<tr>
<td>Hepatitis A virus</td>
<td>1.8</td>
<td>68-590</td>
<td>1.7</td>
<td>-</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>0.01-0.05</td>
<td>3810-6480</td>
<td>0.2-2.1</td>
<td>0.006-0.006</td>
</tr>
<tr>
<td><em>Giardia lamblia</em> cysts</td>
<td>47-&gt;160</td>
<td>-</td>
<td>-</td>
<td>0.5-0.6</td>
</tr>
<tr>
<td><em>Giardia mumps</em> cysts</td>
<td>30-630</td>
<td>-</td>
<td>7.2-18.5</td>
<td>1.8-2.0</td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em> oocysts</td>
<td>-</td>
<td>-</td>
<td>6.5-6.9</td>
<td>&lt;3.9-6.4</td>
</tr>
<tr>
<td><em>Cryptosporidium parvum</em> oocysts from human faeces</td>
<td>7.7 x 10^5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Calculated from data in references 10-12

It is thus clear that great care is needed to ensure that the treatment processes preceding terminal disinfection are operated correctly to ensure the effective removal of pathogens. There are many instances of disinfection failure when turbidity was 5 NTU or more. It is therefore axiomatic that, for successful disinfection, turbidity should always be less than 5 NTU and preferably less than 1 NTU.

As with chemical disinfection, ultraviolet disinfection is more effective against vegetative bacteria than against viruses and bacterial spores, while protozoal cysts are the most resistant. Data show that the minimum dosage recommended, 16 mW.s/cm², is sufficient to inactivate more than 99.9% of vegetative
bacteria but not the other agents (10). Disinfection by ultraviolet radiation is applicable only to clear waters, since appreciable turbidity or dissolved organic carbon will attenuate the radiation. Although there is no residual effect of disinfection by ultraviolet radiation, this is not a drawback when the water has been treated to a high standard to remove biodegradable organic carbon and where the water distribution system is well maintained.

11.3 Choice of treatment

11.3.1 Microbiological conditions

In rural areas supplying small communities, protection of the source of water may be the only "treatment" possible. Such supplies are considered in detail in Volume 3 of Guidelines for drinking-water quality. In large communities, the demand for water is high and can often be met only by using sources of poor microbiological quality.

Two considerations are of paramount importance: firstly, the quality of drinking-water is totally dependent on protection of the source, treatment of the water, and maintenance of the integrity of the distribution system; and secondly, microbiological monitoring can influence water quality only if its findings, and those of the agency responsible for surveillance, are made known to the water engineer and any remedial measures necessary are implemented.

11.3.2 Treatment of groundwater

Groundwater extracted from well protected aquifers is usually free from pathogenic microorganisms, and the distribution of such groundwater without treatment is common practice in many countries. However, the catchment area must be protected by effective regulatory measures and the distribution system adequately protected against secondary contamination of the drinking-water. If the water, in its passage from source to consumer, cannot be protected at all times, disinfection and the maintenance of adequate chlorine residuals are imperative.

11.3.3 Treatment of surface water

The extent to which faecal bacteria, viruses, and parasites are removed by properly designed and operated equipment for flocculation, coagulation, sedimentation, and rapid filtration is equivalent to that achieved by slow sand filtration.

Additional treatment, such as ozonation, will have a considerable disinfecting action besides converting part of the total organic carbon into a biodegradable form. If it is followed by activated carbon treatment or other biological filtration stage, some of the biodegradable organic carbon will be removed by microbial activity, thus reducing the potential for aftergrowth of nuisance bacteria in distribution networks.
Disinfection should be regarded as obligatory for all piped supplies of surface water, even if derived from high-quality, unpolluted sources, since there should always be more than one barrier against the transmission of infection by a water supply. In large, properly run waterworks, the criteria for the absence of E. coli and coliform bacteria can then be met with a very high degree of probability.

Table 11.2 shows that conventional urban water treatment relies on pretreatment and terminal disinfection to remove much of the microbial contamination. Nevertheless, conventional treatment can be effectively operated as a three-stage multiple-barrier system involving: (1) coagulation and settling or flotation; (2) rapid filtration; and (3) terminal disinfection.

Table 11.2 An example to illustrate the level of performance that can be achieved in removal of turbidity and thermotolerant coliform bacteria in conventional urban water treatment

<table>
<thead>
<tr>
<th>Stage and process</th>
<th>Turbidity</th>
<th>Thermotolerant coliform bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Removal(^a)</td>
<td>Average loading (NTU)(^b)</td>
</tr>
<tr>
<td>Micro-stratification</td>
<td>NA(^c)</td>
<td>NA</td>
</tr>
<tr>
<td>Pretreatment(^d)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Coagulation/settling(^e)</td>
<td>90</td>
<td>50</td>
</tr>
<tr>
<td>Rapid filtration(^f)</td>
<td>&gt;80</td>
<td>5</td>
</tr>
<tr>
<td>Terminal chlorination</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>Mains distribution</td>
<td>NA</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

\(^a\) Required performance
\(^b\) NTU, nephelometric turbidity units
\(^c\) NA, not applicable. Process not designed to remove turbidity and/or bacteria. Micro-stratification removes micro-algae and zooplankton
\(^d\) Pretreatments that can result in significant reductions in thermotolerant coliform bacteria are storage in reservoirs for 3-4 weeks, and pre-disinfection
\(^e\) Taken together, coagulation, settling, and rapid filtration should be expected to remove 99.9% of thermotolerant coliform bacteria

Another approach to the application of the multiple-barrier principle has been applied in urban areas for supplies derived from rivers. It involves: (1) raw water storage (or plain sedimentation); (2) rapid sand filtration; (3) slow sand filtration; and (4) terminal disinfection. Steps 1–3 remove turbidity, while 1, 3 and 4 remove microbes. Infiltration, which is highly effective in removing bacteria, viruses, and organic carbon, has been used, notably in the Netherlands, as an additional process following storage and rapid filtration.

11.3.4 Small-scale treatment of surface water

The multiple-barrier concept, as applied in the treatment of surface water for urban supplies, can be adapted for use in rural and remote regions. A typical series of processes would include: (1) storage, sedimentation, or screening; (2)
triple-stage gravel prefiltration; (3) slow sand filtration; and (4) disinfection. Table 11.3 lists typical performance objectives for the removal of turbidity and thermotolerant coliform bacteria in such plants.

A detailed account of water treatment and supply for small remote communities is given in Volume 3 of Guidelines for drinking-water quality.

### Table 11.3 An example of performance objectives for removal of turbidity and thermotolerant coliform bacteria in small-scale water treatment

<table>
<thead>
<tr>
<th>Stage and process</th>
<th>Turbidity</th>
<th>Thermotolerant coliform bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Removal (%)</td>
<td>Average loading (NTU)</td>
</tr>
<tr>
<td>Screening</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Plain sedimentation</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Gravel pre-filters</td>
<td>80</td>
<td>30</td>
</tr>
<tr>
<td>Slow sand filter</td>
<td>&gt;90</td>
<td>&gt;60</td>
</tr>
<tr>
<td>Disinfection</td>
<td>NA</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Distributed water</td>
<td>NA</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

a Required performance  
b NTU, nephelometric turbidity units  
c NA, not applicable. Process not designed to remove turbidity and/or bacteria

### 11.4 Distribution networks

A distribution network transports water from the place of treatment to the consumer. Its design and size will be governed both by the topography and the location and size of the community. The aim is always to ensure that the consumer receives a sufficient and uninterrupted supply and that contamination is not introduced in transit. The shape of the network will be influenced by the location of consumers.

Distribution systems are especially vulnerable to contamination when the pressure falls, particularly in the intermittent supplies of many cities in developing countries. Suction is often created by direct pumping from the mains to private storage tanks, a practice which should be prohibited.

The bacteriological quality of water can deteriorate during distribution, and there are a number of places where contamination can be introduced. If the water contains significant assimilable organic carbon and adequate residual levels of disinfectant are not maintained, or if water mains are not flushed and cleaned frequently enough, growth of nuisance bacteria and other organisms can occur. Where the water contains appreciable assimilable organic carbon and where the water temperature exceeds 20 °C, a chlorine residual of 0.25 mg/litre may be required to prevent the growth of Aeromonas and other nuisance bacteria. Attached microorganisms may grow even in the presence of residual chlorine.
Underground storage tanks and service reservoirs must be inspected for deterioration and for infiltration of surface water and groundwater. It is desirable for the land enclosing underground storage tanks to be fenced off, both to prevent access by people and animals and to prevent damage to the structures.

Repair work on mains provides another opportunity for contamination to occur. Local loss of pressure may result in back-siphonage of contaminated water unless check valves are introduced into the consumers’ water system at sensitive points, such as supplies to ornamental pools, garden irrigation, urinals, and water closets. When repairs to mains have been completed or when new mains are installed, it is essential that the pipes are cleaned, disinfected, and then emptied and refilled with mains water. The water should then be tested bacteriologically after 24 hours, and new mains should not be brought into service until the water quality is bacteriologically satisfactory. If the main has been damaged and water from a fractured sewer or drain may have entered, the situation is most serious and, in addition to chlorination of the water in the repaired main, the level of chlorination should be increased and the main not returned to service until the water quality is satisfactory. These and other actions to be taken should be specified both in national codes of practice and in local instructions to waterworks staff.

As already mentioned, microbial contamination can occur as a result of the use of unsuitable materials for items coming into contact with water; such materials include those used for washers, jointing and packing materials, pipe and tank lining compounds, and plastics used in pipes, tanks, and faucets, all of which can deteriorate to form substances that support the growth of microorganisms. Such materials should be subject to approval by the authority responsible for the water-supply system.

References


PART 2

Chemical and physical aspects
12. Chemical and physical aspects: introduction

12.1 Background information used

The assessment of the toxicity of drinking-water contaminants has been made on the basis of published reports from the open literature, information submitted by governments and other interested parties, and unpublished proprietary data. In the development of the guideline values, existing international approaches to developing guidelines were carefully considered. Previous risk assessments developed by the International Programme on Chemical Safety (IPCS) in Environmental Health Criteria monographs, the International Agency for Research on Cancer (IARC), the Joint FAO/WHO Meetings on Pesticide Residues (JMPR), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) were reviewed. These assessments were relied upon except where new information justified a reassessment. The quality of new data was critically evaluated prior to their use in risk assessment.

12.2 Drinking-water consumption and body weight

Global data on the consumption of drinking-water are limited. In studies carried out in Canada, the Netherlands, the United Kingdom, and the United States of America, the average daily per capita consumption was usually found to be less than 2 litres, but there was considerable variation between individuals. As water intake is likely to vary with climate, physical activity, and culture, the above studies, which were conducted in temperate zones, can give only a limited view of consumption patterns throughout the world. At temperatures above 25 °C, for example, there is a sharp rise in fluid intake, largely to meet the demands of an increased sweat rate (1).

In developing the guideline values for potentially hazardous chemicals, a daily per capita consumption of 2 litres by a person weighing 60 kg was generally assumed. The guideline values set for drinking-water using this assumption do, on average, err on the side of caution. However, such an assumption may underestimate the consumption of water per unit weight, and thus exposure, for those living in hot climates as well as for infants and children, who consume more fluid per unit weight than adults.

The higher intakes, and hence exposure, for infants and children apply for only a limited time, but this period may coincide with greater sensitivity to some
toxic agents and less for others. Irreversible effects that occur at a young age will have more social and public health significance than those that are delayed. Where it was judged that this segment of the population was at a particularly high risk from exposure to certain chemicals, the guideline value was derived on the basis of a 10-kg child consuming 1 litre per day or a 5-kg infant consuming 0.75 litre per day. The corresponding daily fluid intakes are higher than for adults on a body weight basis.

12.3 Inhalation and dermal absorption

The contribution of drinking-water to daily exposure includes direct ingestion as well as some indirect routes, such as inhalation of volatile substances and dermal contact during bathing or showering.

In most cases, the data were insufficient to permit reliable estimates of exposure by inhalation and dermal absorption of contaminants present in drinking-water. It was not possible, therefore, to address intake from these routes specifically in the derivation of the guideline values. However, that portion of the total tolerable daily intake (TDI) allocated to drinking-water is generally sufficient to allow for these additional routes of intake (see section 12.4.1). When there is concern that potential inhalation of volatile compounds and dermal exposure from various indoor water uses (such as showering) are not adequately addressed, authorities could adjust the guideline value.

12.4 Health risk assessment

There are two principal sources of information on health effects resulting from exposure to chemicals that can be used in deriving guideline values. The first is studies on human populations. The value of such investigations is often limited, owing to lack of quantitative information on the concentrations to which people are exposed or on simultaneous exposure to other agents. The second, and the one used most often, is toxicity studies using laboratory animals. Such studies are generally limited because of the relatively small number of animals used and the relatively high doses administered. Furthermore, there is a need to extrapolate the results to the low doses to which human populations are usually exposed.

In order to derive a guideline value to protect human health, it is necessary to select the most suitable experimental animal study on which to base the extrapolation. Data from well-conducted studies, where a clear dose–response relationship has been demonstrated, are preferred. Expert judgement was exercised in the selection of the most appropriate study from the range of information available.
12.4.1 Derivation of guideline values using a tolerable daily intake approach

For most kinds or toxicity, it is generally believed that there is a dose below which no adverse effects will occur. For chemicals that give rise to such toxic effects, a tolerable daily intake (TDI) can be derived as follows:

\[ TDI = \frac{NOAEL \text{ or } LOAEL}{UF} \]

where \( NOAEL \) = no-observed-adverse-effect level,
\( LOAEL \) = lowest-observed-adverse-effect level,
\( UF \) = uncertainty factor.

The guideline value (GV) is then derived from the TDI as follows:

\[ GV = \frac{TDI \times bw \times P}{C} \]

where \( bw \) = body weight (60 kg for adults, 10 kg for children, 5 kg for infants),
\( P \) = fraction of the TDI allocated to drinking-water,
\( C \) = daily drinking-water consumption (2 litres for adults, 1 litre for children, 0.75 litre for infants).

**Tolerable daily intake**

The TDI is an estimate of the amount of a substance in food or drinking-water, expressed on a body weight basis (mg/kg or μg/kg of body weight), that can be ingested daily over a lifetime without appreciable health risk (2).

Over many years, JECFA and JMPR have developed certain principles in the derivation of acceptable daily intakes (ADIs). These principles have been adopted where appropriate in the derivation of TDIs used in developing guideline values for drinking-water quality (3, 4).

ADIs are established for food additives and pesticide residues that occur in food for necessary technological purposes or plant protection reasons. For chemical contaminants, which usually have no intended function in drinking-water, the term "tolerable daily intake" is seen as more appropriate than "acceptable daily intake", as it signifies permissibility rather than acceptability (3).

As TDIs are regarded as representing a tolerable intake for a lifetime, they are not so precise that they cannot be exceeded for short periods of time (4). Short-term exposure to levels exceeding the TDI is not a cause for concern, provided the individual's intake averaged over longer periods of time does not appreciably exceed the level set (5). The large uncertainty factors generally involved in establishing a TDI (see page 124) serve to provide assurance that exposure exceeding
the TDI for short periods is unlikely to have any deleterious effects upon health. However, consideration should be given to any potential acute toxic effects that may occur if the TDI is substantially exceeded for short periods of time (4).

The calculated TDI was used to derive the guideline value, which was then rounded to one significant figure. In some instances, ADI values with only one significant figure set by JECFA or JMPR were used to calculate the guideline value (4). The guideline value was generally rounded to one significant figure to reflect the uncertainty in animal toxicity data and exposure assumptions made. More than one significant figure was used for guideline values only where extensive information on toxicity and exposure to humans provided greater certainty.

No-observed-adverse-effect level and lowest-observed-adverse-effect level

The NOAEL is defined as the highest dose or concentration of a chemical in a single study, found by experiment or observation, that causes no detectable adverse health effect. Whenever possible, the NOAEL is based on long-term studies, preferably of ingestion in drinking-water. However, NOAELs obtained from short-term studies and studies using other sources of exposure (e.g., food, air) may also be used.

If a NOAEL is not available, a LOAEL may be used, which is the lowest observed dose or concentration of a substance at which there is a detectable adverse health effect. When a LOAEL is used instead of a NOAEL, an additional uncertainty factor is normally used (see below).

Uncertainty factors

The application of uncertainty factors has been widely used in the derivation of ADIs for food additives, pesticides, and environmental contaminants. The derivation of these factors requires expert judgement and a careful sifting of the available scientific evidence.

In the derivation of the WHO drinking-water quality guideline values, uncertainty factors were applied to the lowest NOAEL or LOAEL for the response considered to be the most biologically significant and were determined by consensus among a group of experts using the approach outlined below:

<table>
<thead>
<tr>
<th>Source of uncertainty</th>
<th>Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interspecies variation (animals to humans)</td>
<td>1–10</td>
</tr>
<tr>
<td>Intraspecies variation (individual variations)</td>
<td>1–10</td>
</tr>
<tr>
<td>Adequacy of studies or database</td>
<td>1–10</td>
</tr>
<tr>
<td>Nature and severity of effect</td>
<td>1–10</td>
</tr>
</tbody>
</table>

Inadequate studies or databases include those that used a LOAEL instead of a NOAEL and studies considered to be shorter in duration than desirable. Situ-
tions in which the nature or severity of effect might warrant an additional uncertainty factor include studies in which the end-point was malformation of a fetus or in which the end-point determining the NOAEL was directly related to possible carcinogenicity. In the latter case, an additional uncertainty factor was applied for carcinogenic compounds for which a guideline value was derived using a TDI approach (see section 12.4.2). Factors lower than 10 were used, for example, for interspecies variation when humans are known to be less sensitive than the animal species studied.

The total uncertainty factor should not exceed 10 000. If the risk assessment would lead to a higher uncertainty factor, then the resulting TDI would be so imprecise as to lack meaning. For substances for which uncertainty factors were greater than 1000, guideline values are designated as provisional in order to emphasize the high level of uncertainty inherent in these values.

The selection and application of uncertainty factors are important in the derivation of guideline values for chemicals, as they can make a considerable difference to the values set. For contaminants for which there is relatively little uncertainty, the guideline value was derived using a small uncertainty factor. For most contaminants, however, there is great scientific uncertainty, and a large uncertainty factor was used. Hence, there may be a large margin of safety above the guideline value before adverse health effects result.

There is considerable merit in using a method that allows a high degree of flexibility. However, it is important that, where possible, the derivation of the uncertainty factor used in calculating a guideline value is clearly presented as part of the rationale. This helps authorities in using the guidelines, as the safety margin in allowing for local circumstances is clear. It also helps in determining the urgency and nature of the action required in the event that a guideline value is exceeded.

**Allocation of intake**

Drinking-water is not usually the sole source of human exposure to the substances for which guideline values have been set. In many cases, the intake from drinking-water is small in comparison with that from other sources such as food and air. Guideline values derived using the TDI approach take into account exposure from all sources by apportioning a percentage of the TDI to drinking-water. This approach ensures that total daily intake from all sources (including drinking-water containing concentrations of the substance at or near the guideline value) does not exceed the TDI.

Wherever possible, data concerning the proportion of total intake normally ingested in drinking-water (based on mean levels in food, air, and drinking-water) or intakes estimated on the basis of consideration of physical and chemical properties were used in the derivation of the guideline values. Where such information was not available, an arbitrary (default) value of 10% for drinking-water was used. This default value is, in most cases, sufficient to account for additional
It is recognized that exposure from various media may vary with local circumstances. It should be emphasized, therefore, that the derived guideline values apply to a typical exposure scenario or are based on default values that may not be applicable for all areas. In those areas where relevant data on exposure are available, authorities are encouraged to develop context-specific guideline values that are tailored to local circumstances and conditions. For example, in areas where the intake of a particular contaminant in drinking-water is known to be much greater than that from other sources (i.e., air and food), it may be appropriate to allocate a greater proportion of the TDI to drinking-water to derive a guideline value more suited to the local conditions. In addition, in cases in which guideline values are exceeded, efforts should be made to assess the contribution of other sources to total intake; if practicable, exposure from these sources should be minimized.

12.4.2 Derivation of guideline values for potential carcinogens

The evaluation of the potential carcinogenicity of chemical substances is usually based on long-term animal studies. Sometimes data are available on carcinogenicity in humans, mostly from occupational exposure.

On the basis of the available evidence, IARC categorizes chemical substances with respect to their potential carcinogenic risk into the following groups (6) (for a detailed description of the classifications, see box on pp. 127–128):

- Group 1: the agent is carcinogenic to humans
- Group 2A: the agent is probably carcinogenic to humans
- Group 2B: the agent is possibly carcinogenic to humans
- Group 3: the agent is not classifiable as to its carcinogenicity to humans
- Group 4: the agent is probably not carcinogenic to humans.

In establishing the present guideline values for drinking-water quality, the IARC classification for carcinogenic compounds was taken into consideration. For a number of compounds, additional information was also available.

It is generally considered that the initiating event in the process of chemical carcinogenesis is the induction of a mutation in the genetic material (DNA) of somatic cells (i.e., cells other than ova or sperm). Because the genotoxic mechanism theoretically does not have a threshold, there is a probability of harm at any level of exposure. Therefore, the development of a TDI is considered inappropriate, and mathematical low-dose extrapolation is applied. On the other hand, there are carcinogens that are capable of producing tumors in animals or humans without exerting genotoxic activity, but acting through an indirect mechanism. It is generally believed that a threshold dose exists for these nongenotoxic carcinogens.

In order to make the distinction with respect to the underlying mechanism of carcinogenicity, each compound that has been shown to be a carcinogen was evaluated on a case-by-case basis, taking into account the evidence of genotox-
Evaluation of carcinogenic risk to humans

IARC considers the body of evidence as a whole in order to reach an overall evaluation of the carcinogenicity for humans of an agent, mixture, or circumstance of exposure.

The agent, mixture, or exposure circumstance is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent, mixture, or exposure circumstance is a matter of scientific judgement, reflecting the strength of the evidence derived from studies in humans and in experimental animals and from other relevant data.

Group 1. The agent (mixture) is carcinogenic to humans. The exposure circumstance entails exposures that are carcinogenic to humans.

This category is used when there is sufficient evidence of carcinogenicity in humans. Exceptionally, an agent (mixture) may be placed in this category when evidence in humans is less than sufficient but there is sufficient evidence of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent (mixture) acts through a relevant mechanism of carcinogenicity.

Group 2

This category includes agents, mixtures, and exposure circumstances for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost sufficient, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents, mixtures, and exposure circumstances are assigned to either group 2A (probably carcinogenic to humans) or group 2B (possibly carcinogenic to humans) on the basis of epidemiological and experimental evidence of carcinogenicity and other relevant data.

Group 2A. The agent (mixture) is probably carcinogenic to humans. The exposure circumstance entails exposures that are probably carcinogenic to humans.

This category is used when there is limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals. In some cases, an agent (mixture) may be classified in this category when there is inadequate evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent, mixture, or exposure circumstance may be classified in this category solely on the basis of limited evidence of carcinogenicity in humans.
**Group 2B. The agent (mixture) is possibly carcinogenic to humans. The exposure circumstance entails exposures that are possibly carcinogenic to humans.**

This category is used for agents, mixtures, and exposure circumstances for which there is limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals. It may also be used when there is inadequate evidence of carcinogenicity in humans but there is sufficient evidence of carcinogenicity in experimental animals. In some instances, an agent, mixture, or exposure circumstance for which there is inadequate evidence of carcinogenicity in humans but limited evidence of carcinogenicity in experimental animals together with supporting evidence from other relevant data may be placed in this group.

**Group 3. The agent (mixture or exposure circumstance) is not classifiable as to its carcinogenicity to humans.**

This category is used most commonly for agents, mixtures, and exposure circumstances for which the evidence of carcinogenicity is inadequate in humans and inadequate or limited in experimental animals.

Exceptionally, agents (mixtures) for which the evidence for carcinogenicity is inadequate in humans but sufficient in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents, mixtures, and exposure circumstances that do not fall into any other group are also placed in this category.

**Group 4. The agent (mixture) is probably not carcinogenic to humans.**

This category is used for agents or mixtures for which there is evidence suggesting lack of carcinogenicity in humans and in experimental animals. In some instances, agents or mixtures for which there is inadequate evidence of carcinogenicity in humans but evidence suggesting lack of carcinogenicity in experimental animals, consistently and strongly supported by a broad range of other relevant data, may be classified in this group.

For carcinogens for which there is convincing evidence to suggest a nongenotoxic mechanism, guideline values were calculated using a TDI approach, as described in section 12.4.1.

In the case of compounds considered to be genotoxic carcinogens, guideline values were determined using a mathematical model, and the guideline values presented in Volume 1 are the concentrations in drinking-water associated with an estimated upper bound excess lifetime cancer risk of $10^{-5}$ (one additional cancer case per 100,000 of the population ingesting drinking-water containing the substance at the guideline value for 70 years). Concentrations associated with estimated excess lifetime cancer risks of $10^{-4}$ and $10^{-6}$ can be calculated by multiplying and dividing, respectively, the guideline value by 10. These values are also
presented in this volume to emphasize the fact that each country should select its own appropriate risk level. In cases in which the concentration associated with a $10^{-5}$ excess lifetime cancer risk is not practical because of inadequate analytical or treatment technology, a provisional guideline value was set at a practicable level and the estimated associated cancer risk presented.

Although several models exist, the linearized multistage model was generally adopted in the development of these guidelines. Other models were considered more appropriate in a few cases.

It should be emphasized, however, that guideline values for carcinogenic compounds computed using mathematical models must be considered at best as a rough estimate of the cancer risk. These models do not usually take into account a number of biologically important considerations, such as pharmacokinetics, DNA repair, or immunological protection mechanisms. However, the models used are conservative and probably err on the side of caution.

To account for differences in metabolic rates between experimental animals and humans—the former are more closely correlated with the ratio of body surface areas than with body weights—a surface area to body weight correction is sometimes applied to quantitative estimates of cancer risk derived on the basis of models for low-dose extrapolation. Incorporation of this factor increases the risk by approximately one order of magnitude (depending on the species upon which the estimate is based) and increases the risk estimated on the basis of studies in mice relative to that in rats. The incorporation of this factor is considered to be overly conservative, particularly in view of the fact that linear extrapolation most likely overestimates risk at low doses; indeed, Crump et al. (7) concluded that “all measures of dose except dose rate per unit of body weight tend to result in overestimation of human risk”. Consequently, guideline values for carcinogenic contaminants were developed on the basis of quantitative estimates of risk that were not corrected for the ratio of surface area to body weight.

### 12.5 Mixtures

Chemical contaminants of drinking-water supplies are present together with numerous other inorganic and organic constituents. The guideline values were calculated separately for individual substances, without specific consideration of the potential for interaction of each substance with other compounds present. However, the large margin of safety incorporated in the majority of guideline values is considered to be sufficient to account for such potential interactions. In addition, the majority of contaminants will not be present at concentrations at or near their guideline value.

There may, however, be occasions when a number of contaminants with similar toxicological effects are present at levels near their respective guideline values. In such cases, decisions concerning appropriate action should be made, taking into consideration local circumstances. Unless there is evidence to the contrary, it is appropriate to assume that the toxic effects of these compounds are additive.
12.6 Format of monographs for chemical substances

The format adopted for the monographs in this publication is shown below. All of the headings may not, however, be required in every monograph.

**General description**
- Identity
- Physicochemical properties
- Organoleptic properties
- Major uses
- Environmental fate

**Analytical methods**

**Environmental levels and human exposure**
- Air
- Water
- Food
- Estimated total exposure and relative contribution of drinking-water

**Kinetics and metabolism in laboratory animals and humans**

**Effects on laboratory animals and in vitro test systems**
- Acute exposure
- Short-term exposure
- Long-term exposure
- Reproductive toxicity, embryotoxicity, and teratogenicity
- Mutagenicity and related end-points
- Carcinogenicity

**Effects on humans**

**Guideline value**

**References**


13.

Inorganic constituents and physical parameters

13.1 Aluminium

13.1.1 General description

Identity

Aluminium is a widespread and abundant element, accounting for some 8% of the earth's crust. It is found as a normal constituent of soils, plants, and animal tissues.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS no.</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>7429-90-5</td>
<td>Al</td>
</tr>
<tr>
<td>Aluminium oxide</td>
<td>1344-28-1</td>
<td>Al₂O₃</td>
</tr>
<tr>
<td>Aluminium chloride</td>
<td>7446-70-0</td>
<td>AlCl₃.6H₂O</td>
</tr>
<tr>
<td>Aluminium sulfate</td>
<td>10043-01-3</td>
<td>Al₂(SO₄)₃</td>
</tr>
<tr>
<td>Aluminium hydroxide</td>
<td>21645-51-2</td>
<td>Al(OH)₃</td>
</tr>
</tbody>
</table>

Physicochemical properties (1)

<table>
<thead>
<tr>
<th>Property</th>
<th>Al</th>
<th>Al₂O₃</th>
<th>AlCl₃.6H₂O</th>
<th>Al₂(SO₄)₃</th>
<th>Al(OH)₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point (°C)</td>
<td>660.37</td>
<td>2072</td>
<td>100 (decomposes)</td>
<td>770 (decomposes)</td>
<td>300</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>2467</td>
<td>2980</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Density at 20 °C</td>
<td>2.702</td>
<td>3.965</td>
<td>2.398</td>
<td>2.71</td>
<td>2.42</td>
</tr>
<tr>
<td>Water solubility</td>
<td>insoluble</td>
<td>insoluble</td>
<td>soluble</td>
<td>31.3 at 0 °C</td>
<td>-</td>
</tr>
</tbody>
</table>

Organoleptic properties

In the presence of aluminium, levels of iron normally too low to cause problems may produce obvious discoloration of water. The incidence of discoloration in distribution system water, and therefore the frequency of consumer complaints, increases if the aluminium level exceeds about 0.1–0.2 mg/litre in the final water.
Major uses

Aluminium has many industrial and domestic applications. Its compounds are used as antacids, antiperspirants, food additives, and vaccine adjuvants. Aluminium salts are also widely used in water treatment as flocculants.

Environmental fate

Aluminium is usually present in treated drinking-water in the form of reactive species of low relative molecular mass; in natural waters, it is usually associated with particulate matter or organic complexes of high relative molecular mass (2).

13.1.2 Analytical methods

Aluminium may be determined by colorimetry (lower detection limit 5 μg/litre) and by inductively coupled plasma emission spectrometry (lower detection limit 40 μg/litre).

13.1.3 Environmental levels and human exposure

Air

Aluminium is present in air at nanogram per cubic metre levels as a result of the weathering of aluminosilicate rocks and emissions from industrial sources, automobiles, and cigarette smoke (3).

Water

Aluminium may be present in natural waters as a consequence of leaching from soil and rock. In a survey of aluminium in raw waters in the USA, ranges of 14–290 μg/litre in groundwater and 16–1170 μg/litre in surface water were reported (4). In the United Kingdom, concentrations of 200–300 μg/litre were associated with low pH levels and 400–600 μg/litre with an afforested catchment (5).

Aluminium salts are used as coagulants in water treatment. Residual aluminium concentrations in finished waters are a function of the aluminium levels in the source water, the amount of aluminium coagulant used, and the efficiency of filtration of the aluminium floc. Where residual concentrations are high, aluminium may be deposited in the distribution system, and a gradual reduction with increasing distance from the treatment plant may then be observed. Disturbance of the deposits by changes in flow rate may increase aluminium levels at the tap and render the water aesthetically unacceptable (6).
Food

The concentrations of aluminium in food vary widely depending on the nature of the foodstuffs. Studies suggest that aluminium leached from tea leaves can make a significant contribution to dietary intake (7). Use of food additives containing aluminium, such as preservatives, fillers, colouring agents, anticaking agents, emulsifiers, and baking powders, also increases dietary intake.

In addition to its presence in food per se, aluminium leaching from cooking utensils may represent a potential source of dietary exposure (8, 9). Use of aluminium by the food industry in containers and packaging constitutes another dietary source (10).

It should be noted that the ubiquitous nature of aluminium makes it difficult to ensure freedom from contamination throughout the various stages of analysis. The reported concentrations of aluminium in foodstuffs such as vegetables, which are readily contaminated with soil, appear to be decreasing as analytical techniques improve.

Pharmaceuticals

The use of antacids, analgesics, and other aluminium-containing medications is a significant source of exposure for some individuals (11, 12).

Estimated total exposure and relative contribution of drinking-water

A value of 20 mg/day has been suggested as a “typical” daily aluminium intake (13, 14). However, because of individual and geographical variations in eating and drinking habits, a range of 5–20 mg of aluminium per day is probably more realistic. Aluminium in drinking-water will usually contribute only a very small proportion of the total daily intake. If a contribution of 20 mg/day from food is assumed, an adult drinking 2 litres of water per day containing 200 µg of aluminium per litre would receive approximately 2% of his or her total intake from drinking-water.

13.1.4 Kinetics and metabolism in laboratory animals and humans

Studies suggest that less than 1% of dietary inorganic aluminium is absorbed (15, 16). Factors affecting absorption may include vitamin D and fluoride (17) and the presence of complexing agents (18). Aluminium, once absorbed, appears to bind to serum proteins (19); it is eliminated from the body by the kidneys (17). Individuals with renal insufficiency tend to accumulate aluminium as a consequence of their inability to excrete it via the kidneys (20).
13.1.5 Effects on laboratory animals and *in vitro* test systems

**Short-term exposure**

Groups of 25 male Sprague-Dawley rats were fed diets containing basic sodium aluminium phosphates or aluminium hydroxide at mean aluminium doses ranging from 67 to 302 mg/kg of body weight per day for 28 days. No treatment-related effects on body weight, organ weights, haematology, and clinical chemistry were observed, and there was no evidence of increased aluminium concentrations in bone (21).

In a study in which rats received drinking-water containing aluminium nitrate for 1 month, elevated aluminium levels were found in the heart and spleen, and histological changes were apparent in the liver and spleen at doses of 54 and 108 mg of aluminium per kg of body weight per day. No adverse effects were observed at a dose level of 27 mg of aluminium per kg of body weight per day (22).

Rats fed diets containing aluminium hydroxide at 257 or 1075 mg of aluminium per kg of diet for 67 days, equivalent to 13 or 54 mg of aluminium per kg of body weight per day, exhibited elevated levels of aluminium in the tibia, liver, and kidneys. Reduced bone strengths were also noted at the highest dose level (23).

Groups of 10 female Sprague-Dawley rats received aluminium nitrate in their drinking-water at doses of 0, 360, 720, or 3600 mg/kg of body weight for 100 days (equivalent to 0, 26, 52, or 260 mg of aluminium per kg of body weight per day). The only effect observed was depression of body weight gain associated with reduced water and feed intake at the highest dose level. Organ weight, histopathology of the brain, heart, lungs, kidneys, liver, and spleen, haematology, and some clinical chemistry indices were also examined (24).

Rats given oral doses of 0.0025, 0.25, or 2.5 mg of aluminium per kg of body weight for 6 months exhibited some changes in behaviour and mild changes in the biochemistry of the testes at the highest dose level, but there were no significant effects at the lower doses (25).

In a study in which dogs (4 per sex per dose) were fed diets containing basic sodium aluminium phosphate at 0, 3000, 10 000, or 30 000 mg/kg (mean daily aluminium doses of 4, 10, 27, or 75 mg/kg of body weight for males and 3, 10, 22, or 80 mg/kg of body weight for females) for 26 weeks, mild histopathological changes were observed in the liver, kidney, and testes of males in the highest-dose group, whereas brain aluminium concentrations were slightly elevated in females given the highest dose. No effects were noted at the lower dose levels (26).

**Long-term exposure**

Chronic oral intake of aluminium in the diet at doses of about 50 or 100 mg/kg of body weight per day decreased locomotor responses and slowed the acquisition of avoidance behaviour in rats (27). In other studies in rats, it was reported that
aluminium produced osteomalacia (28) and microcytic anaemia (29), initiated impairment of kidney function (30), and caused damage to the lysosomes in the liver, spleen, and kidneys (31).

Reproductive toxicity, embryotoxicity, and teratogenicity

An increase in the aluminium content of the placenta and fetus was found when pregnant mice were given oral doses of aluminium chloride (equivalent to 0, 40, or 60 mg of aluminium per kg of body weight per day) on days 7–16 of gestation (32). No evidence of embryotoxicity or teratogenicity was found in a study of pregnant rats fed aluminium chloride in their diet on days 6–19 of gestation at doses of approximately 5 or 10 mg of aluminium per kg of body weight per day (33). A delay in weight gain and neuromotor development was reported in the offspring of rats given oral doses of aluminium chloride (equivalent to 155 or 192 mg of aluminium per kg of body weight per day) from day 8 of gestation to parturition (34).

Mouse dams fed a diet containing 500 or 1000 mg of aluminium per kg (equivalent to 75 or 150 mg/kg of body weight per day) exhibited neurotoxicity, and their offspring showed neurological changes consistent with maternal toxicity and subsequent delayed development (35). In a study in which groups of 10 pregnant rats were given oral doses of aluminium nitrate of 0, 180, 360, or 720 mg/kg of body weight per day from day 14 of gestation to day 21 of lactation, offspring of dams receiving the highest dose exhibited depressed body weight gain (36).

No evidence of impaired reproductive performance was observed in male rats receiving drinking-water containing aluminium chloride at doses of approximately 0.5, 5, or 50 mg of aluminium per kg of body weight per day for 90 days (37).

13.1.6 Effects on humans

In the early 1970s, a syndrome known as dialysis dementia was described in patients on dialysis, characterized by an insidious onset of altered behaviour, dementia, speech disturbance, muscular twitching, and convulsions, usually with a fatal outcome. Patients had markedly elevated serum aluminium levels with increased concentrations in many tissues, including the cerebral cortex (38). Investigations established a correlation between the aluminium concentration in the water used to prepare the dialysate fluid and the incidence of the syndrome (39, 40). Aluminium from other sources, such as phosphate-binding gels, albumin, and peritoneal dialysis fluid, may also result in elevated aluminium levels in patients on dialysis.

Aluminium has been implicated in the etiology of two severe neurodegenerative diseases, amyotrophic lateral sclerosis and parkinsonism dementia, observed at very high incidence among the Chamorro people of Guam. One hypothesis
suggests that chronic nutritional deficiencies of calcium and magnesium might lead to increased absorption of aluminium, resulting in its deposition in neurons (41), with consequent interference with their structure and eventual formation of the neurofibrillary tangles in the brain that characterize these diseases (42).

In Alzheimer disease, the first recognizable symptoms are memory lapses, disorientation, confusion, and frequently depression. These symptoms mark the start of a progressive mental deterioration for which there is no treatment. Aluminium is one of numerous causal factors that have been proposed for this disease. The brains of Alzheimer patients appear to have elevated levels of aluminium in regions containing large numbers of neurofibrillary tangles (43, 44).

Several hypotheses have been put forward to explain the role of aluminium in Alzheimer disease. The normal blood–brain and cytoplasmic barriers to aluminium may be defective, allowing aluminium to enter the nuclei of brain neurons (45). The localization of aluminium in the amyloid plaque cores of Alzheimer brains has led to the proposition that aluminium might in some way be involved in initiating events leading to plaque formation (46). Aluminium present in the DNA-containing structures of nuclei from the affected regions of such brains may reduce transcription and account for disorders in many cellular processes (47). It has also been proposed that aluminium may interfere with calcium metabolism (48).

Few attempts have been made to study the relationship between Alzheimer disease and exposure to aluminium from an epidemiological point of view. Vogt (49) investigated the relationship between concentrations of aluminium in water and the frequency of Alzheimer disease in southern Norway. Rates of mortality associated with dementia listed as the cause of death on death certificates were found to correlate positively with concentrations of aluminium in the water of different geographical areas. However, this study had a number of weaknesses, including the use of data on raw water rather than on distributed supplies, dubious epidemiological statistics, and inadequate adjustment for other confounding factors.

In a further retrospective epidemiological study of Alzheimer disease and aluminium in drinking-water in Norway, it was concluded that a geographical association existed between aluminium in drinking-water and registered death rates from dementia (50). However, rates of dementia were also correlated with population density and other socioeconomic variables, so that the evidence provided by this study must be regarded as very weak.

An epidemiological study has been carried out in the United Kingdom in which exposure to aluminium from drinking-water was calculated from data provided by local water undertakings (51). Rates of Alzheimer disease were estimated from the records of computerized tomographic scanning units. Districts in which aluminium concentrations in drinking-water exceeded 0.01 mg/litre (four subsets: 0.02–0.04 mg/litre, 0.05–0.07 mg/litre, 0.08–0.11 mg/litre, and >0.11 mg/litre) were found to have an approximately 50% greater incidence of Alz-
Alzheimer disease as compared with those in which aluminium concentrations were below 0.01 mg/litre (one set only). A slight but unconvincing increase in the rate of Alzheimer disease was found with increasing aluminium concentrations for patients aged 40–64 but not those aged 40–69. As all four sets of districts with aluminium concentrations above 0.01 mg/litre showed a higher, and similar, incidence of Alzheimer disease as compared with the set with concentrations below 0.01 mg/litre, the conclusions of this study seem to hinge on the ability of the data to represent adequately the incidence of Alzheimer disease in the latter group.

These three studies give some support to the hypothesis of a positive relationship between the concentration of aluminium in drinking-water and the incidence of Alzheimer disease. The results cannot be considered as conclusive, however, as there are particular difficulties in evaluating the relationship between aluminium and Alzheimer disease by epidemiological means, namely doubts as to the reliability of the data for aluminium exposure and the ability to identify accurately the frequency of Alzheimer disease in different areas, and the possibility of unknown confounding factors.

13.1.7 Conclusions

Aluminium is of low toxicity in laboratory animals, and JECFA established a provisional tolerable weekly intake (PTWI) of 7 mg/kg of body weight in 1988 (52). However, this was based on studies of aluminium phosphate (acidic), which is not the chemical form in which aluminium is present in drinking-water.

In some studies, aluminium appeared to be associated with the brain lesions characteristic of Alzheimer disease, and in a few ecological epidemiological studies the incidence of this disease has been associated with aluminium in drinking-water. These ecological analyses must be interpreted with caution and need to be confirmed by analytical epidemiological studies.

While further studies are needed, the balance of epidemiological and physiological evidence does not at present support a causal role for aluminium in Alzheimer disease. No health-based guideline value is therefore derived. However, a concentration of aluminium of 0.2 mg/litre in drinking-water provides a compromise between the practical use of aluminium salts in water treatment and discoloration of distributed water.

References


**13.2 Ammonia**

**13.2.1 General description**

**Identity**

CAS no.: 7664-41-7  
Molecular formula: NH$_3$

In what follows, the term “ammonia” covers both the nonionized form (NH$_3$) and the ammonium cation (NH$_4^+$) unless stated otherwise.

**Physicochemical properties** (1, 2)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>-77.76 °C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>-33.43 °C</td>
</tr>
<tr>
<td>Density of vapour</td>
<td>0.6 g/litre at 20 °C</td>
</tr>
<tr>
<td>Water solubility</td>
<td>421 g/litre at 20 °C; 706 g/litre at 0 °C</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>882 kPa at 20 °C</td>
</tr>
</tbody>
</table>
**Organoleptic properties**

The threshold odour concentration of ammonia in water is approximately 1.5 mg/litre. A taste threshold of 35 mg/litre has been proposed for the ammonium cation (I).

**Major uses**

Ammonia is used in fertilizer and animal feed production and in the manufacture of fibres, plastics, explosives, paper, and rubber. It is used as a coolant, in metal processing, and as a starting product for many nitrogen-containing compounds (3). Ammonia and ammonium salts are used in cleansing agents and as food additives (I, 4), and ammonium chloride is used as a diuretic.2

**Environmental fate**

On dissolution in water, ammonia forms the ammonium cation; hydroxyl ions are formed at the same time. The equilibrium constant of this reaction, $K_B$, is $1.78 \times 10^{-5}$ (3). The degree of ionization depends on the temperature, the pH, and the concentration of dissolved salts in the water.

The environmental cycling of nitrogen relies mainly on nitrate, followed by ammonia and the ammonium cation, which predominates. The ammonium cation is less mobile in soil and water than ammonia and is involved in the biological processes of nitrogen fixation, mineralization, and nitrification (2).

**13.2.2 Analytical methods**

Ammonia and ammonium cation at concentrations between 0.025 and 3 mg/litre can be determined by the indophenol reaction (I, 2, 5, 6). An ammonia-selective electrode can also be used, as can titrimetry, which is less sensitive (2, 5, 6).

**13.2.3 Environmental levels and human exposure**

**Air**

Air in urban areas contains up to 20 µg of ammonia per m³. Air in areas where farm animals are reared intensively may contain levels as high as 300 µg/m³ (7).

---


GUIDELINES FOR DRINKING-WATER QUALITY

Water
Natural levels in groundwaters are usually below 0.2 mg of ammonia per litre. Higher natural contents (up to 3 mg/litre) are found in strata rich in humic substances or iron or in forests (8). Surface waters may contain up to 12 mg/litre (1). Ammonia may be present in drinking-water as a result of disinfection with chloramines.

The presence of ammonia at higher than geogenic levels is an important indicator of faecal pollution (5). Taste and odour problems as well as decreased disinfection efficiency are to be expected if drinking-water containing more than 0.2 mg of ammonia per litre is chlorinated (9), as up to 68% of the chlorine may react with the ammonia and become unavailable for disinfection (10). Cement mortar used for coating the insides of water pipes may release considerable amounts of ammonia into drinking-water and compromise disinfection with chlorine (10).

The presence of elevated ammonia levels in raw water may interfere with the operation of manganese-removal filters because too much oxygen is consumed by nitrification, resulting in mouldy, earthy-tasting water (8). The presence of the ammonium cation in raw water may result in a drinking-water containing nitrite as the result of catalytic action (11) or the accidental colonization of filters by ammonium-oxidizing bacteria.

Food
Ammonium is a natural component of many foods. Minor amounts of ammonium compounds (<0.001–3.2%) are also added to foods as acid regulators, stabilizers, flavouring substances, and fermentation aids (1).

Estimated total exposure and relative contribution of drinking-water
The estimated daily ammonia intake through food and drinking-water is 18 mg, by inhalation less than 1 mg, and through cigarette smoking (20 cigarettes per day) also less than 1 mg. In contrast, 4000 mg of ammonia per day are produced endogenously in the human intestine (1).

13.2.4 Kinetics and metabolism in laboratory animals and humans
Ammonia is a key metabolite in mammals. It has an essential role in acid-base regulation and the biosynthesis of purines, pyrimidines, and non-essential amino acids (2). It is formed in the body by the deamination of amino acids in the liver, as a metabolite in nerve excitation and muscular activity, and in the gastrointestinal tract by the enzymatic breakdown of food components with the assistance
of bacterial flora. About 99% of metabolically produced ammonia is absorbed from the gastrointestinal tract and transported to the liver, where it is incorporated into urea as part of the urea cycle. Urea formed in the liver is absorbed by the blood, transferred to the kidney, and excreted in urine. Of the ammonia found in urine, two-thirds originates from the tubular epithelium of the kidney where, as a product of the glutaminase reaction, it maintains the acid-base equilibrium by the uptake of hydrogen ions.

13.2.5 Effects on laboratory animals and in vitro test systems

**Acute exposure**

Oral LD$_{50}$ values for ammonium salts are in the range 350–750 mg/kg of body weight. Single doses of different ammonium salts at 200–500 mg/kg of body weight resulted in lung oedema, nervous system dysfunction, acidosis, and kidney damage.

**Short-term exposure**

Animals subchronically exposed to different ammonium salts (75–360 mg/kg of body weight as the ammonium ion) in drinking-water exhibited physiological adaptation to induced acidosis, slight organ effects, or increased blood pressure.

**Long-term exposure**

In male Sprague-Dawley rats given drinking-water containing 1.5% ammonium chloride (about 478 mg of ammonium ion per kg of body weight per day) over a period of 330 days, significant decreases were found in bone mass, calcium content, and blood pH. The treated animals also had lower body weights and lower fat accumulation than controls.

**Reproductive toxicity, embryotoxicity, and teratogenicity**

Oral administration of different ammonium compounds at doses of 100–200 mg/kg of body weight to impuberal female rabbits resulted in enlargement of the ovaries and uterus, hypertrophy of the breast with milk secretion, follicular ripening, and formation of the corpus luteum. A dose of 0.9% ammonium chloride (approximately 290 mg of ammonia per kg of body weight per day) in the drinking-water of pregnant rats inhibited fetal growth but had no teratogenic effects.

---

**Mutagenicity and related end-points**

At high concentrations, positive results in the Balb e/3T3-transformation test, the sex-linked dominant/lethal mutation test, and chromosomal aberrations in fibroblasts of Chinese hamsters were observed; other genotoxicity tests gave negative results (2).

**Carcinogenicity**

There is no evidence that ammonia is carcinogenic (2).

13.2.6 Effects on humans

Ammonia has a toxic effect on healthy humans only if the intake becomes higher than the capacity to detoxify.

If ammonia is administered in the form of its ammonium salts, the effects of the anion must also be taken into account. With ammonium chloride, the acidotic effects of the chloride ion seem to be of greater importance than those of the ammonium ion (1). At a dose of more than 100 mg/kg of body weight per day (33.7 mg of ammonium ion per kg of body weight per day), ammonium chloride influences metabolism by shifting the acid–base equilibrium, disturbing the glucose tolerance, and reducing the tissue sensitivity to insulin (2).

13.2.7 Conclusions

Ammonia is not of direct importance for health in the concentrations to be expected in drinking-water. A health-based guideline has therefore not been derived.

Ammonia can, however, indicate faecal contamination, compromise disinfection efficiency, cause taste and odour problems, result in nitrite formation in distribution systems, and cause the failure of filters for the removal of manganese.

References


### 13.3 Antimony

#### 13.3.1 General description

**Identity**

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS no.</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antimony</td>
<td>7440-36-0</td>
<td>Sb</td>
</tr>
<tr>
<td>Potassium antimony tartrate</td>
<td>28300-74-5</td>
<td>KSBOC₄H₄O₆</td>
</tr>
<tr>
<td>Sodium antimony tartrate</td>
<td>34521-09-0</td>
<td>NaSbOC₄H₄O₆</td>
</tr>
<tr>
<td>Sodium antimony bis(pyrocatechol) 2,4-disulfate</td>
<td>15489-16-4</td>
<td>C₁₂H₁₉Na₂O₂₃S₄Sb</td>
</tr>
</tbody>
</table>
Physicochemical properties (1–3)\(^1\)

<table>
<thead>
<tr>
<th>Property</th>
<th>Sb</th>
<th>K(_2)SbOC(_4)H(_4)O(_6)</th>
<th>Na(_2)SbOC(_2)H(_4)O(_6)</th>
<th>C(<em>{12})H(</em>{18})Na(<em>5)O(</em>{23})S(_4)Sb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point (°C)</td>
<td>630.5</td>
<td>100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>1635</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Density at 20 °C (g/cm(^3))</td>
<td>6.691</td>
<td>2.6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vapour pressure at 886 °C (kPa)</td>
<td>0.133</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Water solubility (g/litre)</td>
<td>insoluble</td>
<td>83</td>
<td>666.7</td>
<td>soluble</td>
</tr>
</tbody>
</table>

Organoletic properties

Potassium antimony tartrate is odourless and has a sweet metallic taste (2).

Major uses

Antimony is used in semiconductor alloys, batteries, antifriction compounds, ammunition, cable sheathing, flameproofing compounds, ceramics, glass, pottery, type castings for commercial printing, solder alloys, and fireworks. Some antimony compounds are used for the treatment of parasitic diseases and as pesticides (1–3).

Environmental fate

Antimony may be present in the atmosphere in gaseous, vapour, and particulate forms. In water, it may undergo either oxidation or reduction, depending on the pH and the other ions present. Soluble forms tend to be quite mobile in water, whereas less soluble species are adsorbed onto clay or soil particles. Antimony may be leached from landfills and sewage sludge into groundwater, surface water, and sediment (4), from the last of which it can be released to the atmosphere through microbial activity under anaerobic conditions (5). More than half of the naturally occurring antimony in sediments is bound to extractable iron and aluminium (6). Antimony is only slightly bioaccumulated.

13.3.2 Analytical methods

Antimony can be determined by graphite furnace atomic absorption spectrometry (lower detection limit 0.8 µg/litre, EPA method 204.2) or inductively coupled plasma mass spectrometry (lower detection limit 0.02 µg/litre, EPA method 6020). Following separation of antimony(III) from antimony(V) using

---

13. INORGANIC CONSTITUENTS AND PHYSICAL PARAMETERS

\( N-(p\text{-methoxyphenyl})-2\text{-furyl}acrylohydroxamic\) acid, the two species can be determined by electrothermal atomic absorption spectrometry at concentrations down to 0.01 \( \mu g/litre\) (7).

13.3.3 Environmental levels and human exposure

**Air**

Antimony was present in the air of four of 58 American cities at levels of 0.42–0.85 \( \mu g/m^3\). Three of 29 nonurban areas had concentrations of 1–2 \( ng/m^3\) (8). Smoking can result in antimony contamination of indoor air (9).

**Water**

Antimony has been identified in natural waters in both the antimony(III) and antimony(V) oxidation states and as methyl antimony compounds. It occurs in seawater at a concentration of about 0.2 \( \mu g/litre\) (10, 11). A survey in the USA found antimony in only three of 988 samples of finished drinking-water from groundwater sources, the concentrations ranging from 41 to 45 \( \mu g/litre\) (lower detection limit 9 \( \mu g/litre\)) (12). In a study of 3834 samples of drinking-water, antimony was found in 16.5% of samples, at concentrations ranging from 0.6 to 4 \( \mu g/litre\) (mean 1.87 \( \mu g/litre\)) (13).

**Food**

Trace quantities of antimony are present in the food supply, the concentration in the diet of a typical adult male being 9.3 \( \mu g/kg\) dry weight based on the analysis of food composites (14).

**Estimated total exposure and relative contribution of drinking-water**

The average intake of antimony by ingestion of food is about 18 \( \mu g/day\) (14); the corresponding figure for drinking-water will usually be less than 8 \( \mu g/day\).

13.3.4 Kinetics and metabolism in laboratory animals and humans

Antimony is not readily absorbed from the gastrointestinal tract, regardless of valence state (15), absorption ranging from less than 5% in cows (16) to 15% in rats (17). Most of the antimony absorbed accumulates in the spleen, liver, and bone (18, 19). Transfer of antimony from maternal to fetal blood has been demonstrated (20). Trivalent antimony readily enters red blood cells, but pentavalent antimony does not (21, 22). Available data are insufficient to determine whether antimony(V) compounds are reduced to antimony(III) in vivo. Parenterally administered trivalent antimony was excreted via the faeces and urine in mice, white rats, hamsters, guinea-pigs, rabbits, dogs, and humans (23). Pentavalent
antimony was excreted primarily in the urine. In cows, orally administered antimony trichloride was excreted primarily in the faeces (16). In adult males, 21.6–70% of the antimony administered daily was excreted in urine, only low levels (0.8–8.4%) being present in faeces. Pentavalent antimony was excreted in urine more rapidly than trivalent antimony (22, 24).

13.3.5 Effects on laboratory animals and in vitro test systems

**Acute exposure**

The acute oral LD$_{50}$ values for potassium antimony tartrate in mice and rats range from 115 to 600 mg of antimony per kg of body weight, whereas an oral LD$_{50}$ of 15 mg of antimony per kg of body weight has been reported for rabbits (4).

**Short-term exposure**

Four rabbits were given potassium antimony tartrate at 15 mg/kg of body weight per day (5.6 mg of antimony per kg of body weight per day) for 7–22 days (25). Small increases in nonprotein nitrogen in blood and urine and in mean urine ammonia nitrogen were observed, which the author interpreted as evidence of increased protein catabolism. Gross and microscopic examination showed haemorrhagic lesions in the stomach and small intestine, liver atrophy with fat accumulation and congestion, haemorrhage in the renal cortex, with some tubular necrosis. This study suggests a LOAEL of 5.6 mg/kg of body weight per day, based on minimal histological injury in tissues.

Male and female Wistar rats were given two antimony-containing pigments in the diet at concentrations up to 10 000 mg/kg (36 and 22 mg/kg of body weight per day, respectively) for 91 days. No effects on behaviour, food consumption, growth, mortality, haematology, clinical data, or organ weights were observed (26). No toxic effects were observed in rats given potassium antimony tartrate, potassium antimonate, antimony trioxide, or antimony pentoxide in food at doses ranging from 0.1 to 4 mg/day for 107 days (1).

**Long-term exposure**

Potassium antimony tartrate (0 or 5 mg of antimony per litre) was administrated in drinking-water to male and female Charles River CD mice from the time of weaning until death (27). Weight loss was observed in males after 18 months and decreased weight gain in females at 12 and 18 months, and life spans were decreased in females but not in males. No significant fatty degeneration of the liver was observed. This study suggests a LOAEL of 0.5 mg/kg of body weight per day.

In a companion study, potassium antimony tartrate (0 or 5 mg of antimony per litre) was administrated in drinking-water to Long-Evans rats (50 per sex per dose) from the time of weaning until death (28). This corresponds to an
average dose of 0.43 mg of antimony per kg of body weight per day, assuming a body weight of 0.35 kg and water consumption of 30 ml/day. No significant effects on glucosuria, proteinuria, fasting blood glucose levels, body weight, heart weight, or heart-to-body-weight ratio were observed. Mean longevity decreased in both sexes. Serum cholesterol levels were increased in male rats and decreased in female rats. Nonfasting blood glucose levels were lower in both sexes. Antimony accumulated in kidney, liver, heart, lung, and spleen, increasing with age and dose. This study identified a LOAEL of 0.43 mg of antimony per kg of body weight per day based on decreased longevity and altered blood glucose and serum cholesterol levels.

**Reproductive toxicity, embryotoxicity, and teratogenicity**

Four ewes fed antimony potassium tartrate at a dose level of 2 mg/kg of body weight for 45 days or throughout gestation gave birth to normal, full-term lambs (29). Sterility and fewer offspring were observed in rats following inhalation exposures to antimony trioxide over 2 months (1, 30). In female rabbits and mice injected with sodium antimony tartrate or an unknown organic antimony compound over a period ranging from 16 to 77 days, some contraception, abortion, and fetal damage occurred, but sterility in male mice was not observed (31). No abnormalities were reported in Wistar rat fetuses whose mothers were given intramuscular injections of antimony dextran glycoside during gestation (19).

**Mutagenicity and related end-points**

Antimony trichloride, antimony pentachloride, and antimony trioxide were mutagenic in the *Bacillus subtilis* (H17 and M45) rec-assay (1, 32, 33). Potassium and sodium antimony tartrate induced chromosomal aberrations in cultured human leukocytes (34, 35). Piperazine and potassium antimony tartrate induced chromosomal aberrations in rat bone marrow cells *in vivo* (36).

**Carcinogenicity**

The effect of lifetime exposure to antimony on tumour frequency was investigated in Charles River CD mice given potassium antimony tartrate (0 or 5 mg of antimony per litre) in drinking-water from the time of weaning until death (27). Tumours (benign and malignant) were found in 34.8% of control animals (no explanation given) and 18.8% of the antimony-treated animals. The authors concluded that antimony exposure had no effect on the incidence or type of spontaneous tumours. In a companion study (28), no significant effects of antimony exposure on tumour frequency were observed in male or female rats.

Antimony trioxide and antimony ore concentrate were found to cause lung tumours in female rats exposed by inhalation (1, 37, 38).
GUIDELINES FOR DRINKING-WATER QUALITY

13.3.6 Effects on humans

Acute antimony poisoning may result in vomiting, diarrhoea, and death (39, 40). Sodium stibogluconate given intravenously in a daily dose of 600 mg of antimony(V) for 10 days to 16 patients with skin lesions caused by parasitic protozoa did not adversely affect either glomerular or renal function (41). Trivalent and pentavalent antimony compounds affected the ECGs of patients being treated for schistosomiasis (42, 43).

Six adult males who had worked in an antimony smelter for 2–13 years exhibited no signs of adverse cardiac, bladder, kidney, or haematological effects, nor were there any reported effects on general health (1, 44). Workmen in a plant where antimony trisulfide was used exhibited increased blood pressure (14 of 113), significant changes in their ECGs (37 of 75), and ulcers (7 of 111 as compared with 15 out of 1000 in the total plant population) (1, 45). Female workers employed in an antimony plant showed an increased incidence of spontaneous late abortions (12.5%) as compared with female workers not exposed to antimony dust (4.1%) (30).

Workers exposed for 9–31 years to dust containing a mixture of antimony trioxide and antimony pentoxide in an antimony smelting plant (1, 46) exhibited symptoms such as chronic coughing, bronchitis, and emphysema, conjunctivitis, staining of frontal tooth surface, inactive tuberculosis, and pleural adhesions. “Antimony dermatitis” characterized by vesicular or pustular lesions was seen in more than half the exposed workers.

13.3.7 Provisional guideline value

In its overall evaluation based on inhalation exposure, IARC concluded that antimony trioxide is possibly carcinogenic to humans (Group 2B) and antimony trisulfide is not classifiable as to its carcinogenicity to humans (Group 3) (1).

In a limited lifetime study in which rats were exposed to antimony in drinking-water at a single dose level of 0.43 mg of antimony per kg of body weight per day, decreased longevity and altered blood levels of glucose and cholesterol were observed (28). The incidence of benign or malignant tumours was not affected. Using an uncertainty factor of 500 (100 for inter- and intranspecies variation and 5 for the use of a LOAEL instead of a NOAEL), a TDI of 0.86 µg/kg of body weight can be calculated. An allocation of 10% of the TDI to drinking-water gives a concentration of 0.003 mg/litre (rounded figure), which is below the practical limit of quantitative analysis.

A provisional guideline value for antimony has therefore been set at a practical quantification level of 0.005 mg/litre. This results in a margin of safety of approximately 250-fold for potential health effects, based on the LOAEL of 0.43 mg/kg of body weight per day observed in the limited lifetime study in rats.
References


### 13.4 Arsenic

#### 13.4.1 General description

**Identity**

Arsenic exists in oxidation states of -3, 0, 3, and 5. It is widely distributed throughout the earth's crust, most often as arsenic sulfide or as metal arsenates and arsenides.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS no.</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>7440-38-2</td>
<td>As</td>
</tr>
<tr>
<td>Arsenic trioxide</td>
<td>1327-53-3</td>
<td>As₂O₃</td>
</tr>
<tr>
<td>Arsenic pentoxide</td>
<td>1303-28-2</td>
<td>As₂O₅</td>
</tr>
<tr>
<td>Arsenic sulfide</td>
<td>1303-33-9</td>
<td>As₂S₃</td>
</tr>
<tr>
<td>Dimethylarsinic acid</td>
<td>75-60-5</td>
<td>(CH₃)₂AsO(OH)</td>
</tr>
<tr>
<td>Lead arsenate</td>
<td>10102-48-4</td>
<td>PbHAsO₄</td>
</tr>
<tr>
<td>Potassium arsenate</td>
<td>7784-41-0</td>
<td>KH₂AsO₄</td>
</tr>
<tr>
<td>Potassium arsenite</td>
<td>10124-50-2</td>
<td>KAsO₂·HAsO₂</td>
</tr>
</tbody>
</table>
**Physicochemical properties** *(1, 2)*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Density (g/cm³)</th>
<th>Water solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>613</td>
<td>-</td>
<td>5.727</td>
<td>insoluble</td>
</tr>
<tr>
<td>As₂O₃</td>
<td>312.3</td>
<td>465</td>
<td>3.738</td>
<td>37 at 20 °C</td>
</tr>
<tr>
<td>As₂O₅</td>
<td>315</td>
<td>-</td>
<td>4.32</td>
<td>1500 at 16 °C</td>
</tr>
<tr>
<td>As₂S₃</td>
<td>300</td>
<td>707</td>
<td>3.43</td>
<td>5 x 10⁻⁴</td>
</tr>
<tr>
<td>(CH₃)₂AsO(OH)</td>
<td>200</td>
<td>-</td>
<td>-</td>
<td>829 at 22 °C</td>
</tr>
<tr>
<td>PbHAsO₄</td>
<td>720</td>
<td>-</td>
<td>5.79</td>
<td>very slightly soluble</td>
</tr>
<tr>
<td>KH₂AsO₄</td>
<td>288</td>
<td>-</td>
<td>2.867</td>
<td>190 at 6 °C</td>
</tr>
<tr>
<td>K₂AsO₃HAsO₂</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>soluble</td>
</tr>
</tbody>
</table>

**Major uses**

Arsenicals are used commercially and industrially as alloying agents in the manufacture of transistors, lasers, and semiconductors, as well as in the processing of glass, pigments, textiles, paper, metal adhesives, wood preservatives, and ammunition. They are also used in the hide tanning process and, to a limited extent, as pesticides, feed additives, and pharmaceuticals.

**Environmental fate**

Arsenic is introduced into water through the dissolution of minerals and ores, from industrial effluents, and via atmospheric deposition *(3–5)*. In well oxygenated surface waters, arsenic(V) is generally the most common species present *(6, 7)*; under reducing conditions, such as those often found in deep lake sediments or groundwaters, the predominant form is arsenic(III) *(8, 9)*. An increase in pH may increase the concentration of dissolved arsenic in water *(10)*.

**13.4.2 Analytical methods**

A silver diethyldithiocarbamate spectrophotometric method is available for the determination of arsenic: the detection limit is about 1 µg/litre *(11)*. Graphite furnace atomic absorption spectroscopy in combination with high-pressure liquid chromatography can also be used to determine various arsenic species *(6)*.
13.4.3 Environmental levels and human exposure

**Air**

Arsenic concentrations in air range from 0.4 to 30 ng/m$^3$ (12–14); higher concentrations are present in the vicinity of industrial sources (12, 15).

**Water**

The level of arsenic in natural waters generally varies between 1 and 2 μg/litre (3). Concentrations may be elevated, however, in areas containing natural sources; values as high as 12 mg/litre have been reported (16).

**Food**

Fish and meat are the main sources of dietary intake of arsenic (17); levels ranging from 0.4 to 118 mg/kg have been reported in marine fish sold for human consumption, and concentrations in meat and poultry can be as high as 0.44 mg/kg (18).

The mean daily intake of arsenic in food for adults has been estimated to range from 16.7 to 129 μg (17, 19–21); the corresponding figures for infants and children are 1.26–15.5 μg (22, 23).

On the basis of data on the arsenic content of various foodstuffs (20, 24), it can be estimated that approximately 25% of the intake of arsenic from food is inorganic and 75% is organic.

**Estimated total exposure and relative contribution of drinking-water**

The estimated mean daily intake of arsenic from food is approximately 40 μg, about 10 μg of which is inorganic arsenic. The mean daily intake of arsenic from drinking-water will generally be less than 10 μg, based on a concentration of arsenic in drinking-water in areas without natural sources of less than 5 μg/litre and an average daily consumption of 2 litres of drinking-water. The estimated intake from air is generally less than 1 μg.

13.4.4 Kinetics and metabolism in laboratory animals and humans

Ingested elemental arsenic is poorly absorbed and largely eliminated unchanged. Soluble arsenic compounds are rapidly absorbed from the gastrointestinal tract (3); arsenic(V) and organic arsenic are rapidly and almost completely eliminated via the kidneys (25–27). Inorganic arsenic may accumulate in skin, bone, and muscle (28); its half-life in humans is between 2 and 40 days (29).

Arsenic(III) is eliminated from the body by the rapid urinary excretion of nonmethylated arsenic in both the trivalent and pentavalent forms and by detoxification by sequential methylation of arsenic(III) in the liver to mono-
methylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) (30, 31). Limited short-term studies on humans indicate that the capacity to methylate inorganic arsenic is progressively, but not completely, saturated when daily intake exceeds 0.5 mg (32).

In humans, inorganic arsenic does not appear to cross the blood–brain barrier; however, transplacental transfer of arsenic in humans has been reported (33).

13.4.5 Effects on laboratory animals and \textit{in vitro} test systems

\textbf{Long-term exposure}

There were significant reductions in cardiac output and stroke volume in male Wistar rats and female New Zealand rabbits ingesting drinking-water containing 50 µg of arsenic(III) per ml for 18 and 10 months, respectively. In contrast, there was no effect on cardiac function in rats following ingestion of the same concentration of arsenic(V) for 18 months (34).

\textbf{Reproductive toxicity, embryotoxicity, and teratogenicity}

Teratogenic effects of arsenic in chicks, golden hamsters, and mice have been reported (35, 36). Arsenate was teratogenic in the offspring of pregnant hamsters following exposure on days 4–7 of gestation by minipump implantation (37); the threshold blood level for teratogenesis was 4.3 µmol/kg (38). The specific form of arsenic responsible for teratogenesis is not known, but it may be arsenite (39).

\textbf{Mutagenicity and related end-points}

Arsenic does not appear to be mutagenic in bacterial and mammalian assays, although it can induce chromosome breakage, chromosomal aberrations, and sister chromatid exchange in a linear, dose-dependent fashion in a variety of cultured cell types, including human cells (24, 40). Arsenic(III) is about an order of magnitude more potent than arsenic(V) in this respect (24).

\textbf{Carcinogenicity}

Arsenic has not been found to be carcinogenic in animal bioassays, with one exception. In a study of the potential of arsenic compounds to act as promoters, a significant increase in the incidence of kidney tumours was observed in male Wistar rats injected intraperitoneally with a single dose of diethylnitrosamine (30 mg/kg) and, from day 7, given the maximum tolerated dose (160 mg/litre) of arsenic(III) in drinking-water for 25 weeks (41).
13.4.6 Effects on humans

Although the results of available studies indicate that arsenic may be an essential element for several animal species (e.g., goats, rats, and chicks), there is no evidence that it is essential for humans (24).

The acute toxicity of arsenic compounds in humans is predominantly a function of their rate of removal from the body. Arsine is considered to be the most toxic form, followed by the arsenites [arsenic(III)], the arsenates [arsenic(V)] and organic arsenic compounds. Lethal doses in humans range from 1.5 mg/kg of body weight (diarsenic trioxide) to 500 mg/kg of body weight (DMAA) (42). Acute arsenic intoxication associated with the ingestion of well-water containing 1.2 and 21.0 mg of arsenic per litre has been reported (43, 44).

Early clinical symptoms of acute intoxication include abdominal pain, vomiting, diarrhoea, muscular pain, and weakness, with flushing of the skin. These symptoms are often followed by numbness and tingling of the extremities, muscular cramping, and the appearance of a papular erythematous rash (45). Within a month, symptoms may include burning paraesthesias of the extremities, palmar-plantar hyperkeratosis, and progressive deterioration in motor and sensory responses (45-47).

Signs of chronic arsenicalism, including dermal lesions, peripheral neuropathy, skin cancer, and peripheral vascular disease, have been observed in populations ingesting arsenic-contaminated drinking-water (48-55). Dermal lesions were the most commonly observed symptoms, occurring after minimum exposure periods of approximately 5 years. Effects on the cardiovascular system were observed in children consuming arsenic-contaminated water (mean concentration 0.6 mg/litre) for an average of 7 years (51, 52).

In a large study conducted in China (Province of Taiwan), a population of 40,421 was divided into three groups based on the arsenic content of their well-water (high, >0.60 mg/litre; medium, 0.30-0.59 mg/litre; and low, <0.29 mg/litre) (48). There was a clear dose-response relationship between exposure to arsenic and the frequency of dermal lesions, “blackfoot disease” (a peripheral vascular disorder), and skin cancer. However, several methodological weaknesses (e.g., investigators were not “blinded”) complicate the interpretation of the results. In addition, the possibility that other compounds present in the water supply might have been responsible for blackfoot disease was not considered. It has been suggested, for example, that humic acid in artesian well-water is the cause of the disease, not arsenic (56).

In a study in which cancer mortality was examined in relation to the arsenic content of contaminated drinking-water in the same villages of China (Province of Taiwan) and at the same three levels, there were significant dose-response relationships for age-adjusted rates for cancers of the bladder, kidney, skin, and lung in both sexes and cancers of the prostate and liver in males (57). A study in which the ecological correlations between the arsenic level of well-water and mortality from various malignant neoplasms in China (Province of Taiwan) were
examined demonstrated a significant association with the arsenic level in well-water for cancers of the liver, nasal cavity, lung, skin, bladder, and kidney in both males and females and for prostate cancer in males (58).

In an investigation of the association between cancer incidence and the ingestion of arsenic-contaminated water in a limited area of China (Province of Taiwan), standardized mortality ratios (SMRs) for cancers of the bladder, kidney, skin, lung, liver, and colon were significantly elevated in the area of arsenic contamination. The SMRs for all but colon cancer also correlated well with the prevalence rate for blackfoot disease (59). In a case–control study of 204 subjects who died of cancer (69 of bladder, 76 of lung, and 59 of liver cancer) and 368 community controls matched for age and sex, the odds ratios of developing these cancers for those who had used artesian well-water for 40 or more years were 3.90, 3.39, and 2.67, respectively. Dose–response relationships were observed for all three cancer types by duration of exposure, and the odds ratios were not changed significantly when several other risk factors were taken into consideration in logistic regression analysis (60). A Technical Panel on Arsenic established by the US Environmental Protection Agency concluded that, although these studies demonstrated a qualitative relationship between the ingestion of arsenic-contaminated water and internal cancers, the data were not sufficient to enable the dose–response relationship to be assessed (24).

In a study conducted in Mexico, the health status of the populations of two rural towns was examined, the towns differing in the average arsenic concentration of their water supplies, which was 0.41 ± 0.114 mg/litre ("exposed") in the first and 0.005 ± 0.007 mg/litre ("control") in the second (54). The prevalence of nonspecific symptoms, such as nausea, abdominal pain, and diarrhoea, was significantly higher in the "exposed" population; the relative risks for these symptoms ranged from 1.9 to 4.8, while that of developing cutaneous lesions ranged from 3.6 to 36. The prevalence of skin cancer in the "exposed" population in Mexico was 6.4%, as compared with 1.06% in the population with similar exposure in China (Province of Taiwan) (0.30–0.59 mg/litre group) (48). This study suffered from methodological weaknesses; for example, the investigators were not blinded and drinking-water was assumed to be the only source of arsenic.

In a case–control study of 270 children with congenital heart disease and 665 healthy children, maternal consumption during pregnancy of drinking-water containing detectable arsenic concentrations was associated with a threefold increase in the occurrence of coarctation of the aorta (the prevalence odds ratio adjusted for all measured contaminants and source of drinking-water was 3.4 with 95% confidence limits of 1.3–8.9) (35). However, no adjustment was made for maternal age, socioeconomic status, or previous reproductive history, and exposure was not determined directly.

In a case–control study in Massachusetts of 286 women with spontaneous abortions and 1391 women with live births, elevated odds ratios for miscarriages were associated with exposure to arsenic in drinking-water (61). The odds ratio for spontaneous abortion, adjusted for maternal age, educational level, and histo-
ry of prior spontaneous abortion, for women exposed to undetectable concentrations, 0.8–1.3 \(\mu g/litre\), and 1.4–1.9 \(\mu g/litre\) of arsenic in their drinking-water were 1.0, 1.1, and 1.5, respectively. Again, however, exposure was determined indirectly, and it would be desirable to follow up these preliminary results in studies designed to assess exposure more accurately.

### 13.4.7 Provisional guideline value

Inorganic arsenic compounds are classified by IARC in Group 1 (carcinogenic to humans) on the basis of sufficient evidence for carcinogenicity in humans and limited evidence for carcinogenicity in animals (62). No adequate data on the carcinogenicity of organic arsenicals were available. The guideline value has been derived on the basis of estimated lifetime cancer risk.

Data on the association between internal cancers and ingestion of arsenic in drinking-water are limited and insufficient for quantitative assessment of an exposure–response relationship (24). However, based on the increased incidence of skin cancer observed in the population in China (Province of Taiwan), the US Environmental Protection Agency has used a multistage model that is both linear and quadratic in dose to estimate the lifetime skin cancer risk associated with the ingestion of arsenic in drinking-water. With this model and data on males (24), the concentrations of arsenic in drinking-water associated with estimated excess lifetime skin cancer risks of \(10^{-4}\), \(10^{-5}\), and \(10^{-6}\) are 1.7, 0.17, and 0.017 \(\mu g/litre\), respectively.

It should be noted, however, that these values may overestimate the actual risk of skin cancer because of possible simultaneous exposure to other compounds in the water and possible dose-dependent variations in metabolism that could not be taken into consideration. In addition, the concentration of arsenic in drinking-water at an estimated skin cancer risk of \(10^{-5}\) is below the practical quantification limit of 10 \(\mu g/litre\).

A value of 13 \(\mu g/litre\) may be derived (assuming a 20% allocation to drinking-water) on the basis of the provisional maximum tolerable daily intake (PMTDI) of inorganic arsenic of 2 \(\mu g/kg\) of body weight set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1983 and confirmed as a provisional tolerable weekly intake (PTWI) of 15 \(\mu g/kg\) of body weight in 1988 (63). JECFA noted, however, that the margin between the PTWI and intakes reported to have toxic effects in epidemiological studies was narrow.

With a view to reducing the concentration of arsenic in drinking-water, a provisional guideline value of 0.01 \(mg/litre\) is recommended. The estimated excess lifetime risk of skin cancer associated with exposure to this concentration is \(6 \times 10^{-4}\).
References


13.5 Asbestos

13.5.1 General description

Identity

Asbestos is a general term for fibrous silicate minerals containing iron, magnesium, calcium, or sodium. These can be divided into two main groups, namely serpentine (e.g. chrysotile) and amphibole (e.g. amosite, crocidolite, and tremolite).
**Physicochemical properties**

Chrysotile is easily degraded by strong acids, whereas amphiboles are more resistant. The various forms of asbestos are generally resistant to alkali. The chemical nature and crystalline structure of asbestos impart to it a number of characteristics, including high tensile strength, durability, flexibility, and resistance to heat and chemicals (1).

**Major uses**

Asbestos, particularly chrysotile, is used in a large number of applications, particularly in construction materials, such as asbestos-cement (A/C) sheet and pipe, electrical and thermal insulation, and friction products, such as brake linings and clutch pads (1).

13.5.2 Analytical methods

The method of choice for the quantitative determination of asbestos in ambient air and water is transmission electron microscopy (TEM) with identification by energy-dispersive X-ray analysis and selected-area electron diffraction (TEM/SAED). However, TEM/SAED is costly, and preliminary screening with TEM alone (2), which has a detection limit of below 0.1 million fibres per litre (MFL) in water (3), is therefore often used.

13.5.3 Environmental levels and human exposure

**Air**

Mean chrysotile concentrations at 24 locations in southern Ontario (Canada) ranged from <2 to 11 fibres longer than 5 \( \mu m \) per litre. Concentrations at 10 remote rural locations were all below the detection limit in this study (<2 fibres/litre) (1, 4). Levels in samples from downtown and suburban locations in Stockholm (Sweden) were in the range 1–3 fibres longer than 5 \( \mu m \) per litre (1, 4).

Airborne asbestos may be released from tapwater in the home. Mean airborne asbestos concentrations were significantly higher (1.7 ng/m\(^3\)) in three homes with water containing elevated concentrations of asbestos than in three control homes (0.31 ng/m\(^3\)); however, the difference in concentration was due primarily to increased numbers of short fibres (<1 \( \mu m \)), which are considered to pose little health risk. Moreover, all the fibre concentrations found in this limited study were within the range of those measured in indoor and outdoor air in other investigations (5). Negligible amounts of asbestos fibres were released to air from water containing 40 ± 10 MFL via a conventional drum-type humidifier (6).
**Water**

Asbestos is introduced into water by the dissolution of asbestos-containing minerals and ores as well as from industrial effluents, atmospheric pollution, and A/C pipes in water-distribution systems. Exfoliation of asbestos fibres from A/C pipes is related to the aggressiveness of the water supply (3). Although A/C piping is used in about 19% of water-distribution systems in Canada, erosion of such piping appeared to contribute measurably to the asbestos content of water supplies at only two of 71 locations surveyed (7). In contrast, high levels of asbestos have been recorded in association with the severe deterioration of A/C pipe containing chrysotile and crocidolite in Woodstock, New York (USA) (8).

Chrysotile was the predominant type of asbestos detected in a national survey of the water supplies of 71 communities in Canada; concentrations varied from not detectable (<0.1 MFL) to 2000 MFL, while median fibre lengths were in the range 0.5–0.8 μm. It was estimated that concentrations were >1 MFL in the water supplies of 25% of the population, >10 MFL for 5% of the population, and >100 MFL for 0.6% of the population. Concentrations were higher in raw than in treated water (7).

The results of a number of surveys indicate that most of the population of the USA consumes drinking-water containing asbestos in concentrations below 1 MFL (9). In 1974, concentrations of optically visible fibres up to 33 MFL were detected in drinking-water supplies in the Netherlands (10). The results of a survey of asbestos concentrations in raw and treated waters in the United Kingdom suggest that most drinking-waters contain asbestos fibres in concentrations varying from not detectable up to 1 MFL (11).

**Food**

The asbestos content of solid foodstuffs has not been well studied because of the lack of a simple, reliable analytical method. Foods that contain soil particles, dust, or dirt probably contain asbestos fibres; crude estimates suggest that the intake of asbestos in food may be significant in comparison with that in drinking-water (12). Concentrations of 0.151 MFL and 4.3–6.6 MFL in beer and 1.7–12.2 MFL in soft drinks have been reported (13).

13.5.4 Kinetics and metabolism in laboratory animals and humans

Information on the transmigration of ingested asbestos through the gastrointestinal tract to other tissues is contradictory (7, 3). Available data indicate that penetration, if it occurs at all, is extremely limited.
13.5.5 Effects on laboratory animals and in vitro test systems

**Reproductive toxicity, embryotoxicity, and teratogenicity**

Administration of 4–400 mg of chrysotile per kg of body weight to CD-1 mice on days 1–15 of pregnancy did not affect the survival of the progeny. In vitro administration did not interfere with implantation on transfer of exposed blastocysts to recipient females but did result in a decrease in post-implantation survival. The authors concluded that asbestos was not teratogenic in these studies (14).

**Mutagenicity and related end-points**

Although not mutagenic, all types of asbestos have induced chromosomal aberrations in in vitro studies (15). In in vivo studies, a single oral administration of chrysotile did not increase the frequency of micronuclei in mice, and there was no increase in chromosomal aberrations in monkeys following oral administration of chrysotile by gavage (10).

**Carcinogenicity**

Although the carcinogenicity of inhaled asbestos is well established, there is no conclusive evidence that ingested asbestos is carcinogenic (1, 3, 16). In a series of extensive investigations involving treatment groups of 250 animals of each sex (17–19), no treatment-related increases in tumour incidence were observed in Syrian golden hamsters fed 1% amosite or short-range (98% shorter than 10 μm) or intermediate-range (65% longer than 10 μm) chrysotile, or in Fischer 344 rats fed 1% tremolite or amosite or short-range chrysotile in the diet over their lifetime. Although the incidence of benign epithelial neoplasms in the gastrointestinal tract in male Fischer 344 rats fed 1% intermediate-range chrysotile was significantly increased as compared with that in pooled controls from contemporary lifetime asbestos feeding studies in the same laboratory, the increase was not statistically significant in comparison with the data for concurrent controls and was limited to one sex.

13.5.6 Effects on humans

The health hazards associated with the inhalation of asbestos in the occupational environment have long been recognized and include asbestosis, bronchial carcinoma, malignant mesothelioma of the pleura and peritoneum, and possibly cancers of the gastrointestinal tract and larynx. In contrast, little convincing evidence has been found of the carcinogenicity of ingested asbestos in epidemiological studies of populations supplied with drinking-water containing high concentrations of asbestos (1, 15, 19–26). Moreover, the ability of asbestos fibres ingested in drinking-water to migrate through the walls of the gastrointestinal tract is very limited.
tract in sufficient numbers to cause adverse local or systemic effects is the subject of considerable disagreement (1, 27, 28).

In ecological population studies (1, 20, 22–25) (i.e. studies in which individual exposures were not estimated and population mobility was not adequately addressed), no consistent evidence was found of an association between cancer mortality or incidence and the ingestion of asbestos in drinking-water. In an analytical epidemiological (case-control) study that was inherently more sensitive than the ecological studies, there was no consistent evidence of a cancer risk associated with the ingestion of asbestos in drinking-water in Puget Sound, where levels up to 200 MFL were observed (26).

13.5.7 Conclusions

Although asbestos is a known human carcinogen by the inhalation route, available epidemiological studies do not support the hypothesis that an increased cancer risk is associated with the ingestion of asbestos in drinking-water. Moreover, in extensive feeding studies in animals, asbestos has not consistently increased the incidence of tumours of the gastrointestinal tract. There is therefore no consistent, convincing evidence that ingested asbestos is hazardous to health, and it is concluded that there is no need to establish a guideline value for asbestos in drinking-water.

References

6. Meranger JC, Reid WW, Davey ABC. The transfer of asbestos from water to air via a portable drum-type home humidifier. Canadian journal of public health, 1979, 70:276-278.


172
13.6 Barium

13.6.1 General description

Identity

Barium is present as a trace element in both igneous and sedimentary rocks. Although it is not found free in nature (1), it occurs in a number of compounds, most commonly barium sulfate (barite) and, to a lesser extent, barium carbonate (witherite).


GUIDELINES FOR DRINKING-WATER QUALITY

**Compound** | **CAS no.** | **Molecular formula**
---|---|---
Barium sulfide | 21109-95-5 | BaS
Barium chloride | 10361-37-2 | BaCl₂
Barium oxide | 1304-28-5 | BaO
Barium hydroxide | 17194-00-2 | Ba(OH)₂
Barium bromide | 10553-31-8 | BaBr₂
Barium nitrate | 10022-31-8 | Ba(NO₃)₂
Barium nitrite | 13465-94-6 | Ba(NO₂)₂
Barium sulfate | 7727-43-7 | BaSO₄
Barium acetate | 543-80-6 | Ba(C₂H₃O₂)₂

**Physicochemical properties (2, 3)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Melting point (°C)</th>
<th>Boiling point (°C)</th>
<th>Density (g/cm³)</th>
<th>Water solubility (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaS</td>
<td>1200</td>
<td>--</td>
<td>4.25</td>
<td>readily soluble</td>
</tr>
<tr>
<td>BaCl₂</td>
<td>960</td>
<td>1560</td>
<td>3.856 at 24°C</td>
<td>310 at 0°C</td>
</tr>
<tr>
<td>BaO</td>
<td>1923</td>
<td>2000</td>
<td>5.32–5.72</td>
<td>15 at 0°C</td>
</tr>
<tr>
<td>Ba(OH)₂</td>
<td>77.9</td>
<td>800</td>
<td>2.18 at 16°C</td>
<td>38.9 at 20°C</td>
</tr>
<tr>
<td>BaCl₂</td>
<td>847</td>
<td>decomposes</td>
<td>4.781 at 24°C</td>
<td>980 at 0°C</td>
</tr>
<tr>
<td>Ba(NO₃)₂</td>
<td>592</td>
<td>decomposes</td>
<td>3.24 at 23°C</td>
<td>92 at 20°C</td>
</tr>
<tr>
<td>Ba(NO₂)₂</td>
<td>217</td>
<td>decomposes</td>
<td>3.23</td>
<td>675 at 20°C</td>
</tr>
<tr>
<td>BaSO₄</td>
<td>1580</td>
<td>--</td>
<td>4.50 at 15°C</td>
<td>0.00285 at 30°C</td>
</tr>
<tr>
<td>Ba(C₂H₃O₂)₂</td>
<td>--</td>
<td>--</td>
<td>2.47</td>
<td>770 at 26°C</td>
</tr>
</tbody>
</table>

**Major uses**

Barium compounds, including barium sulfate and barium carbonate, are used in the plastics, rubber, electronics, and textiles industries, in ceramic glazes and enamels, in glass-making, brick-making, and paper-making, as a lubricant additive, in pharmaceuticals and cosmetics, in case-hardening of steel, and in the oil and gas industry as a wetting agent for drilling mud (4, 5).

**Environmental fate**

Barium in water comes primarily from natural sources. The acetate, nitrate, and halides are soluble in water, but the carbonate, chromate, fluoride, oxalate, phosphate, and sulfate are quite insoluble. The solubility of barium compounds increases as the pH level decreases (1).

Organic barium compounds are ionic and are hydrolysed in water (6). The concentration of barium ions in natural aquatic systems is limited by the presence of naturally occurring anions and possibly also by the adsorption of these ions onto metal oxides and hydroxides (7).
13.6.2 Analytical methods

Barium concentrations in water may be determined by atomic absorption spectroscopy, using either direct aspiration into an air-acetylene flame (detection limit 2 μg/litre) or atomization in a furnace (detection limit 100 μg/litre) (1). Barium in water may also be determined by inductively coupled plasma atomic emission spectrometry, the detection limits being equivalent or superior to those of flame atomic absorption spectroscopy (8).

13.6.3 Environmental levels and human exposure

Air

Barium is generally present in air in particulate form as a result of industrial emissions, particularly in combustion of coal and diesel oil and waste incineration. Concentrations ranging from 0.0015 to 0.95 μg/m³ have been reported. The estimated respiratory intake for an adult male is in the range 0.03–22 μg/day (US Environmental Protection Agency, unpublished data, 1984).

Water

The concentration of barium in groundwater in the Netherlands was measured at 60 locations; the mean and maximum concentrations were 0.23 and 2.5 mg/litre, respectively (9).

Barium concentrations in distributed drinking-water in Canada were found to range from not detectable (detection limit 5 μg/litre) to 600 μg/litre, with a median value of 18 μg/litre; in 86% of the 122 locations surveyed, the concentrations were below 100 μg/litre (10). In 83% of 262 locations surveyed in the Netherlands in 1983, barium concentrations in drinking-water were below 50 μg/litre; the maximum concentration found was below 200 μg/litre (11). In a study of the water supplies of cities in the USA, a median value of 43 μg/litre was reported; in 94% of all determinations the concentrations found were <100 μg/litre (12). Levels of barium in municipal water supplies in Sweden varied from 1 to 20 μg/litre (12).

If an average daily water consumption of 2 litres and a concentration of about 30 μg/litre are assumed, the daily intake of barium from drinking-water is approximately 60 μg.

Food

Most foods contain less than 0.002 mg of barium per gram (13). Some cereal products and nuts may contain high levels: e.g. bran flakes, 0.0039 mg/g; pecans, 0.0067 mg/g; and Brazil nuts, up to 4 mg/g (14).
The long-term mean dietary barium intake for adults has been found to be 0.75 mg/day (range 0.44–1.8 mg/day), including food and fluids (15); 0.6 mg/day from total diet (12); and 1.24 mg/day (range 0.65–1.8 mg/day) for food only (16).

**Estimated total exposure and relative contribution of drinking-water**

On the basis of the above considerations, the mean daily intake of barium from food, water, and air is estimated to be slightly more than 1 mg/day, food being the primary source for the non-occupationally exposed population. However, where barium levels in water are high, drinking-water may contribute significantly to barium intake.

13.6.4 Kinetics and metabolism in laboratory animals and humans

Soluble barium salts are most readily absorbed, although insoluble compounds may also be absorbed to a significant extent (17, 18). The degree of absorption of barium from the gastrointestinal tract also depends on the animal species, the contents of the gastrointestinal tract, diet, and age (17–19).

Barium is rapidly transported in blood plasma, principally to bone (20). Elevated barium/calcium ratios were found in the teeth of children exposed to drinking-water containing 10 mg of barium per litre (21). It has been reported that barium crosses the placental barrier in humans (16).

The faecal route of excretion of barium is the most important in humans and animals (22); in humans, 20% of an ingested dose is excreted in the faeces and 7% in the urine within 24 hours (12, 20).

13.6.5 Effects on laboratory animals and in vitro test systems

**Acute exposure**

The oral LD₅₀ of barium chloride in rats is reported to be 118 mg/kg of body weight (23).

**Short-term exposure**

No effects on blood pressure were seen in Sprague-Dawley rats exposed to 100 mg of barium per litre as barium chloride in drinking-water (equivalent to 1.5 mg/kg of body weight per day) for up to 20 weeks (24). In the same series of studies, no changes were seen in blood pressure in hypertension-susceptible Dahl and uninephrectomized rats exposed for 16 weeks to up to 1000 mg of barium per litre in distilled water or 0.9% saline. At 1000 mg/litre, however, ultracellular changes in the glomeruli of the kidney were discernible by electron micros-
copy. In addition, no significant electrocardiographic changes during (-)-nor­nepinephrine challenge were observed in Sprague-Dawley rats ingesting drinking-water containing 250 mg of barium per litre for 5 months (24).

**Long-term exposure**

In a study on the lifetime exposure of Long-Evans rats to 5 mg of barium per litre as barium acetate in drinking-water, the only significant effect reported was an increase in proteinuria in males (25). In a similar study in which 5 mg of barium per litre as barium acetate was administered in drinking-water to Charles River CD mice over their entire life span, there was a slight reduction in the survival of males, but no effects on body weight gain, oedema, or blanching of incisor teeth (26). No histopathological effects were found in 34 tissues of male and female Sprague-Dawley rats exposed to 1, 10, 100, or 250 mg of barium per litre as barium chloride in drinking-water for up to 68 weeks (24).

Groups of female Long-Evans rats were exposed to 1, 10, or 100 mg of barium per litre as barium chloride in drinking-water for 1, 4, or 16 months (27), equivalent to average doses of 0.051, 0.51, and 5.1 mg of barium per kg of body weight per day (2). Mean systolic pressure remained unchanged in animals exposed to the lowest dose for 16 months. At the intermediate dose, there were mean increases in blood pressure of 0.533–0.933 kPa (4–7 mmHg) by 8 months, which persisted thereafter. In rats receiving the highest dose, significant and persistent increases in mean systolic pressure of 1.60 kPa (12 mmHg) were seen after only 1 month, gradually increasing to a mean of 2.13 kPa (16 mmHg) after 16 months of exposure. Rates of cardiac contraction, electrical excitability, and high-energy phosphate and phosphorylation potential were decreased. As increases in systolic pressure of 0.533–0.933 kPa (4–7 mmHg) are deemed small enough not to constitute an adverse effect, the NOAEL can be considered to be 0.51 mg of barium per kg of body weight per day, and the LOAEL is 5.1 mg of barium per kg of body weight per day.

It has been estimated that a 0.1–1% increase in the clinical incidence of coronary heart disease in the USA over a 6-year period could result from a 2 kPa (15 mmHg) increase in mean systolic blood pressure. Although a 0.67 kPa (5 mmHg) increase in systolic blood pressure would have virtually no short-term clinical implications for those aged 35 years and younger, such an increase may become a difference of nearly 1.33 kPa (10 mmHg) by age 65, which would increase the average risk of a heart attack by 14% in the USA (28).

**Reproductive toxicity, embryotoxicity, and teratogenicity**

No studies on the reproductive, embryotoxic, or teratogenic effects of the ingestion of barium in food or drinking-water were found. In contrast, the inhalation of barium carbonate dust adversely affected spermatogenesis in male rats exposed
to 22.6 mg/m³, and shortened the estrous cycle and disturbed the morphological structure of the ovaries in female rats exposed to 13.4 and 3.1 mg/m³ for 4 months (29).

**Mutagenicity and related end-points**

Barium chloride did not increase the frequency of mutation in repair-deficient strains of *Bacillus subtilis* (30) or induce errors in viral DNA transcription *in vitro* (31).

**Carcinogenicity**

In extremely limited lifetime bioassays of rats and mice exposed to 5 mg/litre in drinking-water, no evidence was found on gross examination at autopsy to show that barium is carcinogenic (25, 26).

13.6.6 Effects on humans

Barium is not considered to be an essential element for human nutrition (16).

At high concentrations, barium causes vasoconstriction by its direct stimulation of arterial muscle, peristalsis as a result of the violent stimulation of smooth muscle, and convulsions and paralysis following stimulation of the central nervous system (32). Depending on the dose and solubility of the barium salt, death may occur in a few hours or a few days. The acute toxic oral dose of barium chloride for humans is 0.2–0.5 g; the estimated acute lethal oral dose is between 3 and 4 g (33). Repeated exposures to barium chloride in table salt are believed to have caused recurrent outbreaks of “pa-ping” disease (a transient paralysis resembling familial periodic paralysis) in China (34), but recovery was usually rapid (12).

The prevalence of dental caries was reported to be significantly lower in 39 children from a community ingesting drinking-water containing 8–10 mg of barium per litre as compared with that in 36 children from another community ingesting drinking-water containing <0.03 mg/litre (35). However, the study population was small, and dental examinations were not conducted in a blind manner.

Associations between the barium content of drinking-water and mortality from cardiovascular disease have been observed in several ecological epidemiological studies. Significant negative correlations between barium concentrations in drinking-water and mortality from atherosclerotic heart disease (36) and total cardiovascular disease (37) have been reported. Conversely, significantly higher sex- and age-adjusted death rates for “all cardiovascular diseases” and “heart disease” have been reported in an unspecified number of Illinois communities with high concentrations of barium in drinking-water (2–10 mg/litre) as compared with those with low concentrations (<0.2 mg/litre) in 1971–75 (38). There were,
however, several confounding factors; although the communities were matched for demographic characteristics and socioeconomic status, population mobility differed between the communities with high and low barium levels. Moreover, it was not possible to control for the use of water softeners in the home (39).

The results of the ecological study in Illinois were not confirmed in a cross-sectional study of the prevalence of cardiovascular disease in 1175 adult residents of West Dundee, Illinois (mean barium concentration in drinking-water 7.3 mg/litre, range 2–10 mg/litre), as compared with 1203 adult residents of McHenry (mean concentrations of barium in drinking-water 0.1 mg/litre) (40). The socioeconomic status and demographic characteristics of the populations in the two towns were similar. Blood pressures of all participants were measured, and data on the occurrence of cardiovascular, cerebrovascular, and renal disease and possible confounding factors were obtained by means of questionnaires administered by trained survey workers. There were no significant differences between the two populations in the prevalence of hypertension, stroke, and heart and kidney disease, even when the use of water softeners, medication, duration of exposure, smoking, and obesity were taken into account. The authors concluded that blood pressure in adults does not appear to be adversely affected even following prolonged ingestion of drinking-water containing more than 7 mg of barium per litre.

In a recent clinical study, 11 “healthy” men were exposed to barium (as barium chloride) in drinking-water (0 mg/litre for 2 weeks, 5 mg/litre for the next 4 weeks, and 10 mg/litre for the last 4 weeks) (41). Attempts were made to control several of the risk factors for cardiovascular disease, including diet, exercise, smoking, and alcohol consumption, throughout the study period (although subjects were not continuously monitored in this regard). No consistent indication of any adverse effects was found. There was, however, a trend towards an increase in serum calcium between 0 and 5 mg/litre, which persisted at 10 mg/litre; for total calcium, normalized for differences in albumin level, this increase was statistically significant. The authors suggested that the increase would not be expected to be clinically important. The lack of adverse effects observed in this study may be attributable to the small number of subjects included or the short period of exposure.

13.6.7 Guideline value

As there is no evidence that barium is carcinogenic (12), the guideline value for barium in drinking-water is derived using the TDI approach.

In the most sensitive epidemiological study conducted to date, there were no significant differences in blood pressure or in the prevalence of cardiovascular disease between a population drinking water containing a mean barium concentration of 7.3 mg/litre and one whose water contained a concentration of 0.1 mg/litre (40). Using the NOAEL of 7.3 mg/litre obtained from this study and an
uncertainty factor of 10 to account for intraspecies variation, a guideline value of 0.7 mg/litre (rounded figure) can be derived for barium in drinking-water.

This value is close to that derived from the results of toxicological studies in animal species. A TDI of 51 μg/kg of body weight can be calculated, based on a NOAEL of 0.51 mg/kg of body weight per day in a chronic study in rats (27) and incorporating uncertainty factors of 10 for intraspecies variation and 1 for interspecies variation, as the results of a well-conducted epidemiological study (40) indicate that humans are not more sensitive than rats to barium in drinking-water. The value derived from this TDI, based on a 20% allocation to drinking-water, is 0.3 mg/litre (rounded figure).

References


13.7 Beryllium

13.7.1 General description

**Identity**

Beryllium is an alkaline earth metal and a constituent of many common minerals, such as beryl and beryllonite (1).

**Physicochemical properties (2)**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical state</td>
<td>Grey solid</td>
</tr>
<tr>
<td>Melting point</td>
<td>1278 °C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>2970 °C</td>
</tr>
<tr>
<td>Density</td>
<td>1.848 g/cm³ at 20 °C</td>
</tr>
</tbody>
</table>

**Organoleptic properties**

A taste detection threshold of 0.24 g/litre has been reported for beryllium chloride (3).

**Major uses**

Beryllium and its alloys have a number of important uses, mostly based on their heat resistance; these include use in space vehicles, X-ray equipment, and electrical components (1).

13.7.2 Analytical methods

Beryllium is determined directly in acidified aqueous samples by electrothermal atomization atomic absorption spectrophotometry. Other metals, such as iron, magnesium, aluminium, and manganese, can interfere with the method. The detection range for beryllium is 0.22–4 µg/litre (4). Other methods include
graphite furnace and flame atomic absorption spectroscopy (5) and gas chromatography with electron capture detection (6).

13.7.3 Environmental levels and human exposure

**Air**

Beryllium is released to air principally as a result of the combustion of fossil fuels (7). However, it is infrequently detected in the atmosphere, and concentrations are usually less than 5 ng/m³ (8).

**Water**

Beryllium enters natural waters through the weathering of rocks, atmospheric fallout, and industrial and municipal discharges (7). Concentrations in natural waters are generally less than 1 μg/litre (1, 7). Beryllium is rarely detected in drinking-water and then only at very low concentrations. In a large-scale survey in the United States, mean and maximum concentrations of 0.2 and 1.2 μg/litre, respectively, were reported (9).

**Food**

The beryllium content of various foodstuffs has been reported to be in the range 0.06–0.17 mg/kg (10). A typical dietary intake has been reported as 100 μg/day, although in a study in the United Kingdom it was estimated that the dietary intake could be less than 15 μg/day (1).

**Estimated total exposure and relative contribution of drinking-water**

The major route of exposure of the general population to beryllium is through food, the contribution from air being negligible in comparison. If a daily intake in the diet of 100 μg of beryllium in adults and a beryllium concentration in drinking-water of 1 μg/litre are assumed, the total contribution from water would be 2% (1). At lower dietary levels, the relative contribution from drinking-water would be increased.

13.7.4 Kinetics and metabolism in laboratory animals and humans

Beryllium and its compounds are not readily absorbed via the oral route, as they tend to form insoluble compounds at physiological pH (11). Following exposure to the chloride and sulfate, less than 1% and 20% respectively of ingested beryllium was absorbed in experimental animals (1, 12). Negligible amounts of beryllium are absorbed through the intact skin (1). Absorbed oral doses are distributed to the gastrointestinal tract, liver, blood, and kidney, and are ultimately stored in bone (12, 13). Inhaled or ingested beryllium is excreted mainly in the faeces (14).
13.7.5 Effects on laboratory animals and in vitro test systems

**Acute exposure**

In general, beryllium compounds are less acutely toxic in animals via the oral route than via other routes of administration (1). Oral LD$_{50}$s of 18–200 mg/kg of body weight have been reported in rodents for a number of beryllium compounds (1).

**Long-term exposure**

In a lifetime study, Long-Evans male and female rats were exposed to 5 mg of beryllium sulfate per litre in drinking-water. Other than a slight depression in the growth of males from 2 to 6 months of age, there were no changes in gross or microscopic pathology, clinical chemistry, or urine analysis (15). Similarly, in a lifetime study in Charles River mice given beryllium sulfate at 5 mg/litre in their drinking-water, slight effects on female body weight were seen. No other treatment-related effects were observed (16).

**Reproductive toxicity, embryotoxicity, and teratogenicity**

No studies are available on the reproductive toxicity of beryllium following ingestion. However, the chloride and oxide have been found to be fetotoxic after intratracheal administration and embryotoxic after intravenous administration (17).

**Mutagenicity and related end-points**

Beryllium does not appear to be mutagenic in various *Salmonella typhimurium* strains (18). It has been shown to interact with DNA (19) and to cause gene mutations (20), chromosomal aberrations, and sister chromatid exchange in cultured mammalian somatic cells (21). Sister chromatid exchange has also been reported in mouse macrophages (22). Dose-dependent positive transformation was induced in mammalian cell lines (23), although beryllium did not induce DNA repair in rat hepatocytes (24). It did not increase the incidence of micronucleated polychromic erythrocytes in the bone marrow of mice in vivo (18).

**Carcinogenicity**

Beryllium compounds administered by injection or inhalation can induce malignant tumours in laboratory animals (1). However, animal studies are inadequate to evaluate whether beryllium compounds are carcinogenic by oral administration. In two lifetime studies, rats and mice ingesting beryllium in drinking-water at a concentration of 5 mg/litre did not show a significant increase in tumours as compared with controls (15,16). In another study in which Wistar rats were fed beryllium at 5, 50, or 500 mg/kg of diet, no treatment-related increase in tumours as compared with controls was observed (1).
13.7.6 Effects on humans

No studies are available on the health effects of ingested beryllium. However, as gastrointestinal absorption is poor, toxicity is expected to be low via this route. Inhalation of beryllium compounds during occupational exposure has been shown to cause acute pneumonitis and chronic pulmonary granulomatosis, also known as chronic beryllium disease (25). There is evidence that the chronic disease is immunologically mediated (25). When insoluble beryllium compounds have become embedded in the skin, allergic-type dermatitis and granulomatous skin ulcerations have been reported, as also has conjunctivitis (1).

IARC has classified beryllium and beryllium compounds as being probably carcinogenic to humans (Group 2A), on the basis of occupational exposure and inhalation studies in laboratory animals (25). However, the epidemiological studies that led to this conclusion have been criticized (26).

13.7.7 Conclusions

There are no suitable oral data on which a toxicologically supportable guideline value could be based. However, the very low concentrations of beryllium normally found in drinking-water seem unlikely to pose a hazard to consumers.

References


13. INORGANIC CONSTITUENTS AND PHYSICAL PARAMETERS


13. Watanabe K et al. [Biotoxicity and beryllium distribution in organs by oral administration of beryllium compounds for long periods. II. Experimental study on oral administration of beryllium compounds.] Rodo kagaku, 1985, 61(5):235-246 (in Japanese). (Chemical abstracts, CA103(13)99963w).


13.8 Boron

13.8.1 General description

*Identity*

Boron is widely distributed in the environment, borax, kernite, and tourmaline being three of the more commonly mined boron minerals. The chemical forms of boron in nature include boric acid and more condensed species such as tetraborate (1).

*Physicochemical properties (2)*

<table>
<thead>
<tr>
<th>Property</th>
<th>Boron</th>
<th>Boric acid (H₃BO₃)</th>
<th>Borax (Na₂B₄O₇·10H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical state</td>
<td>solid</td>
<td>solid</td>
<td>solid</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>2300</td>
<td>169</td>
<td>75</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>2.35</td>
<td>1.43</td>
<td>1.73</td>
</tr>
<tr>
<td>Water solubility</td>
<td>insoluble</td>
<td>63.5</td>
<td>20.1 (cold)</td>
</tr>
</tbody>
</table>
Major uses

Elemental boron and its carbides are used in composite structural materials, high-temperature abrasives, special-purpose alloys, and steel-making. Boron halides are used as catalysts in the manufacture of magnesium alloy products, metal refining, and rocket fuels. Boron hydrides are used as reductants, to control heavy metal discharges in wastewater, as catalysts, and in jet and rocket fuels (1, 2).

Boric acid and borates are used in glass manufacture and as wood and leather preservatives, flame retardants, cosmetic products, and neutron absorbers for nuclear installations. Boric acid, borates, and perborates have been used as mild antiseptics or bacteriostats in eyewashes, mouthwashes, burn dressings, and nappy rash powders, although boric acid is not now regarded as effective for this purpose. Borax is used extensively as a cleaning compound, and borates are applied as agricultural fertilizers. Boron compounds are also used as algicides, herbicides, and insecticides (1, 2).

Environmental fate

The environmental chemistry of boron is not well understood. In water, the predominant species is probably boric acid, which does not dissociate readily at physiological pH (3).

13.8.2 Analytical methods

Boron can be determined by atomic absorption using either direct aspiration into a flame or a furnace technique, the latter having greater sensitivity. Inductively coupled plasma atomic emission spectroscopy can also be used. Detection limits range from 1.25 to 5 μg/litre (2, 4). At concentrations between 0.01 and 1 mg/litre, boron can be determined by spectrometric techniques (5).

13.8.3 Environmental levels and human exposure

Air

The presence of boron in the atmosphere has been attributed to sea spray, volcanic activity, accumulation in dust, and industrial pollution. Boron concentrations of 0.17 μg/m³ have been reported in ocean air (3).

Water

The concentration of boron in seawater ranges from 4 to 5 mg/litre as boric acid. In Canadian coastal waters, boron levels are in the range 3.7–4.3 mg/litre. Estuarine waters are rich in boron, as it can be transported from the sea (3). Boron concentrations in surface waters in the USA range from 0 to 6.5 mg/litre, al-
though most are below 1 mg/litre (3). In northern Italy, boron concentrations in lake waters are below 0.09 mg/litre and, in about 65% of river water samples, close to natural background levels (0.1 mg/litre) (6).

In selected drinking-water supplies in the USA, boron levels were found to be between 0 and 0.74 mg/litre (median 0.12 mg/litre). High levels were attributed to seawater intrusion and fertilizers (3). Average boron concentrations in 3842 samples of treated and distributed water in Canada surveyed in 1987-89 ranged from 0.042 to 235 µg/litre, the maximum being 570 µg/litre (7). In the Netherlands, levels of boron in all drinking-water plants in 1984 varied from <0.005 to 0.61 mg/litre (median 0.02 mg/litre) (4). In selected drinking-water supplies in the former USSR, boron levels were 0-6.0 mg/litre (8). In Sierra Leone, boron levels in nine different drinking-water sources were in the range 4.6-18.1 mg/litre. The highest levels were found in pipe-borne untreated and stream water (9). Levels of boron in 37 brands of mineral water ranged from <0.005 to 4.2 mg/litre; in seven samples, the level was above 1 mg/litre (10).

**Food**

As a constituent of foodstuffs, boron occurs mainly in plant tissues, legumes containing the highest concentrations (25-50 µg/g dry weight), followed by fruits and vegetables (5-20 µg/g) and cereals and grains (1-5 µg/g). In animal muscle and soft tissues, concentrations are well below 1 µg/g. Cow’s milk normally contains 0.5-1.0 mg/litre, the level depending on the boron intake of the cow. Beverages contain variable amounts of boron: coffee, 0.16 mg/litre; apple juice, 1.2 mg/litre; orange juice, 0.53 mg/litre; and lemon juice, 0.59 mg/litre (3, 11).

**Estimated total exposure and relative contribution of drinking-water**

The total daily boron intake in normal human diets was reported to vary from 2.1 to 4.3 mg/day in 1965 and from 1.3 to 4.4 mg/day in 1972 (2). In Canada, this intake was estimated to be 1-3 mg for an adult, depending on the number of boron-containing vegetables in the diet. The contribution from drinking-water was estimated at 0.24 mg/day, based on the median value measured in the USA (3). The contribution to boron intake from air is negligible. The total daily intake can therefore be estimated to be between 1 and 5 mg.

13.8.4 Kinetics and metabolism in laboratory animals and humans

Boron in food or administered as soluble borate (borax) or boric acid is rapidly and almost completely absorbed. Over 93% of a single oral dose of 750 mg of boric acid administered to human volunteers was recovered in the urine within 96 h (2). Between 50% and 66% of boric acid administered orally to rabbits
Absorption through intact skin is poor but is much greater through damaged skin (12). Transplacental distribution has been reported (2).

13.8.5 Effects on laboratory animals and in vitro test systems

**Acute exposure**

Boric acid and borax have a low acute oral toxicity; LD$_{50}$ values for mice, rats, and dogs range from 2000 to over 6000 mg/kg of body weight (7,13). Signs of acute toxicity for both borax and boric acid include depression, ataxia, convulsions, and death; kidney degeneration and testicular atrophy are also observed (1).

**Short-term exposure**

After repeated oral administration of borax or boric acid to rats and dogs, growth inhibition, organ weight changes, and testicular atrophy were the most striking effects. In 90-day studies with rats and dogs at doses ranging from 17.5 to 5250 mg of boron per kg of food (as borax or boric acid), no clear NOAEL could be established (13).

In a 13-week study, mice were fed diets containing boric acid at 1200–20000 mg/kg of food. At high doses (≥5000 mg/kg of food), increased mortality and degeneration or atrophy of the seminiferous tubules were observed. In all dose groups, extramedullary haematopoiesis of the spleen of minimal to mild severity was seen (14).

**Long-term exposure**

No effects were observed on body weight and longevity in a limited lifetime study in which Swiss mice received 5 mg of boron per litre (as sodium metaborate) in their drinking-water (2).

In a study in which B6C3F1 mice received 0, 2500, or 5000 mg of boric acid per kg in the diet for 103 weeks, mortality was significantly increased in both treatment groups. At the highest dose, testicular atrophy and interstitial cell hyperplasia were observed in male mice (14).

Male and female Sprague-Dawley rats were fed diets containing 0, 117, 350, or 1170 mg of boron per kg of food (as borax or boric acid) for 2 years. At the highest dose, increased brain and thyroid weights, decreased body and testes weights, and histopathological alterations of the testes were observed. The NOAEL in this study was 350 mg of boron per kg of food (equivalent to 17.5 mg of boron per kg of body weight per day) (13).

When dogs were fed 0, 58, 117, or 350 mg of boron per kg in the diet (as borax or boric acid) for 2 years, no effects were observed on body weight, food consumption, organ weights, clinical parameters, and histopathology. Dogs fed a
diet containing 1170 mg of boron per kg of food (as borax) for 38 weeks exhibited severe testicular atrophy and spermatogenic arrest by week 26. The NOAEL in this study was 350 mg of boron per kg of food (equivalent to 8.8 mg of boron per kg of body weight per day) (13).

Reproductive toxicity, embryotoxicity, and teratogenicity

In a 90-day drinking-water study with male rats, the highest dose of 6.0 mg of boron per litre (as borax) (0.426 mg of boron per kg of body weight per day) had no effects on fertility and reproduction or on the weight of the testes, prostate, or seminal vesicles. Fructose, zinc, and acid phosphatase levels in the prostate were unaltered (15).

In a 90-day feeding study in rats with borax (dose levels 0, 25, 50, and 100 mg of boron per kg of body weight per day), a LOAEL of 25 mg of boron per kg of body weight per day was established based on dose-dependent tubular germinal aplasia (16).

In a multigeneration study, 0, 117, 350, or 1170 mg of boron per kg of food (as borax or boric acid) was administered to male and female rats (13). At the highest dose, rats were found to be sterile, males showed atrophied testes in which spermatozoa were absent, and females showed decreased ovulation (NOAEL 350 mg of boron per kg of food, equivalent to 17.5 mg of boron per kg of body weight per day).

Mutagenicity and related end-points

The mutagenic activity of boric acid was examined in the Salmonella typhimurium and mouse lymphoma assays with negative results. No induction of sister chromatid exchange or chromosomal aberrations was observed in Chinese hamster ovary cells (14). Sodium borate did not cause gene mutations in the S. typhimurium preincubation assay (2). Borax was not mutagenic in cell transformation assays with Chinese hamster cells, mouse embryo cells, and human fibroblasts (17).

Carcinogenicity

Tumour incidence was not enhanced in studies in which B6C3F1 mice received 0, 2500, or 5000 mg of boric acid per kg of food for 103 weeks (14) or Sprague-Dawley rats were fed diets containing 0, 117, 350, or 1170 mg of boron per kg of food (as borax or boric acid) for 2 years (13).
13.8.6 Effects on humans

Acute boron poisoning has been reported after the application of dressings, powders, or ointments containing borax and boric acid to large areas of burned or abraded skin; the lowest reported dermal lethal dose of boric acid is 8600 mg/kg of body weight (1509 mg of boron per kg of body weight). Ingestion has also been the cause of acute boron poisoning; the lowest reported oral dose of boric acid causing such poisoning is 640 mg/kg of body weight (112 mg of boron per kg of body weight) (1,16). Symptoms of boron poisoning include gastrointestinal disturbances, erythematous skin eruptions, and signs of central nervous system stimulation followed by depression (1,3).

Chronic exposure to boric acid and tetraborates such as borax leads to gastrointestinal irritation, with loss of appetite, nausea, and vomiting, and the appearance of an erythematous rash (12,16). In a human nutrition study in postmenopausal women in which basal diets supplying 0.25 mg of boron per day were supplemented with 3 mg of boron per day for 119 days, reduced urinary calcium and magnesium excretion and elevated steroid levels were reported (2).

13.8.7 Guideline value

As mutagenicity studies gave negative results and carcinogenicity has not been observed, a TDI of 88 µg/kg of body weight was derived by applying an uncertainty factor of 100 (for intra- and interspecies variation) to a NOAEL for testicular atrophy of 8.8 mg of boron per kg of body weight per day in a 2-year diet study in dogs (13). This gives a guideline value for boron of 0.3 mg/litre (rounded figure) if 10% of the TDI is allocated to drinking-water. It should be noted, however, that the intake of boron from food is poorly characterized and that drinking-water treatment does not appear to be very effective in removing it.

References


13.9 Cadmium

13.9.1 General description

Identity

Cadmium is a metal with an oxidation state of +2. It is chemically similar to zinc and occurs naturally with zinc and lead in sulfide ores.

Physicochemical properties (1–3)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical state</td>
<td>Soft white solid</td>
</tr>
<tr>
<td>Density</td>
<td>8.64 g/cm³</td>
</tr>
<tr>
<td>Melting point</td>
<td>320.9 °C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>765 °C at 100 kPa</td>
</tr>
<tr>
<td>Solubility</td>
<td>Soluble in dilute nitric and concentrated sulfuric acids</td>
</tr>
</tbody>
</table>

Major uses

Cadmium metal is used mainly as an anticorrosive, electroplated on to steel. Cadmium sulfide and selenide are commonly used as pigments in plastics. Cadmium compounds are used in electric batteries, electronic components, and nuclear reactors (2, 4).

Environmental fate

Fertilizers produced from phosphate ores constitute a major source of diffuse cadmium pollution. The solubility of cadmium in water is influenced to a large degree by its acidity; suspended or sediment-bound cadmium may dissolve when there is an increase in acidity (2). In natural water, cadmium is found mainly in bottom sediments and suspended particles (4).

13.9.2 Analytical methods

Cadmium can be determined by atomic absorption spectroscopy using either direct aspiration into a flame or a furnace spectrometric technique. The detection limit is 5 µg/litre with the flame method and 0.1 µg/litre with the furnace procedure (1, 5).

13.9.3 Environmental levels and human exposure

Air

Cadmium is present in ambient air in the form of particles in which cadmium oxide is probably an important constituent (4). Annual average concentrations in four cities in Germany in 1981–82 were 1–3 ng/m³. In the Netherlands,
annual average concentrations in 1980–83 were 0.7–2 ng/m³. Levels are generally higher in the vicinity of metallurgical plants. In industrial areas in Belgium, annual average levels in 1985–86 were 10–60 ng/m³ (2). For the general population not living in such areas, cadmium intakes from air are unlikely to exceed 0.8 μg/day (6).

Cigarette smoking increases cadmium concentrations inside houses. The average daily exposure from cigarette smoking (20 cigarettes a day) is 2–4 μg of cadmium (2).

**Water**

Cadmium concentrations in unpolluted natural waters are usually below 1 μg/litre (4). Median concentrations of dissolved cadmium measured at 110 stations around the world were <1 μg/litre, the maximum value recorded being 100 μg/litre in the Rio Rimao in Peru (7). Average levels in the Rhine and Danube in 1988 were 0.1 μg/litre (range 0.02–0.3 μg/litre) (8) and 0.025 μg/litre (9), respectively. In the sediments near Rotterdam harbour, levels in mud varied from 1 to 10 mg/kg dry weight in 1985–86, down from 5–19 mg/kg dry weight in 1981 (2).

Contamination of drinking-water may occur as a result of the presence of cadmium as an impurity in the zinc of galvanized pipes or cadmium-containing solders in fittings, water heaters, water coolers, and taps. Drinking-water from shallow wells in areas of Sweden where the soil has been acidified contained concentrations of cadmium approaching 5 μg/litre (4). In Saudi Arabia, mean concentrations of 1–26 μg/litre were found in samples of potable water, some of which were taken from private wells or cold corroded pipes (10). Levels of cadmium could be higher in areas supplied with soft water of low pH, as this would tend to be more corrosive in plumbing systems containing cadmium. In the Netherlands, in a survey of 256 drinking-water plants in 1982, cadmium (0.1–0.2 μg/litre) was detected in only 1% of the drinking-water samples (2).

**Food**

Food is the main source of cadmium intake from nonoccupationally exposed people. Crops grown in polluted soil or irrigated with polluted water may contain increased concentrations, as may meat from animals grazing on contaminated pastures (3). Animal kidneys and livers concentrate cadmium. Levels in fruit, meat, and vegetables are usually below 10 μg/kg, in liver 10–100 μg/kg, and in kidney 100–1000 μg/kg. In cereals, levels are about 25 μg/kg wet weight. In 1980–88, average cadmium levels in fish were 20 μg/kg wet weight. High levels were found in shellfish (200–1000 μg/kg) (11).

Based on cadmium levels measured in 1977–84, the estimated daily intake in food by the Netherlands population is 20 μg/person (3). The dietary daily intake of cadmium has also been estimated to be in the range 10–35 μg (11). In con-
13.9.4 Kinetics and metabolism in laboratory animals and humans

Absorption via the gastrointestinal tract is influenced by the solubility of the cadmium compound concerned. In healthy persons 3–7% of the cadmium ingested is absorbed; in iron-deficient people, this figure can reach 15–20% (13). Absorbed cadmium enters the bloodstream and is transported to other parts of the body. After binding to metallothionein, it is filtered in the kidney through the glomerulus into the primary urine, then reabsorbed in the proximal tubular cells, where the cadmium–metallothionein bond is broken. The unbound cadmium stimulates the production of new metallothionein, which binds cadmium in the renal tubular cells, thereby preventing the toxic effects of free cadmium. If the metallothionein-producing capacity is exceeded, damage to the proximal tubular cells occurs, the first sign of this effect being low-molecular-weight proteinuria (4).

Tissue cadmium concentrations increase with age. Both kidney and liver act as cadmium stores; 50–85% of the body burden is stored in kidney and liver, 30–60% being stored in the kidney alone. The biological half-life in humans is in the range 10–35 years. Because of the considerable age-related accumulation of cadmium in the body, only a small part of the cadmium absorbed will be excreted in the urine. About 0.007% of the body burden is excreted daily by adults, but individual variation is large (6, 12, 13).

13.9.5 Effects on laboratory animals and in vitro test systems

Acute exposure

Cadmium compounds have a moderate acute oral toxicity; oral LD$_{50}$ values for mice and rats range from 60 to over 5000 mg/kg of body weight. Major effects are desquamation of epithelium, necrosis of the gastric and intestinal mucosa, and dystrophic changes of liver, heart, and kidneys (13).
**Short-term exposure**

After repeated oral administration, the critical effect in animals is a characteristic lesion of the proximal tubules of the kidneys resulting in impaired tubular resorption and consequent urinary excretion of low-molecular-weight proteins. In rhesus monkeys, a NOAEL of 3 mg of cadmium per kg of diet (given as cadmium chloride) was found for these effects, which were also produced by repeated oral administration to rats of doses of 10 mg of cadmium per litre in drinking-water or 10 mg/kg of diet (given as cadmium chloride) and above. Effects on bone (osteoporosis) were also frequently seen at doses of 10–30 mg of cadmium per kg of diet or 10 mg/litre and above in drinking-water. Effects on the liver, haematopoietic system, and immune system have also been reported (13).

**Reproductive toxicity, embryotoxicity, and teratogenicity**

Studies on oral exposure have not provided evidence of teratogenic effects at dose levels below those that were toxic to maternal animals. Ferotoxic and embryotoxic effects were also observed only at toxic dose levels. In a multigeneration study in rats, dose levels up to 100 mg/kg of diet did not cause effects on reproduction. In four-generation studies, 1 mg of cadmium per litre in drinking-water and 0.125 mg of cadmium per kg in the diet caused effects on fertility in mice and rats, respectively. Mild testicular changes in rats were seen after oral administration of 50 mg of cadmium per kg of body weight for 15 months. No effects were seen at 5 mg/kg of body weight or when rats were exposed to 70 mg/litre in their drinking-water for 70 days (13).

**Mutagenicity and related end-points**

Both negative and positive results have been reported with regard to DNA degradation, decreased fidelity of DNA synthesis, microbial DNA repair, gene mutations, and chromosomal abnormalities in mammalian cell cultures, higher plants, and intact animals. It should be noted that the positive results were often weak and seen at high concentrations that also caused cytotoxicity (13).

**Carcinogenicity**

An oral carcinogenicity study in rats with cadmium chloride (1–50 mg of cadmium per kg of diet) did not reveal significantly increased tumour incidence. In long-term oral toxicity studies in rats, no increase in tumour incidence was seen (13). Lung tumours were induced in rats following the inhalation of inorganic cadmium compounds (13,14).
13.9.6 Effects on humans

The estimated lethal oral dose for humans is 350–3500 mg of cadmium; a dose of 3 mg of cadmium has no effects on adults (13).

With chronic oral exposure, the kidney appears to be the most sensitive organ. Cadmium affects the resorption function of the proximal tubules, the first symptom being an increase in the urinary excretion of low-molecular-weight proteins, known as tubular proteinuria (13) (see also section 13.9.4). Intakes of 140–255 μg of cadmium per day have been associated with low-molecular-weight proteinuria in the elderly; the minimum (critical) level of cadmium in the human renal cortex, related to the first sign of tubular dysfunction, varied from 100 to 450 mg/kg wet weight (6). The estimated critical concentration in the renal cortex at which the prevalence of low-molecular-weight proteinuria would reach 10% in the general population is about 200 mg/kg; this would be reached after a daily dietary intake of about 175 μg per person for 50 years, as calculated by regression analysis of cadmium intake and mean kidney cadmium concentration in various countries (6). It was estimated that a daily intake of 100 μg of cadmium per person would lead to the critical cadmium concentration in the renal cortex being exceeded in 2% of the population (6). More severe cadmium damage may also involve the glomeruli, giving rise to increased inulin clearance. Other possible effects include aminoaciduria, glucosuria, and phosphaturia. Disturbances in the renal handling of phosphorus and calcium may cause resorption of minerals from bone, which can result in the development of kidney stones and osteomalacia.

Many cases of itai-itai disease (osteomalacia with various grades of osteoporosis accompanied by severe renal tubular disease) and low-molecular-weight proteinuria have been reported among people living in contaminated areas in Japan and exposed to cadmium via food and drinking-water. The daily intake of cadmium in the most heavily contaminated areas amounted to 600–2000 μg/day; in other less heavily contaminated areas, daily intakes of 100–390 μg/day have been found (12). A relationship between chronic occupational exposure to cadmium or chronic oral exposure to cadmium via the diet in contaminated areas and hypertension could not be demonstrated (13).

Epidemiological studies of people chronically exposed to cadmium via the diet as a result of environmental contamination have not shown an increased cancer risk. The results of studies of chromosomal aberrations in the peripheral lymphocytes of patients with itai-itai disease exposed chronically to cadmium via the diet were contradictory. No reliable studies on reproductive, teratogenic, or embryotoxic effects in humans are available. Epidemiological studies of humans exposed by inhalation to relatively high cadmium concentrations in the workplace showed some evidence of an increased lung cancer risk, but a definite conclusion could not be reached (13).
13.9.7 Guideline value

There is some evidence that cadmium is carcinogenic by the inhalation route, and IARC has classified cadmium and cadmium compounds in Group 2A (15). However, there is no evidence of carcinogenicity by the oral route, and no clear evidence that cadmium is genotoxic.

On the assumption of an absorption rate for dietary cadmium of 5% and a daily excretion rate of 0.005% of body burden, JECFA concluded that, if levels of cadmium in the renal cortex are not to exceed 50 mg/kg, the total intake of cadmium should not exceed 1 μg/kg of body weight per day. The provisional tolerable weekly intake (PTWI) was therefore set at 7 μg/kg of body weight in 1989 (6), and reconfirmed in 1993 (16). It is recognized that the margin between the PTWI and the actual weekly intake of cadmium by the general population is small, namely less than 10-fold, and that this margin may be even smaller in smokers. A guideline value for cadmium of 0.003 mg/litre is established based on an allocation of 10% of the PTWI to drinking-water.

References


13.10 Chloride

13.10.1 General description

**Identity**

Chlorides are widely distributed in nature as salts of sodium (NaCl), potassium (KCl), and calcium (CaCl₂).

**Physicochemical properties (1)**

<table>
<thead>
<tr>
<th>Salt</th>
<th>Solubility in cold water (g/litre)</th>
<th>Solubility in hot water (g/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>357</td>
<td>391</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>344</td>
<td>567</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>745</td>
<td>1590</td>
</tr>
</tbody>
</table>
**Organoleptic properties**

The taste threshold of the chloride anion in water is dependent on the associated cation. Taste thresholds for sodium chloride and calcium chloride in water are in the range 200–300 mg/litre (2). The taste of coffee is affected if it is made with water containing a chloride concentration of 400 mg/litre as sodium chloride or 530 mg/litre as calcium chloride (3).

**Major uses**

Sodium chloride is widely used in the production of industrial chemicals such as caustic soda, chlorine, sodium chlorite, and sodium hypochlorite. Sodium chloride, calcium chloride, and magnesium chloride are extensively used in snow and ice control. Potassium chloride is used in the production of fertilizers (4).

**Environmental fate**

Chlorides are leached from various rocks into soil and water by weathering. The chloride ion is highly mobile and is transported to closed basins or oceans.

13.10.2 Analytical methods

A number of suitable analytical techniques are available for chloride in water, including silver nitrate titration with chromate indicator (5), mercury(II) nitrate titration with diphenylcarbazone indicator, potentiometric titration with silver nitrate, automated iron(III) mercury(II) thiocyanate colorimetry, chloride ion-selective electrode, silver colorimetry, and ion chromatography. Limits of detection range from 50 µg/litre for colorimetry to 5 mg/litre for titration (6).

13.10.3 Environmental levels and human exposure

**Air**

Exposure to chloride in air has been reported to be negligible (4).

**Water**

Chloride in surface and groundwater originates from both natural and anthropogenic sources, such as run-off containing road de-icing salts, the use of inorganic fertilizers, landfill leachates, septic tank effluents, animal feeds, industrial effluents, irrigation drainage, and seawater intrusion in coastal areas (4).

The mean chloride concentration in several rivers in the United Kingdom was in the range 11–42 mg/litre during 1974–81 (7). Evidence of a general increase in chloride concentrations in groundwater and drinking-water has been found (8), but exceptions have also been reported (9). In the USA, aquifers prone to seawater intrusion have been found to contain chloride at concentra-
tions ranging from 5 to 460 mg/litre (10), whereas contaminated wells in the Philippines have been reported to have an average chloride concentration of 141 mg/litre (11). Chloride levels in unpolluted waters are often below 10 mg/litre and sometimes below 1 mg/litre (4).

Chloride in water may be considerably increased by treatment processes in which chlorine or chloride is used. For example, treatment with 40 g of chlorine per m³ and 0.6 mol of iron chloride per litre, required for the purification of groundwater containing large amounts of iron(II), or surface water polluted with colloids, has been reported to result in chloride concentrations of 40 and 63 mg/litre, respectively, in the finished water (8).

**Food**

Chloride occurs naturally in foodstuffs at levels normally less than 0.36 mg/g. An average intake of 100 mg/day has been reported when a salt-free diet is consumed. However, the addition of salt during processing, cooking, or eating can markedly increase the chloride level in food, resulting in an average dietary intake of 6 g/day, which may rise to 12 g/day in some cases (4).

**Estimated total exposure and relative contribution of drinking-water**

If a daily water consumption of 2 litres and an average chloride level in drinking-water of 10 mg/litre are assumed, the average daily intake of chloride from drinking-water would be approximately 20 mg per person (4), but a figure of approximately 100 mg/day has also been suggested (8). Based on these estimates and the average dietary (not salt-free) intake of 6 g/day, drinking-water intake accounts for about 0.33-1.6% of the total intake.

13.10.4 Kinetics and metabolism in laboratory animals and humans

In humans, 88% of chloride is extracellular and contributes to the osmotic activity of body fluids. The electrolyte balance in the body is maintained by adjusting total dietary intake and by excretion via the kidneys and gastrointestinal tract. Chloride is almost completely absorbed in normal individuals, mostly from the proximal half of the small intestine. Normal fluid loss amounts to about 1.5-2 litres/day, together with about 4 g of chloride per day. Most (90–95%) is excreted in the urine, with minor amounts in faeces (4–8%) and sweat (2%) (4).
13.10.5 Effects on laboratory animals and \textit{in vitro} test systems

**Acute exposure**

The oral LD$_{50}$ values for calcium chloride, sodium chloride, and potassium chloride in the rat have been reported as 1000, 3000, and 2430 mg/kg of body weight, respectively (8).

**Short-term exposure**

The toxicity of chlorides depends on the cation present; that of chloride itself is unknown. Although excessive intake of drinking-water containing sodium chloride at concentrations above 2.5 g/litre has been reported to produce hypertension (12), this effect is believed to be related to the sodium ion concentration.

13.10.6 Effects on humans

A normal adult human body contains approximately 81.7 g of chloride. On the basis of a total obligatory loss of chloride of approximately 530 mg/day, a dietary intake for adults of 9 mg of chloride per kg of body weight has been recommended (equivalent to slightly more than 1 g of table salt per person per day). For children up to 18 years of age, a daily dietary intake of 45 mg of chloride per kg of body weight should be sufficient (4). A dose of 1 g of sodium chloride per kg of body weight was reported to have been lethal in a 9-week-old child (8).

Chloride toxicity has not been observed in humans except in the special case of impaired sodium chloride metabolism, e.g. in congestive heart failure (13). Healthy individuals can tolerate the intake of large quantities of chloride provided that there is a concomitant intake of fresh water. Little is known about the effect of prolonged intake of large amounts of chloride in the diet. As in experimental animals, hypertension associated with sodium chloride intake appears to be related to the sodium rather than the chloride ion (4).

13.10.7 Other considerations

Chloride increases the electrical conductivity of water and thus its corrosivity. In metal pipes, chloride reacts with metal ions to form soluble salts (8), thus increasing levels of metals in drinking-water. In lead pipes, a protective oxide layer is built up, but chloride enhances galvanic corrosion (14). It can also increase the rate of pitting corrosion of metal pipes (8).
13.10.8 Conclusions

Chloride concentrations in excess of about 250 mg/litre can give rise to detectable taste in water, but the threshold depends on the associated cations. Consumers can, however, become accustomed to concentrations in excess of 250 mg/litre. No health-based guideline value is proposed for chloride in drinking-water.

References


13.11 Chromium

13.11.1 General description

*Identity*

Chromium is widely distributed in the earth's crust. It can exist in oxidation states of +2 to +6. Soils and rocks may contain small amounts of chromium, almost always in the trivalent state.

**Physicochemical properties (1-4)**

<table>
<thead>
<tr>
<th>Property</th>
<th>Cr</th>
<th>CrCl₃</th>
<th>K₂CrO₄</th>
<th>Cr₂O₃</th>
<th>CrO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point (°C)</td>
<td>1857</td>
<td>1152</td>
<td>968.3</td>
<td>2266</td>
<td>196</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>2672</td>
<td></td>
<td></td>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>Solubility (g/litre)</td>
<td>insoluble</td>
<td>slightly soluble</td>
<td>790</td>
<td>insoluble</td>
<td>624</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>7.14</td>
<td>2.76</td>
<td>2.73</td>
<td>5.21</td>
<td>2.70</td>
</tr>
</tbody>
</table>

**Major uses**

Chromium and its salts are used in the leather tanning industry, the manufacture of catalysts, pigments and paints, fungicides, the ceramic and glass industry, and in photography, and for chrome alloy and chromium metal production, chrome plating, and corrosion control (1, 3, 4).

**Environmental fate**

The distribution of compounds containing chromium(III) and chromium(VI) depends on the redox potential, the pH, the presence of oxidizing or reducing compounds, the kinetics of the redox reactions, the formation of chromium(III) complexes or insoluble chromium(III) salts, and the total chromium concentration. In the environment, chromium(VI) occurs mostly as CrO₄²⁻ or HCrO₄⁻, and chromium(III) as Cr(OH)₆³⁻. In soil, chromium(III) predominates. Chromium(VI) can easily be reduced to chromium(III) by organic matter, for example, and its occurrence in soil is often the result of human activities. In water,
chromium(III) is a positive ion that forms hydroxides and complexes, and is adsorbed at relatively high pH values. In surface waters, the ratio of chromium(III) to chromium(VI) varies widely, and relatively high concentrations of the latter can be found locally. In general, chromium(VI) salts are more soluble than those of chromium(III), making chromium(VI) relatively mobile.

In air, chromium is present in the form of aerosols. It can be removed from the atmosphere by wet and dry deposition. Both trivalent and hexavalent chromium are released into the air. Because of analytical difficulties, data on chromium speciation in ambient air are rarely available, but the proportion present as chromium(VI) has been estimated as 0.01–30%, based on one study (4).

13.11.2 Analytical methods

Methods for the determination of chromium in biological and environmental samples are developing rapidly, and all early results (especially for the lower chromium levels) should be interpreted with caution.

Many techniques can be used for the determination of total chromium, including atomic absorption spectroscopy, emission spectroscopy, X-ray fluorescence, and neutron activation analysis. Detection limits for atomic absorption spectroscopy are in the range 0.05–0.2 μg/litre (5).

For determining chelated chromium or the hexavalent or trivalent form only, such methods as gas chromatography (with various detection techniques), polarography, and spectrophotometry can be used (3–5). The determination of chromium species is currently a very sophisticated procedure, and few analytical data are available (4).

13.11.3 Environmental levels and human exposure

Air

In arctic air, chromium concentrations of 5–70 pg/m³ have been measured. Ambient air at most stations in the USA contained very little chromium; mean levels were generally below 300 ng/m³, and median levels less than 20 ng/m³ (6). In non-industrialized areas, concentrations above 10 ng/m³ are uncommon (7). Concentrations in urban areas are 2–4 times higher than regional background concentrations (8). The mean concentration of total chromium in air in the Netherlands varied from 2 to 5 ng/m³ (4).

As a result of smoking, indoor air concentrations can be 10–400 times greater than outdoor concentrations (approximately 1000 ng/m³).
**Water**

The average concentration of chromium in rainwater is in the range 0.2–1 μg/litre \((4, 9-11)\). Natural chromium concentrations in seawater of 0.04–0.5 μg/litre have been measured \((3)\). In the North Sea, a concentration of 0.7 μg/litre was found \((4)\).

The natural total chromium content of surface waters is approximately 0.5–2 μg/litre and the dissolved chromium content 0.02–0.3 μg/litre \((4, 10, 12)\). Chromium concentrations in antarctic lakes increase with depth from <0.6 to 30 μg/litre \((13)\). Most surface waters contain between 1 and 10 μg of chromium per litre. In general, the chromium content of surface waters reflects the extent of industrial activity. In surface waters in the USA, levels up to 84 μg/litre have been found \((1)\); in central Canada, surface water concentrations ranged from 0.2 to 44 μg/litre.\(^1\) In the Rhine, chromium levels are below 10 μg/litre \((14)\), and in 50% of the natural stream waters in India the concentration is below 2 μg/litre \((9)\).

In general, the chromium concentration in groundwater is low (<1 μg/litre). In the Netherlands, a mean concentration of 0.7 μg/litre has been measured, with a maximum of 5 μg/litre \((4)\). In India, 50% of 1473 water samples from dug wells contained less than 2 μg/litre \((9)\). In groundwater in the USA, levels up to 50 μg/litre have been reported; in shallow groundwater, median levels of 2–10 μg/litre have been found \((1, 15)\). Most supplies in the USA contain less than 5 μg/litre. In 1986, levels in 17 groundwater supplies and one surface water supply exceeded 50 μg/litre \((1)\).

Approximately 18% of the population of the USA are exposed to drinking-water levels between 2 and 60 μg/litre and <0.1% to levels between 60 and 120 μg/litre \((1)\). In the Netherlands, the chromium concentration of 76% of the supplies was below 1 μg/litre and of 98% below 2 μg/litre \((16)\). A survey of Canadian drinking-water supplies gave an overall median level of 2 μg of chromium per litre, with maxima of 14 μg/litre (raw water) and 9 μg/litre (treated water) \((17)\).

**Food**

Food contains chromium at concentrations ranging from <10 to 1300 μg/kg \((4, 18, 19)\). Highest concentrations have been found in meat, fish, fruit, and vegetables \((18)\). Utensils used in the preparation of food may contribute to chromium levels.

**Estimated total exposure and relative contribution of drinking-water**

Mean chromium intakes from food and water range from 52 to 943 μg/day \((3)\). The estimated total intake of chromium from air, water, and food by the general

---

\(^1\) Data from the National Water Quality Data Bank (NAQUADAT), Inland Waters Directorate, Environment Canada, 1985.
population in the United Kingdom is in the range 78–106 μg/day. Food contributed 93–98% of the total intake and water 1.9–7%. The contribution from air was negligible (18). In the Netherlands, the estimated mean daily chromium intake is 100 μg, with a range of 50–200 μg (4).

In general, food appears to be the major source of intake. Drinking-water intake can, however, contribute substantially when total chromium levels are above 25 μg/litre.

13.11.4 Kinetics and metabolism in laboratory animals and humans

Oral exposure studies in animals found that <0.5–6% of chromium compounds was absorbed; in human studies, the corresponding figure could be as much as 10%. Absorption depends on chromium speciation; chromium(VI) appears to be absorbed from the gastrointestinal tract to a greater extent than chromium(III). Tissue chromium levels of rats exposed to chromium(VI) (as potassium chromate) in drinking-water were 4–15 times higher than those of rats exposed to chromium(III) (as the trichloride). The absorption of chromium(VI) is lowered by partial intragastric reduction to chromium(III) (20). Mean fractional absorption values of 5% and 25% have been estimated for the gastrointestinal absorption of chromium(III) and chromium(VI) species and of organic chromium in food ("biologically incorporated"), respectively (21). A fractional absorption value of 5% is considered to be a good estimate for the gastrointestinal absorption of soluble inorganic chromium compounds, but 0.5% is more appropriate for that of insoluble inorganic chromium compounds such as chromium trioxide pigment (20).

Once absorbed, the fate of chromium will depend on the oxidation state. Chromium(VI) readily penetrates cell membranes, but chromium(III) does not. Chromium is therefore found in both erythrocytes and plasma after gastrointestinal absorption of chromium(VI) but exclusively in the plasma after that of chromium(III). Once transported through the cell membrane, chromium(VI) is rapidly reduced to chromium(III), which subsequently binds to macromolecules. In animal studies, chromium was found to accumulate mainly in liver, kidneys, spleen, and bone marrow after both oral and parenteral administration of different compounds, the distribution depending on the speciation. In humans, the highest concentrations are found in hilar lymph nodes and lungs, followed by spleen, liver, and kidneys (20), and tissue chromium levels decline with age. In both laboratory animals and humans, water-soluble compounds can be converted into insoluble compounds with long residence times.

After oral exposure to chromium compounds, especially those of chromium(III), chromium is recovered almost entirely in the faeces because of the poor absorption rate. Animal studies show that urine is the major route of elimi-
nation of absorbed chromium. In a 1-year balance study in which two humans had mean daily dietary intakes of 200 and 290 µg of chromium, 60% and 40% of the total amount excreted were recovered in the urine and faeces, respectively (20).

13.11.5 Effects on laboratory animals and in vitro test systems

Acute exposure

Oral LD₅₀ values in rats were in the range 20–250 mg of chromium(VI) per kg of body weight and 185–615 mg of chromium(III) per kg of body weight, based on tests with dichromates and chromic compounds, respectively (20).

Short-term exposure

Three-month-old inbred BD rats (5–14 per sex per dose) were exposed for 90 days, 5 days per week, to 0, 2%, or 5% of insoluble, nonhydrated chromium(III) oxide (Cr₂O₃) pigment in feed (22). The dose levels are equivalent to 0, 480, and 1210 mg of chromium(III) per kg of body weight per day (20). Survival, feed intake, body and organ weights, blood analysis, and the macroscopic and microscopic appearance of major organs were not affected. The only effect observed was a dose-related decrease in liver and spleen weights, ranging from 15% to 35% (22).

Long-term exposure

Chromium(III)

In a 1-year study, 5-week-old Sprague-Dawley albino rats (9 males and 12 females) were exposed to 25 mg of chromium(III) per litre (as chromium tri­chloride, CrCl₃) in drinking-water, equivalent to 2.5 mg of chromium(III) per kg of body weight per day. Feed consumption, body weight gain, and the gross and microscopic appearance of tissues were not affected. The only effect observed was some accumulation of chromium in various tissues (23).

Chromium(VI)

In a 1-year study, 5-week-old albino Sprague-Dawley rats (8–12 per sex per dose) were exposed to dose levels up to 25 mg of chromium(VI) per litre (as potassium chromate) in drinking-water. The highest dose is equivalent to 2.5 mg of chromium(VI) per kg of body weight per day. Feed consumption, body weight gain, blood parameters, and the gross and microscopic appearance of organs were not affected. The only effects observed were decreased water consumption (20%) and accumulation of chromium in various tissues (23).

In a limited lifetime toxicity study in which Swiss mice of the Charles River CD strain (54 per sex) were exposed from weaning until death to 5 mg of chromium(VI) per litre (as potassium chromate) in drinking-water, survival parameters and body weight were not affected (24). Exposure of NMRI mice in a
29-month three-generation study to 135 mg of chromium(VI) per litre (as potassium chromate) in drinking-water did not affect survival or growth (25).

**Reproductive toxicity, embryotoxicity, and teratogenicity**

In a 90-day study with limited numbers of 3-month-old inbred BD rats, exposure of male and female animals for 60 days prior to mating and through gestation to dose levels of 0, 2%, or 5% insoluble, nonhydrated chromium(II) oxide pigment in feed did not result in embryotoxicity or fetotoxicity or teratogenicity (22). In studies with hamsters and mice, parenteral administration of chromium(III) or chromium(VI) during gestation did result in embryotoxicity or fetotoxicity and teratogenicity. These effects appear to be associated with maternal toxicity, but definitive conclusions cannot be reached (20).

**Mutagenicity and related end-points**

Chromium(VI) compounds cause mutations and allied effects such as chromosomal aberrations in a wide range of prokaryotic and eukaryotic test systems, both *in vitro* and *in vivo*. Chromium(III) compounds are not active in similar systems, or only at high, cytotoxic concentrations. It has therefore been concluded that chromium(VI) is mutagenic, whereas chromium(III) is not.

The mutagenic activity of chromium(VI) is decreased or abolished by reducing agents such as human gastric juice and rat liver microsomal fraction. Inactive chromium(III) compounds are not converted into mutagens by biological systems, but only by treatment with strong oxidizing agents. The difference between the mutagenic action of chromium(VI) and chromium(III) can be explained by differences in physicochemical properties. Although chromium(VI), which readily penetrates cell membranes, is the causative agent, there are strong indications that chromium(III) or intermediates such as chromium(V) formed during the intracellular reduction of chromium(VI) are the genetically active agents that form ligands with macromolecules such as DNA (20).

**Carcinogenicity**

In a lifetime carcinogenicity study in which 3-month-old inbred male and female BD rats (60 per dose) were exposed, 5 days per week for 600 days, to 0, 2%, or 5% of insoluble, nonhydrated chromium(III) oxide pigment in feed, tumour incidence was not affected (22). The highest dose is equivalent to 1210 mg of chromium(III) per kg of body weight per day (20).

In a limited lifetime carcinogenicity study, Swiss mice of the Charles River CD strain (54 per sex) were exposed from weaning until death to 5 mg of chromium(VI) per litre (as potassium chromate) in drinking-water. According to the authors (24), the study suggested that chromium(VI) is carcinogenic, but the very limited data reported do not allow evaluation (20).
Exposure of NMRI mice in a 29-month three-generation study to 135 mg of chromium(VI) per litre (as potassium chromate) in drinking-water did not result in carcinogenic activity in the stomach (25).

The carcinogenicity of chromium, especially with regard to lung tumours, has also been investigated in a number of inhalation studies; in other studies, the chromium was administered by implantation or injection. Based on all the available studies, it has been concluded that there is sufficient evidence in experimental animals for the carcinogenicity of calcium, lead, strontium, and zinc chromates (chromium(VI)); limited evidence for the carcinogenicity of chromium trioxide (chromic acid) and sodium dichromate; and inadequate evidence for the carcinogenicity of other chromium(VI) and chromium(III) compounds and of metallic chromium (2, 26).

13.11.6 Effects on humans

Requirements

The daily chromium requirement for adults is estimated to be 0.5–2 μg of absorbable chromium(III). If a fractional absorption value of 25% for "biologically incorporated" chromium(III) in food is assumed, this is provided by a daily dietary intake of 2–8 μg of chromium(III), equivalent to 0.03–0.13 μg of chromium(III) per kg of body weight per day for a 60-kg adult (20).

Acute exposure

Ingestion of 1–5 g of "chromate" (not further specified) resulted in severe acute effects such as gastrointestinal disorders, haemorrhagic diathesis, and convulsions. Death may occur following cardiovascular shock (20).

Mutagenicity

In some occupational studies, increased incidences of genotoxic effects such as chromosomal aberrations and sister chromatid exchanges have been found in workers exposed to chromium(VI) compounds (20).

Carcinogenicity

In epidemiological studies, an association has been found between occupational exposure to chromium(VI) compounds and mortality due to lung cancer. On the basis of these studies, it has been concluded that there is sufficient evidence of respiratory carcinogenicity in humans exposed to chromium(VI) in these occupational settings. Data on lung cancer risk in other chromium-associated occupational settings and for cancer at sites other than the lungs are considered to be insufficient. The epidemiological data do not allow an evaluation of the relative contributions to carcinogenic risk of metallic chromium, chromium(III), and
chromium(VI) or of soluble versus insoluble chromium compounds, but it appears that exposure to a mixture of chromium(VI) compounds of different solubilities results in the highest risk to humans \( (2, 26) \).

IARC has classified chromium(VI) in Group 1 (carcinogenic to humans) and metallic chromium and chromium(III) in Group 3 (not classifiable as to their carcinogenicity to humans) \( (2, 26) \).

### 13.11.7 Provisional guideline value

In principle, because the health effects are determined largely by the oxidation state, different guideline values for chromium(III) and chromium(VI) should be derived. However, current analytical methods and the variable speciation of chromium in water favour a guideline value for total chromium.

Because of the carcinogenicity of chromium(VI) by the inhalation route and its genotoxicity, the current guideline value of 0.05 mg/litre has been questioned, but the available toxicological data do not support the derivation of a new value. As a practical measure, 0.05 mg/litre, which is considered to be unlikely to give rise to significant risks to health, has been retained as the provisional guideline value until additional information becomes available and chromium can be re-evaluated.

**References**


25. Borneff I et al. [Carcinogenic substances in water and soil. XXII. Mouse drinking study with 3,4-benzpyrene and potassium chromate.] Archiv für Hygiene, 1968, 152(68):45-53 (in German).


13.12 Colour

13.12.1 General description

Identity

The appearance of colour in water is caused by the absorption of certain wavelengths of normal light by coloured substances ("true" colour) and by the scattering of light by suspended particles; combined, these constitute "apparent" colour (1-3). Treatment removes much of the suspended matter from drinking-water, and most of the remaining discoloration arises from true colour, which is generally substantially less than apparent colour (4).

Organoleptic properties

It has been suggested that the organic matter (primarily humic and fulvic acids) usually responsible for the colour of drinking-water give it an earthy smell and taste, but there is no conclusive evidence for this. Highly coloured polluted water will frequently have an objectionable taste, but the precise causal relationship is unknown. It is known that the organic colouring material in water stimulates the growth of many aquatic microorganisms, some of which are directly responsible for the production of odour in water (5).
13.12.2 Analytical methods

There are essentially two methods for the measurement of colour intensity in potable water: visual comparison with standards, and absorbance analysis (6).

In the visual comparison method, colour is measured in true colour units (TCU, or Hazen units), 1 TCU being defined as the colour produced by 1 mg of platinum per litre (as chloroplatinic acid) in the presence of 2 mg of cobalt(II) chloride per litre (4, 6). The colour of a filtered water sample is measured by visual comparison with a series of standards of known TCU. This method was designed for use in the determination of the colour of naturally (yellow-brown) coloured water and is difficult to apply to other colours. As the colour of natural surface waters generally increases with increasing pH (1), it is recommended that the pH of a colour sample be recorded together with the colour measurement (4, 6).

Absorbance analysis involves filtration through a cellulose-acetate membrane and subsequent spectrophotometric measurement of the absorbance of the filtrate (6).

13.12.3 Environmental levels and human exposure

Water

As already mentioned, colour in natural waters is due mainly to organic matter, particularly dissolved humic and fulvic acids, which originate from soil, peat, and decaying vegetation. In addition, inorganic iron and manganese are present in some groundwaters and surface waters and may impart a red and black hue, respectively. Highly coloured wastewaters, in particular from the pulp, paper, dye, and textile industries, can also produce coloured waters.

Discoloration of potable water may arise from the dissolution of iron (red) or copper (blue) in distribution pipes, which can be enhanced by bacteriological processes. Microbiological action can also produce "red water", resulting from the oxidation of iron(II) to iron(III) by "iron bacteria". Similarly, black discoloration may result from the action of bacteria capable of oxidizing dissolved manganese to give insoluble forms.

13.12.4 Effects on laboratory animals and in vitro test systems

Short-term exposure

A low-ash preparation of soil fulvic acid was supplied in drinking-water at concentrations of 10, 100, or 1000 mg/litre to male rats for periods of up to 90 days (Becking & Yagminas, 1978, unpublished data). No significant changes in body weight, food and water intake, organ/body weight ratios, or tissue histology were observed. The same fulvic acid preparation given daily for 14 days to rats by gavage at a dosage of 1000 mg/kg of body weight was not lethal but did reduce the rate of weight gain and cause slight changes in kidney enzyme concentrations.
Drinking-water containing nonchlorinated (total organic carbon (TOC) concentration 1.0 g/litre) and chlorinated humic substances (TOC 0.1, 0.5, 1.0 g/litre) was administered to groups of male Sprague-Dawley rats for a period of 90 days (7). The average body weight gain and terminal body weight were decreased significantly by 1.0 g/litre chlorinated humic substances and slightly by 0.5 g/litre chlorinated humic and 1.0 g/litre nonchlorinated humic substances. No significant differences were observed in food consumption. However, fluid consumption was significantly decreased by 1.0 g/litre nonchlorinated and 1.0 or 0.5 g/litre chlorinated humic substances, namely by 14%, 16%, and 17%, respectively. The most significant finding of this study was the increased incidence and severity of haematuria in the group receiving 1.0 g/litre chlorinated humic substances. These studies suggest that there is minimal risk from exposure to chlorination by-products of humic acids as far as target organ effects are concerned.

**Mutagenicity and related end-points**

Chlorinated and nonchlorinated humic acids (TOC 1 g/litre in distilled water) were tested for mutagenic activity by both in vitro and in vivo assays. In the Ames test, the results showed positive mutagenic activity only after chlorination, whereas induction of sister chromatid exchange was observed with both chlorinated and nonchlorinated humic acids. In contrast, in the in vivo studies, no evidence was found of mutagenic activity for both chlorinated and nonchlorinated samples (8).

13.12.5 Effects on humans

Colour-producing organic substances are not themselves thought to be harmful to health. However, they can react with chlorine to produce undesirable levels of chlorination by-products, including trihalomethanes.

Most metals readily form complexes with humic substances in water, which can greatly increase their solubility (9,10). For example, naturally occurring humic substances in water may increase the solubility of iron by a factor of up to $10^9$ (10).

The bioavailability and human toxicity of complexes between humic material and such toxic metals as aluminium, copper, cadmium, and mercury have been investigated in only a small number of studies (11).

13.12.6 Conclusions

The colour of drinking-water is usually due to the presence of coloured organic acids (humic and fulvic) associated with the humus fraction of soil. Colour is strongly influenced by the presence of iron and other metals, either as natural impurities or as corrosion products. It may also result from the contamination of...
the water source with industrial effluents and may be the first indication of a hazard­
ous situation. The source of colour in a water supply should be investigated, particu­larly if a substantial change takes place. Colours below 15 TCU are usually accep­table to consumers, but acceptability may vary according to local circumstances. No health-based guideline value is proposed for colour in drinking-water.

References


13.13 Copper

13.13.1 General description

Identity

Widely used copper compounds include CuCl₂·2H₂O (CAS no. 7447-39-4), Cu(NO₃)₂·3H₂O (CAS no. 10031-43-3), and CuSO₄·5H₂O (CAS no. 7758-99-8).

Physicochemical properties (1, 2)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Water solubility (g/litre)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuCl₂·2H₂O</td>
<td>710 at 0 °C; 1080 at 100 °C</td>
</tr>
<tr>
<td>Cu(NO₃)₂·3H₂O</td>
<td>1380 at 0 °C; 12700 at 100 °C</td>
</tr>
<tr>
<td>CuSO₄·5H₂O</td>
<td>320 at 0 °C; 2030 at 100 °C</td>
</tr>
</tbody>
</table>

Organoleptic properties

Dissolved copper imparts a colour and an unpleasant astringent taste to drinking-water (3). Staining of laundry and plumbing fixtures occurs when copper concentrations in water exceed 1 mg/litre. The taste threshold is above 5 mg/litre, although taste is detectable in distilled water at 2.6 mg/litre (4). The organoleptic threshold of dissolved Cu²⁺ is 0.8–1.0 mg/litre in mineral water and 2.4–3.2 mg/litre in 5 mmol/litre saccharose (5).

Major uses

Copper is an important heat and electrical conductor. It is also used for water pipes, roof coverings, household goods, and chemical equipment, in the arts, and in many alloys (e.g. brass and bronze). Copper oxides, chlorides, sulfates, ethanoates, bromides, and carbonates are widely used in pest control, as inorganic dyes, as feed additives, in photography, in seed disinfectants, as fungicides and algicides, and in electroforming (1, 2, 6).

Environmental fate

Monovalent copper is unstable in aqueous solution. Only those copper(1) compounds that are insoluble in water (Cu₂O, Cu₂S) and certain copper(1) complexes are stable in aqueous environments. Cu(II) forms complexes with both inorganic and organic ligands such as ammonium and chloride ions and humic acids (2).
13.13.2 Analytical methods

The most important methods for the determination of copper and their detection limits are atomic absorption spectrometry with flame detection (1.5 μg/litre) or in the graphite furnace (60 ng/litre); pulse inverse voltammetry (1 ng/litre); spectral photometry (100 μg/litre); neutron activation (0.2 ng of copper per 500 mg solid sample); and emission spectroscopy (0.5 μg/litre) (6, 7).

13.13.3 Environmental levels and human exposure

Air

Copper concentrations in air in rural areas in the USA and Europe are normally below 10 ng/m³. In urban areas, concentrations may be as high as 1500 ng/m³ (6, 8), although levels around 25 ng/m³ have also been found (6).

Water

Natural copper concentrations in drinking-water are around a few micrograms per litre (6). Depending on such properties as hardness, pH, anion concentrations, oxygen concentration, temperature, and the technical conditions of the pipe system (1, 6), water from copper pipes may contain several milligrams of copper per litre (1, 9). In a sample of water for human consumption which had remained stagnant for 12 h, an extreme level of 22 mg of copper per litre was found (10).

Food

Foods especially rich in copper (10–100 mg/kg) are veal and pig, sheep, and calf liver. Chocolate and chocolate products, tea, and coffee (dry) can also contain more than 10 mg of copper per kg. Other foods may contain up to 10 mg/kg (nuts), median values being around 2 mg/kg (1, 11).

Estimated total exposure and relative contribution of drinking-water

Copper is ingested by humans mainly via food and drinking-water. In two 24-h intake studies in the Netherlands, the average daily copper intake per person was 1.2–1.4 mg (1). Intakes of 1.82–2.38 mg/day (11) and less than 0.95 mg/day (duplicate study) (12) have been reported in the western and eastern parts of Germany, respectively; intakes in the USA were 2–4 mg/day (6). Drinking-water can contribute a significant proportion of the daily copper intake if it has flowed through copper installations (1, 6).
13.13.4 Kinetics and metabolism in laboratory animals and humans

Up to 100% copper absorption was observed in newborn rats. After weaning, absorption rates fell in various animal species to below 10% (13). Estimated values for intestinal copper absorption in humans vary between 25% and 65% (13). In adults, the absorption and retention rates of copper depend on the daily intake and, as a consequence, copper overload is unlikely. In a balance study with bottle-fed infants, absorption and retention rates were 23.9% and 21.9% of intake, respectively (14).

Copper is an essential element. Balance studies on adults suggest that a copper intake of 1–5 mg/day, corresponding to 20–80 μg/kg of body weight per day (15, 16) is required. The normal copper content in the adult is 1–2 mg/kg of body weight. In neonates, the liver contains over 90% of the total body copper (4–5 mg/kg of body weight); the copper concentration in the newborn liver is 6–10-fold higher than in the adult liver (17) but decreases during the first 3 months of life (18).

Normal copper concentrations in plasma are 0.9–1.3 mg/litre (17). Of this, 5–10% is bound to albumin and 90–95% specifically to the copper transport protein ceruloplasmin (8, 13). In the liver, copper is bound mainly to metallothionein but also to functionally specific enzymes (19); glutathione serves as a buffer to trap free copper ions that would otherwise be toxic (20). Partial saturation of metallothionein with copper and zinc in the liver of the newborn depends on the cytosolic zinc:copper ratio (18).

About 1 mg of copper per day is transported to the tissues bound to ceruloplasmin (15, 16). Excretion takes place primarily via the faeces; urine contains only 0.5–3% of the daily intake (6, 21, 22).

13.13.5 Effects on laboratory animals and in vitro test systems

**Acute exposure**

Depending on the animal species and the anion of the copper salt administered, oral LD₅₀ values vary between 15 (guinea-pig: CuCl₂) and 416 (rats: Cu(OH)Cl; Cu₂O) mg/kg of body weight (13, 21, 22).

**Short-term exposure**

In most studies with rodents, copper given orally in doses of up to 50 mg/kg of body weight for less than 1 year caused either no effects or adaptation to copper exposure with transient signs of toxicity. No such adaptation was observed in rabbits, pigs, and sheep (13, 21, 22), the last-named being especially sensitive to some of the acute effects of excess copper intake.
Long-term exposure
In two oral studies, NOAELs of 5 mg/kg of body weight per day (1 year; dog) and 160 mg/kg of body weight per day (2 years; rat) were found for the end-points liver functional changes (dog) and various macroscopic and microscopic pathological parameters (rat). In a 16-month rabbit study, a LOAEL of 12 mg/kg of body weight per day was estimated for cirrhosis-like hepatic changes (21, 22).

Reproductive toxicity, embryotoxicity, and teratogenicity
Copper gluconate given orally to mice and rats at 30 mg/kg of body weight per day on days 6–14 and 5–15 of gestation, respectively, was neither embryotoxic nor teratogenic. In another assay with comparable exposure, the fertility of rats was not affected. A much higher NOAEL was reported with copper sulfate for skeletal deformations of fetuses from exposed mothers, but reduced maternal food intake could not be ruled out as the cause (6, 21, 22).

Mutagenicity and related end-points
The results of mutagenicity tests are inconclusive (6). Positive results from in vitro tests using free copper ions are not applicable to the in vivo situation, where copper is always tightly bound to low- and high-molecular-weight ligands (18, 19).

Carcinogenicity
Based on the results of a number of animal studies involving oral and intraperitoneal exposure to various copper compounds, it is generally agreed that copper and its salts are not animal carcinogens (6, 21, 22).

13.13.6 Effects on humans
Acute exposure
The lethal oral dose for adults lies between 50 and 500 mg of copper(II) salt per kg of body weight. Vomiting, diarrhoea, nausea, and some acute symptoms presumably due to local irritation by ingested copper(II) ions have been described in several case reports (6, 21, 22). The estimated concentration of copper(II) in drinking-water or beverages that can lead to symptoms of this type is 30 mg/litre but may vary with the binding and chemical form of copper present.

Short-term exposure
Copper pipes in haemodialysis devices have caused systemic copper poisoning in patients (21, 22). Drinking-water from a new copper kettle used over a period of 3 months for the preparation of food and beverages may have been responsible
for a strongly enhanced serum copper level, behavioural changes, diarrhoea, and progressive loss of strength in a 15-month-old child (23).

A 14-month-old infant died of micronodular liver cirrhosis, probably due to pre- and postnatal exposure to up to 6.8 mg of copper per litre in the very acid water that had flowed through a copper installation and had been used to prepare the infant's feed (24). A total of 22 similar cases of early childhood liver cirrhosis have been described in two limited areas in Germany (25). The estimated daily copper intake that might have triggered the cirrhosis in the infants' early months of life (26) is at least 900 μg/kg of body weight, about 10 times their daily requirement (21, 22).

**Long-term exposure**

In hepatolenticular degeneration (Wilson disease) which is caused by reduced copper excretion in the bile, the normal daily copper intake of a few milligrams is enough to trigger liver cirrhosis and excessive copper accumulation in many organs, but only after several years of exposure (6, 27). The copper status of the healthy liver of neonates during the first few months of life is comparable to that of a person suffering from Wilson disease (28), which may explain why infants are more sensitive to factors that threaten copper homeostasis than are older children and adults (25).

In a recent Finnish report, it was claimed that a positive correlation existed between coronary heart disease incidence and the plasma copper level under conditions of selenium malnutrition (29). The duration and source of the excessive copper exposure in this study were not specified.

**13.13.7 Provisional guideline value**

Based on a NOAEL of 5 mg/kg of body weight per day for the end-point liver toxicity in a rather old 1-year study in dogs and in the light of the essentiality of copper, a provisional maximum tolerable daily intake (PMTDI) of 0.5 mg/kg of body weight was established by JECFA in 1982 (21, 22). An allocation of 10% of the PMTDI to drinking-water gives a guideline value of 2 mg of copper per litre (rounded figure). Although this study did not take into account the differences in copper metabolism in the neonate, a concentration of 2 mg/litre should provide a sufficient margin of safety for bottle-fed infants because their copper intake from other sources is usually low. In view of the remaining uncertainties regarding copper toxicity in humans, however, this guideline value is considered provisional. Copper can give rise to taste problems, but the taste should be acceptable at the health-based provisional guideline value.
GUIDELINES FOR DRINKING-WATER QUALITY

References


13.14 Cyanide

13.14.1 General description

Almost all of the recent literature on cyanide has resulted from interest in the root crop cassava, which provides a major part of the diet for between 300 and 500 million people living in developing countries in the tropics and subtropics. If not properly prepared, cassava can contain very high levels of cyanide, and outbreaks of disease have been associated with its consumption.

13.14.2 Analytical methods

Cyanide can be determined in water by both titrimetric and photometric techniques, with a detection limit of 2 μg/litre (1).

13.14.3 Environmental levels and human exposure

**Water**

Cyanides are occasionally found in drinking-water, primarily as a consequence of industrial contamination.

**Food**

A recent study suggests that dietary exposure to cyanide is considerably greater in developing countries than in developed ones. For a group of 73 subjects in Liberia consuming cassava, the mean daily intake of cyanide ion was calculated to be 0.61 mg/kg of body weight (2). Although insufficient data are available from which to calculate the average daily intake in developed countries, it is very unlikely to be of this magnitude.

13.14.4 Kinetics and metabolism in laboratory animals and humans

Cyanide ion is readily absorbed by the gastrointestinal tract and is rapidly converted into thiocyanate by the enzyme rhodanese. Oral and subcutaneous doses of cyanide in rats are excreted as thiocyanate, primarily in the urine (3, 4). Golden hamsters exposed to cyanide by subcutaneous infusion appeared to excrete...
only a relatively small percentage (10–15%) of the dose as thiocyanate in the urine (5), perhaps because rhodanese activity in hamsters is lower than in rats, and hence they are less able to convert cyanide into thiocyanate (6).

13.14.5 Effects on laboratory animals and in vitro test systems

Short-term exposure

A reduction in feed consumption and body weight gain was noted in a group of six male and two female African rats fed diets containing potassium cyanide at 2500 mg/kg for 84 days. No effects on the pathology of the thyroid, liver, kidney, and spleen or on serum total proteins, albumin, aspartate aminotransferase, and alanine aminotransferase were observed, but serum urea concentration was elevated (7).

Addition of potassium cyanide at a concentration of 200 mg/litre to the drinking-water of a group of seven male Sprague-Dawley rats produced a slight elevation in liver weight but had no effect on body weight gain after 21 days. Addition of potassium cyanide to the diet at 200 mg/kg had no effect on either parameter. However, there was evidence that cyanide added to the diet was quickly lost or bound, and hence actual exposure was likely to have been less than anticipated. This study also showed that cyanide offers some protection against selenium toxicity in rats, possibly through the formation of the SeCN ion (8).

Six weanling pigs were fed a diet containing potassium cyanide (500 mg of cyanide per kg) for 56 days. A reduction in feed intake was noted, but there was no effect on body weight gain. There were no effects on the weights of several organs examined except for some evidence of an increase in thyroid weight. Pathological examination of a range of tissues revealed no treatment-related effects (9).

Pigs were fed diets of cassava containing 0, 96, or 400 mg of cyanide per kg for 72 days. A lowering of serum thyroxine was noted at both dose levels. There were no effects on serum total protein, albumin, alanine aminotransferase, and aspartate aminotransferase, but serum urea was elevated (10).

Erythrocyte glucose-6-phosphate dehydrogenase activity was significantly depressed in miniature swine receiving oral doses of 1.2 mg of cyanide ion per kg of body weight per day from week 0 to week 12, but activity returned to control levels after week 16. At lower doses of 0.4 and 0.7 mg of cyanide ion per kg of body weight per day, enzyme inhibition was initially delayed; by week 20, activity was significantly lower than in controls and the highest dose group (11).

The effects of cyanide on behaviour were studied in pigs given oral doses of 0, 0.4, 0.7, or 1.2 mg of cyanide ion per kg of body weight per day for 6 months. Exposure to cyanide was reported to produce increasing ambivalence and slower response times to stimuli with increasing dose. Behaviours demanding high energy tended to be affected more readily than those demanding low energy. An effect on glucose metabolism was suggested as a possible explanation for this finding. A reduction in serum thyroxine and, more notably, in triiodothyronine levels was
found at all three doses (12). However, clear effects were observed only at the highest dose.

A group of 10 male rats was fed a 10% casein diet containing added methionine, vitamin B₁₂, iodine, and potassium cyanide (1500 mg/kg) for nearly 1 year. Compared with a control group not receiving cyanide, depression of body weight gain was observed throughout the study, but there were no deaths or clinical signs of toxicity. Depression of both plasma thyroxine and the thyroxine secretion rate, suggestive of depressed thyroid function, was evident at 4 months but less so after 1 year. At autopsy, the animals were found to have enlarged thyroids, which may have been the mechanism of adaptation. Some differences in the histopathology of the spinal cord, notably the white matter, were also found between controls and cyanide-treated animals (13).

Chicks were fed diets containing up to 30% cassava root meal for 28 days. The cassava root meal itself contained 300 mg of total cyanide per kg. No effects on survival, feeding performance, body weight gain, or haematology were noted. The additional inclusion of 3% cassava foliage meal (containing 156 mg of total cyanide per kg and 20 μg of aflatoxin per kg) resulted in depression of body weight gain (14).

Reproductive toxicity, embryotoxicity, and teratogenicity

A group of 20 pregnant female rats was fed cassava diets with added potassium cyanide (500 mg/kg) for about 20 days. After parturition, dams and offspring were continued on the diet for the 21-day lactation period. Some pups were also continued on the diet for a further 28 days post-weaning. No effects on gestation, lactation performance, or growth of offspring were seen. However, if offspring from dams not treated with cyanide were exposed during the post-weaning period only, depression of both body weight gain and feed intake as compared with untreated controls was observed (15).

Groups of six pregnant pigs were fed diets of cassava to which cyanide at levels of 0, 250, or 500 mg/kg (as potassium cyanide) was added until parturition, after which sows and offspring returned to a standard diet for a 56-day lactation period. Dietary cyanide had no effect on the numbers or weights of fetuses. A slight elevation of maternal thyroid weight was noted. Pathological changes were also observed in this organ in animals receiving the highest dose level (16). This study suggests that effects may occur in the pig at doses an order of magnitude lower than that found in short-term studies on the rat.

Pregnant golden hamsters were exposed to sodium cyanide (0.126–0.1295 mmol/kg per hour) on days 6–9 of gestation by infusion via subcutaneously implanted osmotic minipumps. High incidences of resorptions and malformations were seen in the offspring, the most common abnormalities being neural tube defects (5).
13.14.6 Effects on humans

Cyanide may lower vitamin B\textsubscript{12} levels and hence exacerbate vitamin B\textsubscript{12} deficiency. It has also been linked to an increased incidence of goitre (cretinism) in Zaire through effects on iodine uptake by the thyroid. Those with nutritional inadequacy or inborn metabolic errors are particularly vulnerable (17). Chronic effects on the thyroid and particularly on the nervous system were observed in some populations as a consequence of the consumption of inadequately processed cassava containing high levels of cyanide. This problem seems to have decreased significantly in the West African populations in which it was widely reported following a change in processing methods and a general improvement in nutritional status.

13.14.7 Guideline value

There are a very limited number of toxicological studies suitable for use in deriving a guideline value. There is, however, some indication in the literature that pigs may be more sensitive than rats. Only one study is available in which a clear effect level was observed, at 1.2 mg/kg of body weight per day, in pigs exposed for 6 months (12). The effects observed were on behavioural patterns and serum biochemistry.

Using the LOAEL from this study and applying an uncertainty factor of 100 to reflect inter- and intraspecies variation (no additional factor for a LOAEL was considered necessary because of doubts over the biological significance of the observed changes), a TDI of 12 µg/kg of body weight was calculated.

An allocation of 20% of the TDI to drinking-water is made because exposure to cyanide from other sources is normally small and because exposure from water is only intermittent. This results in a guideline value of 0.07 mg/litre (rounded figure), which is considered to be protective for both acute and long-term exposure.

References


13.15 Fluoride

13.15.1 General description

Identity

Fluorine is a fairly common element that does not occur in the elemental state in nature because of its high reactivity. It accounts for about 0.3 g/kg of the earth's crust and exists in the form of fluorides in a number of minerals, of which fluor spar, cryolite, and fluorapatite are the most common. The oxidation state of the fluoride ion is -1.

Physicochemical properties (1, 2)

<table>
<thead>
<tr>
<th>Property</th>
<th>Sodium fluoride (NaF)</th>
<th>Hydrogen fluoride (HF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical state</td>
<td>White, crystalline powder</td>
<td>Colourless liquid or gas with biting smell</td>
</tr>
<tr>
<td>Melting point (°C)</td>
<td>993</td>
<td>-83</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>1695 at 100 kPa</td>
<td>19.5</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>2.56</td>
<td>-</td>
</tr>
<tr>
<td>Water solubility</td>
<td>42 g/litre at 10 °C</td>
<td>Readily soluble below 20 °C</td>
</tr>
<tr>
<td>Acidity</td>
<td>-</td>
<td>Strong acid in liquid form; weak acid dissolved in water</td>
</tr>
</tbody>
</table>

Major uses

Inorganic fluorine compounds are used in aluminium production, as a flux in the steel and glass fibre industries, and in the production of phosphate fertilizers (which contain an average of 3.8% fluorine), bricks, tiles, and ceramics. Fluor silicic acid is used in municipal water fluoridation schemes (1).

Environmental fate

Although sodium fluoride is soluble in water (1), aluminium, calcium, and magnesium fluorides are only sparingly so (3).

13.15.2 Analytical methods

Fluoride is usually determined by means of an ion-selective electrode, which makes it possible to measure the total amount of free and complex-bound fluoride dissolved in water. The method can be used for water containing at least 20 μg/litre (2). For rainwater in which fluoride was present at a concentration of 10 μg/litre, a detection limit of 1 μg/litre was reported (4).

A method using a fluoride-selective electrode and an ion analyser to determine fluoride at levels of 0.05–0.4 mg/litre has been described (5). With a slight modification, the method can be used to measure fluoride at 0.4–2.0 mg/litre.
13.15.3 Environmental levels and human exposure

**Air**

Natural background concentrations are of the order of 0.5 ng/m$^3$. If anthropogenic emissions are included, worldwide background concentrations are of the order of 3 ng/m$^3$. In the Netherlands, concentrations in areas without sources are 30–40 ng/m$^3$, rising to 70 ng/m$^3$ in areas with many sources (2). In a survey of fluoride in the air of some communities in the USA and Canada, concentrations were in the range 0.02–2.0 µg/m$^3$ (6). In some provinces of China, fluoride concentrations in indoor air ranged from 16 to 46 µg/m$^3$ owing to the indoor combustion of high-fluoride coal for cooking and for drying and curing food (7).

**Water**

Traces of fluoride are present in many waters; higher concentrations are often associated with underground sources. In seawater, a total fluoride concentration of 1.3 mg/litre has been reported (2). In areas rich in fluoride-containing minerals, well-waters may contain up to about 10 mg of fluoride per litre. The highest natural level reported is 2800 mg/litre. Fluorides may also enter a river as a result of industrial discharges (2). In groundwater, fluoride concentrations vary with the type of rock that the water flows through but do not usually exceed 10 mg/litre (3). In the Rhine in the Netherlands, levels are below 0.2 mg/litre. In the Meuse, concentrations fluctuate (0.2–1.3 mg/litre) as a result of variations in industrial processes (2).

Fluoride concentrations in the groundwater of some villages in China were greater than 8 mg/litre (8, 9). In Canada, fluoride levels in drinking-water of <0.05–0.2 mg/litre (nonfluoridated) and 0.6–1.1 mg/litre (fluoridated) have been reported in municipal waters; in drinking-water prepared from well-water, levels up to 3.3 mg/litre have been reported. In the USA, 0.2% of the population is exposed to more than 2.0 mg/litre (3). In the Netherlands, year-round averages for all drinking-water plants are below 0.2 mg/litre (2). In some African countries where the soil is rich in fluoride-containing minerals, levels in drinking-water are relatively high (e.g. 8 mg/litre in the United Republic of Tanzania) (3).

**Food**

Virtually all foodstuffs contain at least traces of fluoride. All vegetation contains some fluoride, which is absorbed from soil and water. The highest levels in field-grown vegetables are found in curly kale (up to 40 mg/kg fresh weight) and endive (0.3–2.8 mg/kg fresh weight) (2). Other foods containing high levels include fish (0.1–30 mg/kg) and tea (2, 3). High concentrations in tea can be caused by high natural concentrations in tea plants or by the use of additives during growth or fermentation. Levels in dry tea can be 3–300 mg/kg (average
100 mg/kg), so that 2–3 cups of tea contain approximately 0.4–0.8 mg (2, 6). In areas where water with a high fluoride content is used to prepare tea, the intake via tea can be several times greater.

**Dental uses**

For dental purposes, fluoride preparations may contain low (0.25–1 mg per tablet; 1000–1500 mg of fluorine per kg of toothpaste) or high concentrations (liquids containing 10 000 mg/litre and gels containing 4000–6000 mg/kg are used for local applications) (2).

**Estimated total exposure and relative contribution of drinking-water**

Levels of daily exposure to fluoride depend mainly on the geographical area. In the Netherlands, the total daily intake is calculated to be 1.4–6.0 mg of fluoride. Food seems to be the source of 80–85% of fluoride intake; intake from drinking-water is 0.03–0.68 mg/day and from toothpaste 0.2–0.3 mg. For children, the total intake via food and water is decreased because of lower consumption. Intake of food and water relative to body weight is higher, however, and is further increased by the swallowing of toothpaste or fluoride tablets (up to 3.5 mg of fluoride per day) (2).

Daily intakes ranging from 0.46 to 3.6–5.4 mg/day have been reported in several studies (6). Daily exposure in volcanic areas (e.g. the United Republic of Tanzania) may be as high as 30 mg for adults, mainly from drinking-water intake (J. E. M. Smet, personal communication, 1990). In areas with relatively high concentrations in groundwater, drinking-water becomes increasingly important as a source of fluoride. In some counties in China where coal has a high fluoride content, the average daily intake of fluoride ranged from 0.3 to 2.3 mg via air and from 1.8 to 8.9 mg via food (10).

13.15.4 Kinetics and metabolism in laboratory animals and humans

After oral uptake, water-soluble fluorides are rapidly and almost completely absorbed in the gastrointestinal tract. Fluorides less soluble in water are absorbed to a lesser degree. Absorbed fluoride is transported via the blood; with prolonged intake of fluoride from drinking-water, concentrations in the blood are the same as those in drinking-water, a relationship that remains valid up to a concentration in drinking-water of 10 mg/litre. Distribution of fluoride is a rapid process. It is incorporated into teeth and bones; there is virtually no storage in soft tissues. Incorporation into teeth and skeletal tissues is reversible; after cessation of exposure, mobilization from these tissues takes place. Fluoride is excreted via urine, faeces, and sweat (3, 6, 11).
13.15.5 Effects on laboratory animals and in vitro test systems

**Long-term exposure**

Most long-term studies are limited. In drinking-water studies with sodium fluoride, effects on skeletal tissues were observed. In a 2-year study in rats and mice (25 or 175 mg of sodium fluoride per litre of drinking-water), discolouration and dysplasia developed at both dose levels; osteosclerosis in the long bones was seen in the high-dose females only (12). In another recent 2-year oral study in rats, there were effects on the teeth (ameloblastic dysplasia, fractured and malformed incisors, enamel hypoplasia) and bones (subperiosteal hyperkeratosis) at all dose levels, including the lowest of 4 mg of sodium fluoride per kg of body weight per day (13).

**Mutagenicity and related end-points**

Many mutagenicity studies have been carried out with fluorides (usually sodium fluoride). Tests on bacteria and insects were negative, as were in vivo studies (11, 12, 14). In mammalian cells in vitro, fluoride causes genetic damage (including chromosomal aberrations) at cytotoxic concentrations only (>10 mg/litre), the mechanism for which is not known. This genetic effect is probably of limited relevance for practical human exposures (11).

**Carcinogenicity**

IARC evaluated the available studies in 1987 and concluded that the limited data provided inadequate evidence of carcinogenicity in experimental animals (14). In a recent study in which rats and mice were given sodium fluoride in drinking-water at 11, 45, or 79 mg/litre (as fluoride ion), only the incidence of osteosarcomas in the bones of male rats increased (incidences 0/80, 0/51, 1/50, and 3/80 in the controls, low-, mid-, and high-dose groups, respectively). This increase was considered to provide equivocal evidence for a carcinogenic action in male rats; the study yielded no evidence for such an action in female rats or in male or female mice (12). In another recent study, no carcinogenic effect was observed in rats given sodium fluoride in the diet at dose levels of 4, 10, or 25 mg/kg of body weight per day for 2 years (13).

13.15.6 Effects on humans

Fluorine is probably an essential element for both animals and humans. For humans, however, the essentiality has not been demonstrated unequivocally, and no data indicating the minimum nutritional requirement are available. To produce signs of acute fluoride intoxication, minimum oral doses of at least 1 mg of fluoride per kg of body weight were required (11).
Many epidemiological studies of possible adverse effects of the long-term ingestion of fluoride via drinking-water have been carried out. These studies clearly establish that fluoride primarily produces effects on skeletal tissues (bones and teeth). Low concentrations provide protection against dental caries, especially in children. This protective effect increases with concentration up to about 2 mg of fluoride per litre of drinking-water; the minimum concentration of fluoride in drinking-water required to produce it is approximately 0.5 mg/litre.

Fluoride may give rise to mild dental fluorosis (prevalence: 12–33%) at drinking-water concentrations between 0.9 and 1.2 mg/litre (15). This has been confirmed in numerous studies, including a recent large-scale survey carried out in China (16), which showed that, with drinking-water containing 1 mg of fluoride per litre, dental fluorosis was detectable in 46% of the population examined. As a rough approximation, for areas with a temperate climate, manifest dental fluorosis occurs at concentrations above 1.5–2 mg of fluoride per litre of drinking-water. In warmer areas, dental fluorosis occurs at lower concentrations in the drinking-water because of the greater amounts of water consumed (3, 6, 10). It is also possible that, in areas where fluoride intake via routes other than drinking-water (e.g. air, food) is elevated, dental fluorosis develops at concentrations in drinking-water below 1.5 mg/litre (10).

Fluoride can also have more serious effects on skeletal tissues. Skeletal fluorosis (with adverse changes in bone structure) is observed when drinking-water contains 3–6 mg of fluoride per litre. Crippling skeletal fluorosis develops where drinking-water contains over 10 mg of fluoride per litre (6). The US Environmental Protection Agency considers a concentration of 4 mg/litre to be protective against crippling skeletal fluorosis (17).

Several epidemiological studies are available on the possible association between fluoride in drinking-water and cancer rates among the population. IARC evaluated these studies in 1982 and 1987 and considered that they provided inadequate evidence of carcinogenicity in humans (1, 14). The results of several epidemiological studies on the possible adverse effects of fluoride in drinking-water on pregnancy outcome are inconclusive (3, 6, 11).

It is known that persons suffering from certain forms of renal impairment have a lower margin of safety for the effects of fluoride than the average person. The data available on this subject are, however, too limited to allow a quantitative evaluation of the increased sensitivity to fluoride toxicity of such persons (3, 11).

13.15.7 Guideline value

In 1987, IARC classified inorganic fluorides in Group 3 (14). Although there was equivocal evidence of carcinogenicity in one study in male rats, extensive epidemiological studies have shown no evidence of it in humans (12).

There is no evidence to suggest that the guideline value of 1.5 mg/litre set in 1984 needs to be revised. Concentrations above this value carry an increasing risk
of dental fluorosis, and much higher concentrations lead to skeletal fluorosis. The value is higher than that recommended for artificial fluoridation of water supplies (18). In setting national standards for fluoride, it is particularly important to consider climatic conditions, water intake, and intake of fluoride from other sources (e.g. from food and air). In areas with high natural fluoride levels, it is recognized that the guideline value may be difficult to achieve in some circumstances with the treatment technology available.

References


### 13.16 Hardness

#### 13.16.1 General description

**Identity**

Water hardness is the traditional measure of the capacity of water to react with soap. Hard water requiring considerably more soap to produce a lather. It is not caused by a single substance but by a variety of dissolved polyvalent metallic ions, predominantly calcium and magnesium cations, although other cations, e.g. barium, iron, manganese, strontium and zinc, also contribute. Hardness is most commonly expressed as milligrams of calcium carbonate equivalent per litre, water containing less than 60 mg of calcium carbonate per litre generally being considered as soft. Although hardness is caused by cations, it may also be discussed in terms of carbonate (temporary) and noncarbonate (permanent) hardness.
GUIDELINES FOR DRINKING-WATER QUALITY

Sources

The principal natural sources of hardness in water are dissolved polyvalent metallic ions from sedimentary rocks, seepage, and run-off from soils. Calcium and magnesium, the two principal ions, are present in many sedimentary rocks, the most common being limestone and chalk. They are also present in a wide variety of industrial products and are common constituents of food. As mentioned above, a minor contribution to the total hardness of water is also made by other polyvalent ions, e.g. aluminium, barium, iron, manganese, strontium, and zinc.

Organoleptic properties

The taste threshold for the calcium ion is in the range 100–300 mg/litre, depending on the associated anion, but higher concentrations are acceptable to consumers. Hardness levels above 500 mg/litre are generally considered to be aesthetically unacceptable, although this level is tolerated in some communities (1).

13.16.2 Environmental levels and human exposure

Water

Concentrations of up to 100 mg of calcium per litre are fairly common in natural sources of water; sources containing over 200 mg of calcium per litre are rare. Magnesium salts are soluble, natural water sources typically containing concentrations of up to 10 mg/litre. Such sources rarely contain more than 100 mg of magnesium per litre, and it is usually calcium hardness that predominates (2).

In drinking-water, hardness is in the range 10–500 mg of calcium carbonate per litre (3). Estimated daily intakes of 2.3 and 52.1 mg of magnesium in soft- and hard-water areas, respectively, have been reported, based on adults drinking 2 litres of water per day (4).

Food

Virtually all foods contain calcium and magnesium, and dietary intake is the principal route of exposure. Typical diets provide about 1000 mg of calcium per day and 200–400 mg of magnesium per day. Dairy products are a particularly rich source of calcium, whereas magnesium tends to be associated more with meat and foodstuffs of plant origin (4–6).

Estimated total exposure and relative contribution of drinking-water

The typical dietary contribution of calcium and magnesium is over 80% of the total daily intake. Of this, approximately 30% of calcium and 55% of magnesium will be absorbed. For calcium and magnesium, the typical contribution from water is 5–20% (2, 5, 6).
13.16.3 Effects on humans

There does not appear to be any convincing evidence that water hardness causes adverse health effects in humans. In contrast, the results of a number of epidemiological studies have suggested that water hardness may protect against disease. However, the available data are inadequate to prove any causal association.

**Cardiovascular disease**

In most large-scale studies, an inverse relationship between the hardness of drinking-water and cardiovascular disease has been reported (7–13). However, no such association has been found in some studies (14, 15), and in those involving small geographical areas a clear association is often not found (16).

The extent to which confounding variables, such as climatic, socioeconomic, or major risk factors, may account for the inverse relationship is unclear. Nevertheless, in a number of studies, a weak inverse relationship was reported after allowance was made for climatic and socioeconomic factors (17) and after major risk factors such as hypertension, smoking habits, and elevated serum lipids were taken into account (18, 19). An inverse relationship between hardness and cardiovascular disease had been reported in men after allowing for climatic and certain social factors, but only up to about 170 mg of calcium carbonate per litre (20).

A variety of hypotheses have been proposed to explain the possible inverse association (21–27). However, none has been fully substantiated, nor has a particular element been found to be conclusively associated with cardiovascular disease.

**Other health effects**

The results of several studies have suggested that a variety of other diseases are also inversely correlated with the hardness of water, including anencephaly (28, 29) and various types of cancer (30, 31). However, the significance of these results is unclear, and it has been suggested that the associations may reflect disease patterns that can be explained by social, climatological, and environmental factors, rather than by the hardness of the water. Some data suggest that very soft waters with a hardness of less than 75 mg/litre may have an adverse effect on mineral balance, but detailed studies are not available.

13.16.4 Other considerations

Depending on the interaction of other factors, such as pH and alkalinity, water with a hardness above approximately 200 mg/litre may cause scale deposition in the distribution system, as well as increased soap consumption. In contrast, soft water, with a hardness less than about 100 mg/litre, has a greater tendency to
cause corrosion of pipes, resulting in the presence of certain heavy metals, such as cadmium, copper, lead and zinc, in drinking-water (2). The degree to which such corrosion and solubilization of metals occurs also depends on the pH, alkalinity, and dissolved oxygen concentration.

13.16.5 Conclusions

Although a number of epidemiological studies have shown a statistically significant inverse relationship between the hardness of drinking-water and cardiovascular disease, the available data are inadequate to permit the conclusion that the association is causal. No health-based guideline value for water hardness is proposed.

References


### 13.17 Hydrogen sulfide

#### 13.17.1 General description

**Identity**

- CAS no.: 7783-06-4
- Molecular formula: $\text{H}_2\text{S}$

**Physicochemical properties** (1, 2)$^1$

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical appearance</td>
<td>Colourless gas</td>
</tr>
<tr>
<td>Melting point</td>
<td>$-85.5 \degree C$</td>
</tr>
<tr>
<td>Boiling point</td>
<td>$-60.7 \degree C$</td>
</tr>
<tr>
<td>Density</td>
<td>$1.54 \text{ g/litre at } 0 \degree C$</td>
</tr>
<tr>
<td>Water solubility</td>
<td>$4370 \text{ ml/litre at } 0 \degree C$; $1860 \text{ ml/litre at } 40 \degree C$</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>$1875 \text{ kPa at } 20 \degree C$</td>
</tr>
</tbody>
</table>

$^1$ Conversion factor in air: $1 \text{ mg/m}^3 = 0.670 \text{ ppm.}$
Organoleptic properties

Hydrogen sulfide has an offensive “rotten eggs” odour that is detectable at very low concentrations in air, below 8 µg/m³ (3). At concentrations of 50–150 mg/m³ in air, it has a deceptively sweet smell; above this range, it deadens the sense of smell (4). In water, the taste and odour thresholds for hydrogen sulfide are estimated to be between 0.05 and 0.1 mg/litre. The taste and odour threshold for sulfides is about 0.2 mg/litre (5).

Major uses

The major uses of hydrogen sulfide include its conversion into sulfur and sulfuric acid and the manufacture of inorganic sulfides, thiophenes, thiols, thioaldehydes, and thioketones. It is used in dye manufacture, tanning, the production of wood-pulp, chemical processing, and the manufacture of cosmetics. Spring waters that contain elevated concentrations of hydrogen sulfide are used for therapeutic medicinal baths (1).

Environmental fate

Hydrogen sulfide is formed when soluble sulfides are hydrolysed in water. In water, hydrogen sulfide dissociates, forming monohydrogensulfide(1-) (HS⁻) and sulfide (S²⁻) ions. The relative concentrations of these species are a function of the pH of the water, hydrogen sulfide concentrations increasing with decreasing pH. At pH 7.4, about one-third exists as undissociated hydrogen sulfide and the remainder largely as the monohydrogensulfide(1-) anion (6). The sulfide is present in appreciable concentrations above pH 10 (7). In well aerated water, hydrogen sulfide is readily oxidized to sulfates and biologically oxidized to elemental sulfur. In anaerobic water, microbial reduction of sulfate to sulfide can occur (7).

13.17.2 Analytical methods

Hydrogen sulfide is traditionally determined using an acid displacement procedure (8, 9); the hydrogen sulfide is displaced by acidification, followed by analysis by gas chromatography using a flame photometric detector. The procedure has been used for water, sewage, and effluents containing 0–2.0 mg of sulfide per litre with a detection limit of about 0.25 mg of sulfur per litre (9). An estimated lower detection limit of 0.06 mg/litre has been reported for a similar method (10). The methylene blue colorimetric method is another standard analytical procedure for hydrogen sulfide determination, at concentrations ranging between 0.1 and 20 mg/litre (11). A number of methods have been developed for the determination of sulfide (11, 12).
13.17.3 Environmental levels and human exposure

**Air**

Hydrogen sulfide is present in air primarily as a result of natural emissions. Concentrations generally vary from 0.1 to 1 µg/m³ in ambient air, although concentrations above 100 µg/m³ have been reported near industrial plants (3). An estimated daily intake of 2–20 µg can be calculated on the assumption that 20 m³ of air containing hydrogen sulfide at natural concentrations is inhaled.

**Water**

Most of the hydrogen sulfide present in raw waters is derived from natural sources and industrial processes. It is particularly noticeable in some groundwaters, depending on source rock mineralogy and the microorganisms present (13). In the USA, a maximum concentration of 500 µg of undissociated hydrogen sulfide per litre has been reported in fresh water (14).

**Food**

A number of foodstuffs and drinks may contain sulfides. However, estimation of exposure from food is complicated by the formation of sulfides in cooked foods. Levels in heated dairy products range from 0.8 mg/litre in skimmed milk (0.1% fat) to 1.84 mg/litre in cream (30.5% fat). The hydrogen sulfide content of cooked meat ranges from 0.276 mg/kg for beef to 0.394 mg/kg for lamb. Hydrogen sulfide is formed principally from the sulfur-containing amino acids in meat protein, levels being higher in anaerobically packaged meat. Dimethyl sulfide is used in the manufacture of jellies, candy, soft drinks, and cream in the United Kingdom, where the maximum probable intake has been estimated at 1.7 mg/day (15).

13.17.4 Kinetics and metabolism in laboratory animals and humans

Hydrogen sulfide and soluble alkali sulfides are rapidly absorbed following ingestion (7). Inhaled hydrogen sulfide has been shown to be distributed to the brain, liver, kidneys, pancreas, and small intestine (16). It is metabolized mainly by the liver, the two routes being oxidation to sulfate and methylation to methanethiol and dimethyl sulfide (17). Sulfides and sulfates are rapidly excreted via the kidneys in experimental animals, but a small proportion of the sulfides may also be excreted via the lungs. Some metallic sulfides are excreted in the faeces (1).
13.17.5 Effects on laboratory animals and in vitro test systems

**Acute exposure**

Oral LD_{50} values of 205 and 208 mg/kg of body weight were reported in the mouse and rat, respectively, for sodium sulfide (Registry of Toxic Effects of Chemical Substances, 1989, unpublished data).

**Short-term exposure**

Dimethyl sulfide given daily at an oral dose of 250 mg/kg of body weight for 14 weeks was found to produce no ill effects in rats. This dose is equivalent to a daily intake of 15 g by a 60-kg adult. However, hydrogen sulfide has been reported to be more toxic than dimethyl sulfide by a factor of 50 (18).

**Reproductive toxicity, embryotoxicity, and teratogenicity**

The ingestion of “thermal” mineral water containing 4–12 mg of hydrogen sulfide per litre was embryotoxic in rats, whereas water containing 2–3 mg/litre had no effect. However, the significance of these findings is doubtful, as few experimental details were published and the mineral water contained numerous other substances (19). No effects on pregnancy were seen other than a dose-dependent increase in delivery time in female rats exposed to 112 mg/m³ hydrogen sulfide from day 6 of gestation until day 21 postpartum. In addition, no significant effects on the growth and development of pups were seen (20).

**Mutagenicity and related end-points**

Hydrogen sulfide was not mutagenic in *Salmonella typhimurium* strains TA97, TA98, or TA100, with or without metabolic activation (21). Chromosomal aberrations have been reported in the bone marrow of adult rats exposed to 10 mg/m³ for 3–4 months (22). Hydrogen sulfide has been shown to increase the mutagenicity of hydrogen peroxide in *S. typhimurium* strain TA102 (23). This may be significant where hydrogen peroxide is employed as an oxidizing agent in water-treatment processes.

**Carcinogenicity**

In a study in which Charles River CD male and female rats were given 9 or 18 mg of sodium sulfide per kg of body weight in water by gavage in either the presence or absence of a 1% thyroid extract at least twice a week for 78 weeks, no evidence of carcinogenicity was found. Because of the high mortality in all treated and control groups, the validity of the results is questionable (24).
13.17.6 Effects on humans

No data are available on the oral toxicity of hydrogen sulfide. However, alkali sulfides irritate mucous membranes and can cause nausea, vomiting, and epigastric pain following ingestion. The oral dose of sodium sulfide fatal to humans has been estimated at 10–15 g (1).

When inhaled, hydrogen sulfide is highly acutely toxic to humans (25). Its rapid mode of action involves the formation of a complex with the iron(III) ion of the mitochondrial metalloenzyme cytochrome oxidase, thereby blocking oxidative metabolism (4, 25). Other enzymes reported to be inhibited by sulfides are succinate dehydrogenase, adenosinetriphosphatase, DOPA oxidase, carbonic anhydrase, dipeptidase, benzamidase, and some enzymes containing iron such as catalase and peroxidases (1). Reduction of disulfide bridges in proteins has been suggested as a mechanism whereby enzyme function could be altered (3). Irritation of the eyes and respiratory tract can be observed at concentrations of 15–30 mg/m$^3$, and concentrations of 700–1400 mg/m$^3$ can cause unconsciousness and respiratory paralysis resulting in death (3).

Few studies on prolonged exposure to low concentrations of hydrogen sulfide have been undertaken. In one study, the reticulocytes of 17 workers engaged in wood-pulp production who were exposed to low levels of hydrogen sulfide and methylthiols were analysed (26). The activities of a number of enzymes involved in the haem biosynthetic pathway were inhibited, although the mechanism is unclear.

13.17.7 Conclusions

The taste and odour threshold for hydrogen sulfide in water has been estimated to be as low as 0.05 mg/litre. Although oral toxicity data are lacking, it is unlikely that anyone could consume a harmful dose of hydrogen sulfide in drinking-water. Consequently, no health-based guideline value is proposed. However, hydrogen sulfide should not be detectable in drinking-water by taste or odour.

References


### 13.18 Iron

#### 13.18.1 General description

**Identity**

Iron is the second most abundant metal in the earth's crust, of which it accounts for about 5%. Elemental iron is rarely found in nature, as the iron ions Fe\(^{2+}\) and Fe\(^{3+}\) readily combine with oxygen- and sulfur-containing compounds to form oxides, hydroxides, carbonates, and sulfides. Iron is most commonly found in nature in the form of its oxides (1, 2).

**Physicochemical properties** (3)

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>1535 °C</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>7.86 at 25 °C</td>
</tr>
</tbody>
</table>
**Organoletic properties**

Iron (as Fe^{2+}) concentrations of 40 μg/litre can be detected by taste in distilled water. In a mineralized spring water with a total dissolved solids content of 500 mg/litre, the taste threshold value was 0.12 mg/litre. In well-water, iron concentrations below 0.3 mg/litre were characterized as unnoticeable, whereas levels of 0.3–3 mg/litre were found acceptable (E. Dahi, personal communication, 1991).

In drinking-water supplies, iron(II) salts are unstable and are precipitated as insoluble iron(III) hydroxide, which settles out as a rust-coloured silt. Anaerobic groundwaters may contain iron(II) at concentrations of up to several milligrams per litre without discoloration or turbidity in the water when pumped directly from a well, although turbidity and colour may develop in piped systems at iron levels above 0.05–0.1 mg/litre. Staining of laundry and plumbing may occur at concentrations above 0.3 mg/litre (4).

Iron also promotes undesirable bacterial growth ("iron bacteria") in waterworks and distribution systems, resulting in the deposition of a slimy coating on the piping (4).

**Major uses**

Iron is used as constructional material, *inter alia* for drinking-water pipes. Iron oxides are used as pigments in paints and plastics. Other compounds are used as food colours and for the treatment of iron deficiency in humans. Various iron salts are used as coagulants in water treatment.

**Environmental fate**

Aeration of iron-containing layers in the soil can affect the quality of both groundwater and surface water if the groundwater table is lowered or nitrate leaching takes place. Dissolution of iron can occur as a result of oxidation and decrease in pH.

13.18.2 Analytical methods

Iron in water can be determined by atomic absorption spectrometry (detection limit 1 μg/litre) or by colorimetric methods (detection limit 5 μg/litre) (5).

13.18.3 Environmental levels and human exposure

**Air**

In remote areas, iron levels in air are about 50–90 ng/m^3; at urban sites, levels are about 1.3 μg/m^3. Concentrations up to 12 μg/m^3 have been reported in the vicinity of iron- and steel-producing plants (6).
GUIDELINES FOR DRINKING-WATER QUALITY

**Water**

The median iron concentration in rivers has been reported to be 0.7 mg/litre. In anaerobic groundwater where iron is present in the form of iron(II), concentrations will usually be 0.5-10 mg/litre, but concentrations up to 50 mg/litre can sometimes be found (6). Concentrations of iron in drinking-water are normally less than 0.3 mg/litre but may be higher in countries where various iron salts are used as coagulating agents in water-treatment plants and where cast iron, steel, and galvanized iron pipes are used for water distribution.

**Food**

Iron occurs as a natural constituent in plants and animals. Liver, kidney, fish, and green vegetables contain 20-150 mg/kg, whereas red meats and egg yolks contain 10-20 mg/kg. Rice and many fruits and vegetables have low iron contents (1-10 mg/kg).

**Estimated total exposure and relative contribution of drinking-water**

Reported daily intakes of iron in food — the major source of exposure — range from 10 to 14 mg (7, 8). Drinking-water containing 0.3 mg/litre would contribute about 0.6 mg to the daily intake. Intake of iron from air is about 25 μg/day in urban areas.

13.18.4 Kinetics and metabolism in humans

Iron is an essential trace element in living organisms. The data in this section are derived from studies in humans only; laboratory animals are not acceptable models because they have much higher intakes than humans and do not absorb iron compounds in the same way (6).

Most iron is absorbed in the duodenum and upper jejunum (9). Absorption depends on the individual’s iron status and is regulated so that excessive amounts of iron are not stored in the body (10). Total body iron in adult males and females is usually about 50 and 34-42 mg/kg of body weight, respectively (10). The largest fraction is present as haemoglobin, myoglobin, and haem-containing enzymes. The other major fraction is stored in the body as ferritin and haemosiderin, mainly in the spleen, liver, bone marrow, and striated muscle (6).

Daily losses of iron in adults are small (1 mg/day) and due mainly to cell exfoliation. About two-thirds of this loss occurs from the gastrointestinal tract and most of the remainder from the skin. Iron losses in urine and sweat are negligible (11). In adult females, there is an additional iron loss of about 15-70 mg each month in menstrual blood (12).
13.18.5 Effects on laboratory animals and *in vitro* test systems

**Acute exposure**

Wide variations in toxicity have been reported for different iron salts and animal species. Oral LD$_{50}$s for iron salts are about 300–600 mg/kg of body weight in the mouse and about 800–2000 mg/kg of body weight in the rat (13). The effects of toxic doses of iron include depression, rapid and shallow respiration, coma, convulsions, respiratory failure, and cardiac arrest.

**Reproductive toxicity, embryotoxicity, and teratogenicity**

Iron compounds were not teratogenic in the chicken embryo test (14). In a study of iron(II) sulfate and iron(III) sodium diphosphate in mice and rats, neither maternal toxicity nor teratogenic effects were found (14, 15). In an eight-generation reproduction study in rats, iron oxide was not toxic at an estimated intake of 25 mg of iron per day, and reproductive performance was better than expected (14, 15). In a five-generation study, iron dextran administered by intramuscular injection had no effect on litter size or growth (16).

**Mutagenicity and related end-points**

A number of iron(II) and iron(III) salts have been tested for mutagenicity in *Saccharomyces cerevisiae* strain D-4 and *Salmonella typhimurium* strains TA1535, TA1537, and TA1538, with and without metabolic activation. Iron(II) lactate, iron(III) diphosphate, iron(III) orthophosphate, and iron(III) sodium diphosphate were inactive in all systems used. Iron(II) sulfate was active in the suspension tests with activation. Iron(II) gluconate was mutagenic for indicator strain TA1538 in activation tests with primate liver preparations (14). Iron dextran did not induce chromosomal aberrations in human leukocyte cultures (17).

**Carcinogenicity**

Iron dextran complex repeatedly injected subcutaneously or intramuscularly was considered by IARC to be carcinogenic to animals (18).

13.18.6 Effects on humans

Iron is an essential element in human nutrition. Estimates of the minimum daily requirement for iron depend on age, sex, physiological status, and iron bioavailability and range from about 10 to 50 mg/day (12). The average lethal dose of iron is 200–250 mg/kg of body weight, but death has occurred following the ingestion of doses as low as 40 mg/kg of body weight (6). Autopsies have shown haemorrhagic necrosis and sloughing of areas of mu-
cosa in the stomach with extension into the submucosa. Chronic iron overload results primarily from a genetic disorder (haemochromatosis) characterized by increased iron absorption and from diseases that require frequent transfusions (10). Adults have often taken iron supplements for extended periods without detrimental effects (10), and an intake of 0.4–1 mg/kg of body weight per day is unlikely to cause adverse effects in healthy persons (19).

13.18.7 Conclusions

Anaerobic groundwaters may contain iron(II) at concentrations up to several milligrams per litre without discoloration or turbidity in the water when directly pumped from a well. Taste is not usually noticeable at iron concentrations below 0.3 mg/litre, although turbidity and colour may develop in piped systems at levels above 0.05–0.1 mg/litre. Laundry and sanitary ware will stain at iron concentrations above 0.3 mg/litre.

Iron is an essential element in human nutrition. Estimates of the minimum daily requirement for iron depend on age, sex, physiological status, and iron bioavailability and range from about 10 to 50 mg/day.

As a precaution against storage of excessive iron in the body, JECFA established a provisional maximum tolerable daily intake (PMTDI) in 1983 of 0.8 mg/kg of body weight (14), which applies to iron from all sources except for iron oxides used as colouring agents, and iron supplements taken during pregnancy and lactation or for specific clinical requirements. Allocation of 10% of this PMTDI to drinking-water gives a value of about 2 mg/litre, which does not present a hazard to health. The taste and appearance of drinking-water will usually be affected below this level, although iron concentrations of 1–3 mg/litre can be acceptable for people drinking anaerobic well-water.

No health-based guideline value for iron is proposed.

References


13.19 Lead

13.19.1 General description

**Identity**

Lead is the commonest of the heavy elements, accounting for 13 mg/kg of the earth's crust. Several stable isotopes of lead exist in nature, including, in order of abundance, $^{206}$Pb, $^{207}$Pb, $^{208}$Pb, and $^{204}$Pb.

**Physicochemical properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical state</td>
<td>Soft metal</td>
</tr>
<tr>
<td>Melting point</td>
<td>327 °C</td>
</tr>
</tbody>
</table>

**Major uses**

Lead is used in the production of lead acid batteries, solder, alloys, cable sheathing, pigments, rust inhibitors, ammunition, glazes, and plastic stabilizers (1). Tetraethyl and tetramethyl lead are important because of their extensive use as antiknock compounds in petrol, but their use for this purpose has been almost completely phased out in North America and western Europe, though not in eastern Europe or many developing countries. From a drinking-water perspective, the almost universal use of lead compounds in plumbing fittings and as solder in water-distribution systems is important. Lead pipes may be used in older distribution systems and plumbing (2).

13.19.2 Analytical methods

Atomic absorption spectrometry and anodic stripping voltammetry are the methods most frequently used for determining the levels of lead in environmental and biological materials. Detection limits of less than 1 µg/litre can be achieved by means of atomic absorption spectrometry (3). Because corrosion of plumbing systems is an important source of excessive lead in drinking-water, lead levels in water should be measured at the tap, rather than at the drinking-water source, when estimating human exposure.

13.19.3 Environmental levels and human exposure

**Air**

Concentrations of lead in air depend on a number of factors, including proximity to roads and point sources. Annual geometric mean concentrations measured at more than 100 stations across Canada declined steadily from 0.74 µg/m$^3$ in 1973 to 0.10 µg/m$^3$ in 1989 (4, 5), reflecting the decrease in the use of lead ad-
drives in petrol. Typical quarterly averages for urban areas without significant point sources in the USA in 1987 were in the range 0.1-0.3 µg/m³; in the vicinity of major point sources, such as lead smelters and battery plants, air levels typically ranged from 0.3 to 4.0 µg/m³ (6). Levels at three locations in Barcelona (Spain) during the winter of 1985 ranged from 0.9 to 2.5 µg/m³ (7), presumably reflecting heavy use of leaded petrol. The overall means in London and in a rural area of Suffolk in 1984-85 were 0.50 µg/m³ (range 0.23-0.82) and 0.10 µg/m³ (range 0.05-0.17), respectively (8). Levels of lead in 1983 in the Norwegian Arctic, an area remote from urban influences, varied between 0.1-0.3 and 0.3-9.0 ng/m³ (9).

If an average concentration in air of 0.2 µg/m³ is assumed, the intake of lead from air can be calculated to range from 0.5 µg/day for an infant to 4 µg/day for an adult.

**Water**

With the decline in atmospheric emissions of lead since the introduction of legislation restricting its use in fuels, water has assumed new importance as the largest controllable source of lead exposure in the USA (10).

Lead is present in tapwater to some extent as a result of its dissolution from natural sources but primarily from household plumbing systems in which the pipes, solder, fittings, or service connections to homes contain lead. PVC pipes also contain lead compounds that can be leached from them and result in high lead concentrations in drinking-water. The amount of lead dissolved from the plumbing system depends on several factors, including the presence of chloride and dissolved oxygen, pH, temperature, water hardness, and standing time of the water, soft, acidic water being the most highly plumbosolvent (11, 12). Although lead can be leached from lead piping indefinitely, it appears that the leaching of lead from soldered joints and brass taps decreases with time (10). Soldered connections in recently built homes fitted with copper piping can release enough lead (210-390 µg/litre) to cause intoxication in children (13). The level of lead in drinking-water may be reduced by corrosion-control measures such as the addition of lime and the adjustment of the pH in the distribution system from <7 to 8-9 (14,15).

In 1988, it was estimated that a lead level of 5 µg/litre was exceeded in only 1.1% of public water-distribution systems in the USA (16). A more recent review of lead levels in drinking-water in the USA found the geometric mean to be 2.8 µg/litre (10). The median level of lead in drinking-water samples collected in five Canadian cities was 2.0 µg/litre (17). A recent study in Ontario (Canada) found that the average concentration of lead in water actually consumed over a one-week sampling period was in the range 1.1-30.7 µg/litre, with a median level of 4.8 µg/litre (18). In the United Kingdom in 1975-76, there was virtually no lead in the drinking-water in two-thirds of households, but in 10% of homes in England and 33% in Scotland levels were above 50 µg/litre (2). In Glasgow
GUIDELINES FOR DRINKING-WATER QUALITY

(Scotland), where the water was known to be plumbosolvent, the lead concentration in about 40% of the samples exceeded 100 μg/litre (19).

If a concentration of 5 μg/litre in drinking-water is assumed, the total intake of lead from this source can be calculated to range from 3.8 μg/day for an infant to 10 μg/day for an adult.

Food

Prepared food contains small but significant amounts of lead. The lead content is increased when the water used for cooking or the cooking utensils contain lead, or the food, especially if acidic, has been stored in lead-ceramic pottery ware or lead-soldered cans. The intake of lead from lead-soldered cans is declining as the use of lead-free solders becomes more widespread in the food processing industry (2, 20).

A number of estimates based on figures for per capita consumption have been made of the daily dietary lead intake, e.g. 27 μg/day in Sweden (21); 66 μg/day in Finland (22); and 23 μg/day for a 2-year-old in the USA (23). Estimates obtained from duplicate diet studies are in the same range and include a mean dietary intake for all food and drink of about 40 μg/day for mothers and 30 μg/day for children aged 5–7 years in England (8) and 53.8 μg/day (0.8 μg/kg of body weight per day) for the intake of lead from food for adolescents and adults in Canada (17). Lead intakes for adults were 90 μg/day in Belgium, 24 μg/day in Sweden, and 177 μg/day in Mexico, based on faecal monitoring of lead (24). In some countries, dietary intakes as high as 500 μg/day have been reported (20). The regular consumption of wine can also result in a significant increase in lead intake; an average level of 73 μg/litre has been reported (25).

Other routes of exposure

Soil and household dust are significant sources of lead exposure for small children (6, 26, 27), but the levels are highly variable, ranging from <5 μg/g to tens of milligrams per gram in contaminated areas. As lead is immobile, levels in contaminated soils will remain essentially unchanged unless action is taken to decontaminate them (28). The highest lead concentrations usually occur in surface soil at depths of 1–5 cm.

In a 2-year study in England during 1984 and 1985, the geometric mean concentrations of lead in road dust collected in the vicinity of two London schools and in a rural area were 1552–1881 and 83–144 μg/g, respectively. For household dusts in London and in a rural area of Suffolk for 3 consecutive years (1983–85) the geometric mean concentrations were 857 and 333 μg/g, respectively (8). Household dust concentrations were 332 μg/g in an Edinburgh study (29) and 424 μg/g in one in Birmingham (30).

The amount of soil ingested by children aged 1–3 years is about 40–55 mg/day (27, 31, 32). A comprehensive study of a group of 2-year-old urban chil-
Children indicated an intake of lead from dust of 42 μg/day, almost twice the dietary lead intake (30). Studies in inner-city areas in the USA have shown that peeling paint or dust originating from leaded paint during removal may contribute significantly to children's exposure to lead (33).

**Estimated total exposure and relative contribution of drinking-water**

More than 80% of the daily intake of lead is derived from the ingestion of food, dirt, and dust. At 5 μg/litre, the average daily intake of lead from water forms a relatively small proportion of the total daily intake for children and adults but a significant one for bottle-fed infants. Such estimates have a wide margin of error, as it is not known to what extent the general public flushes the system before using tapwater; in addition, the stagnation time (and hence the lead level) is highly variable (10). The contribution of ingested dust and dirt to the total intake is known to vary with age, peaking around 2 years (32).

13.19.4 Kinetics and metabolism in laboratory animals and humans

Adults absorb approximately 10% of the lead contained in food (6), but young children absorb 4-5 times as much (34, 35); the gastrointestinal absorption of lead from ingested soil and dust by children has been estimated to be close to 30% (26). Absorption is increased when the dietary intakes of iron or calcium and phosphorus are low (36–38). Iron status is particularly important, as children from disadvantaged homes are more likely to suffer from anaemia, further increasing their absorption of lead (39).

The principal vehicle for the transport of lead from the intestine to the various body tissues is the red blood cell (40), in which lead is bound primarily to haemoglobin and has a special affinity for the beta, delta and, in particular, fetal gamma chains (41). Following its absorption, lead appears both in a soft tissue pool consisting of the blood, liver, lungs, spleen, kidneys, and bone marrow, which is rapidly turned over, and in a more slowly turned over skeletal pool. The half-life of lead in blood and soft tissues is about 36-40 days for adults (42), so that blood lead concentrations reflect only the intake of the previous 3-5 weeks. In the skeletal pool, the half-life of lead is approximately 17-27 years (42, 43). In adults, some 80-95% of the total body burden of lead is found in the skeleton, as compared with about 73% in children (44, 45). The biological half-life of lead may be considerably longer in children than in adults (46). Under conditions of extended chronic exposure, a steady-state distribution of lead between various organs and systems usually exists (6), and the blood lead concentration can therefore be used as a reasonably good indicator of exposure from all sources (47); the relationship between them is generally thought to be curvilinear in character (2, 19).
Placental transfer of lead occurs in humans as early as week 12 of gestation, and uptake of lead by the fetus continues throughout development (48). The concentration of lead in umbilical cord blood is 80–100% of the maternal blood lead level; the same applies to blood lead in the fetus (49–52).

Inorganic lead is not metabolized in the body. Unabsorbed dietary lead is eliminated in the faeces, and lead that is absorbed but not retained is excreted unchanged via the kidneys or through the biliary tract (53). Metabolic balance studies in infants and young children indicated that, at intakes greater than 5 μg/kg of body weight per day, net retention of lead averaged 32% of intake, whereas retention was negative (i.e. excretion exceeded intake) at intakes less than 4 μg/kg of body weight per day (35). No increases in blood lead were observed in infants with low exposure to other sources of lead and mean dietary intakes of 3–4 μg/kg of body weight per day (54), thus confirming the metabolic data.

13.19.5 Effects on laboratory animals and in vitro test systems

Neurological effects

Research on young primates has demonstrated that exposure to lead results in significant behavioural and cognitive deficits, e.g. impairment of activity, attention, adaptability, learning ability, and memory, as well as increased distractibility. Such effects have been observed following postnatal exposure of monkeys to lead for 29 weeks in amounts resulting in blood lead levels ranging from 10.9 to 33 μg/dl (55). These effects persisted into early adulthood, even after levels in the blood had returned to 11–13 μg/dl, and were maintained for the following 8–9 years (56). Studies on small groups of monkeys dosed continuously from birth onwards with 50 or 100 μg/kg of body weight per day showed that at 7–8 years of age there were still significant deficits in both short-term memory and spatial learning (57).

Reproductive toxicity, embryotoxicity, and teratogenicity

Effects on sperm counts and on the testicles (testicular atrophy) in male rats and on estrous cycles in female rats have been observed at blood lead levels above 30 μg/100 ml (58, 59).

Mutagenicity and related end-points

Results of studies on the genotoxicity of lead are conflicting (54, 60–62), but most suggest that some lead salts are genotoxic. Lead chloride, ethanoate, oxide, and tetroxide were inactive in mutagenicity tests on a number of prokaryotes and fungi, including Salmonella typhimurium and Saccharomyces cerevisiae. In vitro tests on human cells were positive for chromosomal damage in one case and negative in two others. In vivo short-term tests on mice, rats, cattle, and monkeys
were positive in three cases (dominant lethal test and chromosome damage to bone marrow cells) but negative in five others (60, 61).

**Carcinogenicity**

Renal tumours have been induced in rats, mice, and hamsters exposed orally to high levels of lead ethanoate, subacetate, or phosphate in the diet. In one study, 5, 18, 62, 141, 500, 1000, or 2000 mg of lead per kg of diet (about 0.3, 0.9, 3, 7, 27, 56, and 105 mg/kg of body weight per day) were fed to rats for 2 years. Renal tumours (mostly tubular epithelial adenomas) developed in male rats at 500, 1000, and 2000 mg/kg, but only at 2000 mg/kg in female rats (53, 62, 63).

13.19.6 Effects on humans

Lead is a cumulative general poison, infants, children up to 6 years of age, the fetus, and pregnant women being the most susceptible to adverse health effects. Its effects on the central nervous system can be particularly serious.

**Acute and long-term exposure**

Overt signs of acute intoxication include dullness, restlessness, irritability, poor attention span, headaches, muscle tremor, abdominal cramps, kidney damage, hallucinations, and loss of memory, encephalopathy occurring at blood lead levels of 100–120 μg/dl in adults and 80–100 μg/dl in children. Signs of chronic lead toxicity, including tiredness, sleeplessness, irritability, headaches, joint pains, and gastrointestinal symptoms, may appear in adults at blood lead levels of 50–80 μg/dl. After 1–2 years of exposure, muscle weakness, gastrointestinal symptoms, lower scores on psychometric tests, disturbances in mood, and symptoms of peripheral neuropathy were observed in occupationally exposed populations at blood lead levels of 40–60 μg/dl (6).

Renal disease has long been associated with lead poisoning; however, chronic nephropathy in adults and children has not been detected below blood lead levels of 40 μg/dl (64, 65). Damage to the kidneys includes acute proximal tubular dysfunction and is characterized by the appearance of prominent inclusion bodies of a lead–protein complex in the proximal tubular epithelial cells at blood lead concentrations of 40–80 μg/dl (66).

There are indications of increased hypertension at blood lead levels greater than 37 μg/dl (67). A significant association has been established, without evidence of a threshold, between blood lead levels in the range 7–34 μg/dl and high diastolic blood pressure in people aged 21–55, based on data from the second US National Health and Nutrition Examination Survey (NHANES II) (68, 69). The significance of these results has been questioned (70).

Lead interferes with the activity of several of the major enzymes involved in the biosynthesis of haem (6). The only clinically well-defined symptom associat-
ed with the inhibition of haem biosynthesis is anaemia (40), which occurs only at blood lead levels in excess of 40 µg/dl in children and 50 µg/dl in adults (71). Lead-induced anaemia is the result of two separate processes: the inhibition of haem synthesis and an acceleration of erythrocyte destruction (40). Enzymes involved in the synthesis of haem include δ-aminolaevulinate synthetase (whose activity is indirectly induced by feedback inhibition, resulting in accumulation of δ-aminolaevulinate, a neurotoxin) and δ-aminolaevulnic acid dehydratase (δ-ALAD), coproporphyrinogen oxidase, and ferrochelatase, all of whose activities are inhibited (6, 40). The activity of δ-ALAD is a good predictor of exposure at both environmental and industrial levels, and inhibition of its activity in children has been noted at a blood lead level as low as 5 µg/dl (72); however, no adverse health effects are associated with its inhibition at this level.

Inhibition of ferrochelatase by lead results in an accumulation of erythrocyte protoporphyrin (EP), which indicates mitochondrial injury (47). NOAELs for increases in EP levels in infants and children exist at about 15–17 µg/dl (73–75). In adults, the NOAEL for increases in EP levels ranged from 25 to 30 µg/dl (76); for females alone, the NOAEL ranged from 20 to 25 µg/dl, which is closer to that observed for children (74, 77, 78). Changes in growth patterns in infants younger than 42 months of age have been associated with increased levels of EP; persistent increases in levels led initially to a rapid gain in weight but subsequently to a retardation of growth (79). An analysis of the NHANES II data showed a highly significant negative correlation between the stature of children aged 7 years and younger and blood lead levels in the range 5–35 µg/dl (80).

Lead has also been shown to interfere with calcium metabolism, both directly and by interfering with the haem-mediated generation of the vitamin D precursor 1,25-dihydroxycholecalciferol. A significant decrease in the level of circulating 1,25-dihydroxycholecalciferol has been demonstrated in children whose blood lead levels were in the range 12–120 µg/dl, with no evidence of a threshold (81, 82). Tissue lead content is increased in calcium-deficient persons, a fact that assumes great importance in the light of the increased sensitivity to lead exposure that could result from the calcium-deficient status of pregnant women. It has also been demonstrated that interactions between calcium and lead were responsible for a significant portion of the variance in the scores on general intelligence ratings, and that calcium influenced the deleterious effect of lead (83). The regulatory enzyme brain protein, kinase C, is stimulated in vitro by picomolar lead concentrations (an effect similar to that produced by micromolar calcium concentrations), levels that could be expected from environmental exposure (84).

Several lines of evidence demonstrate that both the central and peripheral nervous systems are the principal targets for lead toxicity. The effects include subencephalopathic neurological and behavioural effects in adults, and there is also electrophysiological evidence of effects on the nervous system of children at blood lead levels well below 30 µg/dl. Aberrant electroencephalograph readings were significantly correlated with blood levels down to 15 µg/dl (85, 86). Sig-
significant reductions in maximal motor nerve conduction velocity (MNCV) have been observed in children aged 5-9 years living near a smelter, with a threshold occurring at a blood lead level around 20 \( \mu g/dl \); a 2% decrease in the MNCV was seen for every 10 \( \mu g/dl \) increase in the blood lead level (87). The auditory nerve may be a target for lead toxicity, in view of reports of reduced hearing acuity in children (88). In the NHANES II survey in the USA, the association with blood lead was highly significant at all levels from 5 to 45 \( \mu g/dl \) for children 4-19 years old, with a 10-20% increased likelihood of an elevated hearing threshold for persons with a blood lead level of 20 \( \mu g/dl \) as compared with 4 \( \mu g/dl \) (89). The NHANES II data also showed that blood lead levels were significantly associated with the age at which infants first sat up, walked, and started to speak. Although no threshold existed for the age at which the child first walked, thresholds existed at the 29th and 28th percentile of lead rank for the age at which the child sat up and spoke, respectively (89).

**Reproductive effects**

Gonadal dysfunction in men, including depressed sperm counts, has been associated with blood lead levels of 40-50 \( \mu g/dl \) (90-93). Reproductive dysfunction may also occur in females occupationally exposed to lead (6, 61).

Epidemiological studies have shown that exposure of pregnant women to lead increases the risk of preterm delivery. In a study of 774 pregnant women in Port Pirie who were followed to the completion of their pregnancy, the relative risk of preterm delivery was more than four times higher among women with blood lead levels above 14 \( \mu g/dl \) than in those with 8 \( \mu g \) or less per dl (94).

Elevated cord blood lead levels were associated with minor malformations, such as angiomas, syndactylism, and hydrocele, in about 10% of all babies. The relative risk of malformation doubled at blood lead levels of about 7-10 \( \mu g/dl \), and the incidence of any defect increased with increasing cord lead levels over the range 0.7-35.1 \( \mu g/dl \) (95).

**Mutagenicity**

Cytogenetic studies in humans exposed to lead (blood lead levels > 40 \( \mu g/dl \)) have given conflicting results; chromatid and chromosomal aberrations, breaks, and gaps were reported in 9 of 16 studies but not in the remainder (60, 61).

**Carcinogenicity**

The carcinogenicity of lead in humans has been examined in several epidemiological studies, which either have been negative or have shown only very small excess mortalities from cancers. In most of these studies, there were either concurrent exposures to other carcinogenic agents or other confounding factors such as smoking that were not considered (60, 61). A study on 700 smelter workers
(mean blood level 79.7 μg/litre) and battery factory workers (mean blood level 62.7 μg/litre) indicated an excess of deaths from cancer of the digestive and respiratory systems (96), the significance of which has been debated (97, 98). There was also a nonsignificant increase in urinary tract tumours in production workers. In a study on lead smelter workers in Australia, no significant increase in cancers was seen, but there was a substantial excess of deaths from chronic renal disease (99). IARC considers that the overall evidence for carcinogenicity in humans is inadequate (60).

**Neurological effects in infants and children**

A number of cross-sectional and longitudinal epidemiological studies have been designed to investigate the possible detrimental effects that exposure of young children to lead might have on their intellectual abilities and behaviour. These studies have been concerned with documenting effects arising from exposure to "low" levels of lead (i.e. blood lead < 40 μg/dl), at which overt clinical symptoms are absent. Several factors affect the validity of the conclusions drawn from them (100), including the statistical power of the study, the effect of bias in the selection of study and control populations, the choice of parameter used to evaluate lead exposure, the temporal relationship between exposure measurement and psychological evaluations, the extent to which the neurological and behavioural tests used can be quantified accurately and reproducibly, which confounding covariates are included in any multiple regression analysis, and the effect of various nutritional and dietary factors, such as iron and calcium intake (39).

**Cross-sectional studies**

A number of cross-sectional studies have been carried out in which many of the above factors were taken into account. In one such study in the USA, a group of 58 children aged 6–7 years with "high" dentine lead levels (corresponding to a blood lead level of approximately 30–50 μg/dl) performed significantly less well than 100 children from a "low" lead group (mean blood lead level 24 μg/dl). The children's performance was measured using the Wechsler intelligence test in addition to other visual and auditory tests and teachers' behavioural ratings (101). There was a significant difference of 4 points and a uniform downward shift in IQ scores. Although this study found that a child in the group with "high" dentine lead was three times more likely to have an IQ of 80 or lower than one in the "low" lead group, it was claimed in a 1986 review that the effect was statistically significant only for children with the highest lead levels in dentine (blood lead > 40 μg/dl) (6).

A similar study in which lead in dentine was used as the indicator of exposure was carried out on a cohort of 400 children in the United Kingdom (102). There were several consistent but nonsignificant differences between the high- and low-lead groups similar to those observed in the American study, including
IQ decrements of about 2 points and poorer scores in behaviour indices. In the British study, mean blood lead levels in the "high" exposure group (15.1 µg/dl) were lower than the mean of the "low" group (24 µg/dl) in the American study, which may explain why the results lacked statistical significance. The results of studies on children in Germany (103-105) were similar to those of the British study, in that the effect of lead on behaviour was only of borderline significance.

In another study (106) involving 500 Edinburgh schoolchildren aged 6–9 years, a small (up to 5 points in the British Ability Scales) but significant negative relationship was found between blood lead levels and intelligence scores, reading skills, and number skills. There was a dose–response relationship in the range 5.6–22.1 µg/dl. The effect of lead was small compared with that of several of the other 33 variables considered. A series of studies (107-109) on about 800 children in the United Kingdom with blood lead levels between 4 and 32 µg/dl failed to find any significant associations between lead and indices of intelligence and behaviour after socioeconomic and family characteristics were taken into account. It was suggested that lead might have a noticeable effect only when other factors predisposing to social disadvantage (particularly low socioeconomic status or poor home environment) are present (108-110).

In a cross-sectional study in Lavrion (Greece) involving 509 primary schoolchildren living near a lead smelter, blood lead levels between 7.4 and 63.9 µg/dl (mean 23.7 µg/dl) were recorded (111). When the IQ was measured by means of the revised Wechsler Intelligence Scale for Children and due account taken of 17 potential confounders, a significant association was found between blood lead levels and IQ, with a threshold at about 25 µg/dl. Attentional performance was also associated with blood lead levels in two different tests, but no threshold level was found. This study was part of a multicentre collaborative international study on schoolchildren sponsored by WHO and the Commission of the European Communities (112). A more or less uniform protocol was used, and quality assurance procedures were applied to the exposure analyses. The most consistent associations were for visual–motor integration as measured by the Bender Gestalt test and for reaction performance as measured by the Vienna Reaction Device. The results of many of the remaining tests were inconsistent. The degree of association between lead exposure and outcome was very weak (<0.8%), even in the statistically significant cases.

The cross-sectional studies are, on balance, consistent in demonstrating statistically significant associations between blood lead levels of 30 µg/dl or more and IQ deficits of about 4 points. Although there were associations between lower blood lead levels and IQ deficits of about 2 points, these were only marginally statistically significant, except in the Edinburgh study. It is particularly difficult to determine minimum levels above which significant effects occur.
Longitudinal studies

Longitudinal studies have the advantage as compared with cross-sectional studies that more precise estimates of exposure can be made; in addition, the reversibility of the effects and the temporal sequence of causality can be investigated. However, such studies also have certain disadvantages; for example, repeated psychometric testing may lead to artefactual results, and there may also be problems of bias associated with attrition within the study population.

The possible relationship between low-level lead exposure during the fetal period and in early childhood and later effects on infant and child development has been investigated in at least six prospective studies, in the USA (Boston, Cincinnati, and Cleveland), Australia (Port Pirie, Sydney), and Scotland (Glasgow). Broadly similar methodologies were used in all the studies to facilitate comparisons. The Bayley Scales of Infant Development or subsets of this test were used to evaluate early cognitive development in verbal and performance skills in infants and young children, whereas the McCarthy Scales of Children's Abilities (MSCA) were used in most studies on older children. In all the studies, except that in Glasgow, the average maternal and cord blood lead concentrations were less than 10 μg/dl (range 6.0–9.5 μg/dl).

In the Boston Lead Study, three groups of infants and young children were classified according to umbilical cord blood lead concentrations, the levels in the low-, middle-, and high-lead groups being < 3, 6–7, and 10–25 μg/dl (mean 14.6 μg/dl), respectively. Children were tested twice a year from age 6 months to almost 5 years (113, 114). After controlling for 12 potential confounders, a significant inverse relationship was demonstrated between fetal exposure, measured as lead levels in cord blood, and mental development at age 2, as measured by the Bayley Mental Development Index (MDI). There was no significant correlation with the children's current blood lead levels, all of which were less than 8.8 μg/dl. However, the results of testing at almost 5 years, using the McCarthy Scales, showed an attenuation of this association. At 57 months, only the association between intelligence scores and blood lead 3 years previously, at age 2, remained significant after controlling for confounding variables (114).

In a longitudinal study involving 305 pregnant women in Cincinnati (115), an inverse relationship was found between either prenatal or neonatal blood lead levels and performance in terms both of the Bayley Psychomotor Development Index (PDI) and the Bayley MDI at the ages of 3 and 6 months for both male infants and infants from the poorest families. The mean blood lead levels for neonates and their mothers were 4.6 and 8.2 μg/dl, respectively, and all blood lead levels were below 30 μg/dl. Multiple regression analysis for boys only showed that, for every increment of 1 μg/dl in the prenatal blood lead level, the covariate-adjusted Bayley MDI at 6 months of age decreased by 0.84 points. The inverse relationship between MDI and prenatal blood lead disappeared at age 1, because it was accounted for, and mediated through, the effect of lead on birth weight; however, the Bayley PDI was still significantly related to maternal blood lead (116).
In a prospective study of design similar to that of the Boston study, undertaken at Port Pirie, a lead smelter town in Australia, 537 children were studied from birth to 4 years (117). The cohort was divided into four groups on the basis of maternal and umbilical blood lead, which ranged from a geometric mean of 0.21 to 0.72 μmol/litre (4.3–14.9 μg/dl). The mean blood lead level varied from 9.1 μg/dl at mid-pregnancy to 21.3 and 19 μg/dl at 2 and 4 years, respectively. The integrated postnatal average blood lead level was 19.1 μg/dl. At 6, 15, 24, and 36 months, the developmental status of the child was assessed by means of the Bayley MDI; the MSCA were used at 4 years. At each age, a consistent but weak inverse relationship was found between concurrent postnatal blood lead levels and MSCA scores; no allowance was made for possible confounding factors. No such relationship was found for perinatal blood lead. After 18 covariates considered to be potential confounders were incorporated in the multivariate analysis, the integrated blood lead level showed the strongest inverse relation with the General Cognitive Index (GCI) score (a subset of the McCarthy Scales) at age 4 years, which suggests that the detrimental effect of lead on child development is cumulative during early childhood. Repeated analysis restricted to children whose blood lead levels were below 25 μg/dl showed that the inverse relationship with the GCI score was as strong for this group as for the cohort as a whole, thus demonstrating the absence of a clear threshold below which a detrimental effect of lead on child development does not occur.

A number of prospective studies have failed to show any consistent association between mental development and blood lead, either during the perinatal period or in early childhood. In a study carried out on extremely socially disadvantaged mothers and infants in Cleveland, Ohio (USA), no relationship was found between blood lead at any time and language development, MDI, or the results of the Stanford-Binet IQ test at age 3 years, after confounding factors, the most important of which was the care-giving environment, were taken into account. In this cohort, half the mothers had alcohol-related problems, and the average maternal IQ was 79 (118). In a second Australian study carried out in Sydney on a relatively prosperous population of 318 mothers and children, no association was found between blood lead in the mother or the child at any age and mental or motor deficits at age 4 years, after account was taken of six covariates, including the HOME score (a measure of the care-giving environment) (119). A third negative study was that carried out in Glasgow (Scotland), where the primary exposure was to high lead levels in water which were dramatically reduced by corrosion-control measures shortly after the children were born. The cohort was divided into high, medium, and low groups, on the basis of maternal blood lead, with means of 33.1, 17.7, and 7.0 μg/dl, respectively. Although the expected decrements in scores in the Bayley MDI and PDI were observed at ages 1 and 2 years as lead exposure increased, they could be better accounted for by birth weight, home environment, and socioeconomic status, as shown by stepwise multiple regression analysis (120).
The results of the prospective studies have been somewhat disappointing because of the inconsistency between studies. It appears that prenatal exposure may have early effects on mental development, but that these do not persist up to age 4, at least not as shown by the tests so far used. There are indications that these early effects may be mediated through birth weight or other factors. Several studies have indicated that the generally higher exposures of children in the 18–36-month age range may be negatively associated with mental development, but this, too, has not been confirmed by other studies.

13.19.7 Guideline value

The evidence for the carcinogenicity of lead in humans is inconclusive because of the limited number of studies, the small cohort sizes, and the failure to take adequate account of potential confounding variables. However, an association has been demonstrated experimentally between the ingestion of lead salts and renal tumours. Lead and inorganic lead compounds have therefore been placed in Group 2B of the IARC classification, namely possible human carcinogen (evidence inadequate in humans, sufficient in animals) (60).

As there is evidence from human studies that adverse effects other than cancer may occur at very low lead levels, and that a guideline thus derived would also be protective for carcinogenic effects, it is considered appropriate to derive the guideline using the TDI approach.

In 1986, JECFA established a provisional tolerable weekly intake (PTWI) of 25 µg of lead per kg of body weight (equivalent to 3.5 µg/kg of body weight per day) for infants and children which took account of the fact that lead is a cumulative poison so that any increase in the body burden of lead should be avoided (71). The PTWI was based on metabolic studies in infants (35, 54) showing that a mean daily intake of 3–4 µg/kg of body weight was not associated with an increase in blood lead levels or in the body burden of lead, whereas an intake of 5 µg/kg of body weight or more resulted in lead retention. This PTWI was reconfirmed by JECFA in 1993 and extended to all age groups (121).

On the assumption of a 50% allocation to drinking-water for a 5-kg bottle-fed infant consuming 0.75 litres of drinking-water per day, the guideline value is 0.01 mg/litre. As infants are considered to be the most sensitive subgroup of the population, this guideline value will also be protective for other age groups.

Lead is exceptional in that most lead in drinking-water arises from plumbing in buildings and the remedy consists principally of removing plumbing and fittings containing it. This requires time and money, and it is recognized that not all water will meet the guideline immediately. Meanwhile, all other practical measures to reduce total exposure to lead, including corrosion control, should be implemented.
References


GUIDELINES FOR DRINKING-WATER QUALITY


65. Lilis R et al. Lead effects among secondary lead smelter workers with blood lead below 80 microgram/100 mL. *Archives of environmental health*, 1977, 32:256-266.


13.20 Manganese

13.20.1 General description

**Identity**

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS no.</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese</td>
<td>7439-96-5</td>
<td>Mn</td>
</tr>
<tr>
<td>Manganese(II) chloride</td>
<td>7773-01-5</td>
<td>MnCl₂</td>
</tr>
<tr>
<td>Trimanganese tetroxide</td>
<td>1317-35-7</td>
<td>Mn₃O₄</td>
</tr>
<tr>
<td>Manganese dioxide</td>
<td>1313-13-9</td>
<td>MnO₂</td>
</tr>
<tr>
<td>Potassium permanganate</td>
<td>7722-64-7</td>
<td>KMnO₄</td>
</tr>
</tbody>
</table>

Manganese, one of the more abundant metals in the earth's crust, usually occurs together with iron. The most environmentally important manganese compounds are those that contain Mn²⁺, Mn⁴⁺, and Mn⁷⁺.

**Physicochemical properties (1)**

<table>
<thead>
<tr>
<th>Property</th>
<th>Mn</th>
<th>MnCl₂</th>
<th>Mn₃O₄</th>
<th>MnO₂</th>
<th>KMnO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point (°C)</td>
<td>1244</td>
<td>650</td>
<td>1564</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Boiling point (°C)</td>
<td>1962</td>
<td>1190</td>
<td></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>7.20</td>
<td>2.97</td>
<td>4.86</td>
<td>5.03</td>
<td>2.70</td>
</tr>
<tr>
<td>Water solubility (g/litre)</td>
<td>Insoluble</td>
<td>723</td>
<td>Insoluble</td>
<td>Insoluble</td>
<td>63.8</td>
</tr>
</tbody>
</table>

**Organoleptic properties**

At concentrations exceeding 0.1 mg/litre, the manganese ion imparts an undesirable taste to beverages and stains plumbing fixtures and laundry (2). When manganese(II) compounds in solution undergo oxidation, manganese is precipitated, resulting in encrustation problems. Even at about 0.02 mg/litre, manganese will form coatings on piping that may later slough off as a black precipitate (3). In addition, certain nuisance organisms concentrate manganese and give rise to taste, odour, and turbidity problems in distributed water (2, 4).

**Major uses**

Manganese is used principally in the manufacture of iron, steel, and other alloys. Manganese dioxide and other manganese compounds are used in products such as batteries, glass, and fireworks. Potassium permanganate is used as an oxidant for cleaning, bleaching, and disinfection purposes (5).
Environmental fate

Elemental and inorganic forms of manganese may be present in the atmosphere as suspended particulates (6). In surface waters, manganese occurs in both dissolved and suspended forms. Anaerobic groundwater often contains elevated levels of dissolved manganese. The divalent form predominates in most water at pH 4–7, but more highly oxidized forms may occur at higher pH values or result from microbial oxidation (5). Manganese can be adsorbed onto soil to an extent depending on the organic content and cation exchange capacity of the latter. It can bioaccumulate in lower but not higher organisms, so that biomagnification in food-chains is not significant (1).

13.20.2 Analytical methods

Atomic absorption spectrophotometry is used for determining manganese concentrations in microlitre samples (7). Inductively coupled argon-plasma optical emission spectrometry has a detection limit of around 2 μg/litre for manganese (8). Colorimetric methods are also used in water analysis and have detection limits of about 10 μg/litre (9).

13.20.3 Environmental levels and human exposure

Air

Concentrations of manganese average 5 ng/m³ in the ambient air of nonindustrialized areas and up to 33 ng/m³ in industrialized areas. Source-dominated air levels may reach 0.13 μg/m³ or above (5).

Water

Manganese concentrations in lakes and rivers around the world range from 0.001 to about 0.6 mg/litre (6). Higher levels in aerobic waters are usually associated with industrial pollution. Reducing conditions in groundwater and some lakes and reservoirs are conducive to high levels: up to 1.3 mg/litre in neutral water and 9.6 mg/litre in acidic water (5). In the USA, in a number of public drinking-water surveys, mean manganese levels ranging from 0.004 to 0.03 mg/litre were reported (1, 5). In Germany, the drinking-water supplied to 90% of all households contained less than 0.02 mg of manganese per litre (10).

Food

Manganese was found in dairy products at levels of 0.02–0.5 mg/kg; levels in meats, fish, and eggs were in the range 0.1–4 mg/kg. Higher levels were found in

---

1 Also based on data from the National Water Quality Data Bank (NAQUADAT), Ottawa, Environment Canada, Inland Waters Directorate, 1976.
vegetables (0.41–6.61 mg/kg), grains and cereals (0.41–41 mg/kg), and nuts (18–47 mg/kg). A cup of tea can contain 0.4–1.3 mg of manganese (1).

**Estimated total exposure and relative contribution of drinking-water**

The greatest exposure to manganese is usually from food. Adults consume between 2 and 20 mg/day in the diet, the upper end of the range being associated with a vegetarian diet (11,12). The average daily manganese nutrient requirement for normal physiological function is estimated to be 2–5 mg for adults (13). Infants consume 2.5–25 µg/kg of body weight per day during the first 6 months of life (6).

Manganese intake from drinking-water is normally substantially lower than that from food. At typical drinking-water levels of 4–30 µg/litre, the intake of manganese would range from 8 to 60 µg/day for an adult. Other sources indicate that manganese intake from water can be an order of magnitude higher (5). Exposure to manganese from air is generally several orders of magnitude lower than that from the diet, about 0.1–3 µg, depending on the distance from the source.

13.20.4 Kinetics and metabolism in laboratory animals and humans

Absorption of manganese across the gastrointestinal tract is regulated by normal physiological processes that maintain manganese homoeostasis. Typically, about 3–8% of an ingested dose is absorbed (15), but absorption may be greater for young animals and infants (16). The absorption of manganese is intimately linked to that of iron, iron-deficient diets leading to an increased absorption of both iron and manganese (15). Absorption is inversely related to the level of calcium in the diet (11) and directly related to that of potassium (17).

Manganese is present in all tissues of the body, the highest levels usually being found in the liver, kidney, pancreas, and adrenals (18,19). It accumulates preferentially in certain regions of the brain in young animals and infants (20,21). Manganese can also be detected in human hair (22).

Manganese does not appear to be covalently linked to any organometallic compounds in the body. It may undergo changes in oxidation state (23). Manganese is a constituent of the enzymes pyruvate carboxylase and superoxide dismutase, is required as a cofactor in a number of enzyme systems, and plays a role in flavoprotein function and the synthesis of sulfated mucopolysaccharides, cholesterol, and haemoglobin (24,25).

Manganese is almost entirely excreted in the faeces, only a small proportion (0.1–2%) being eliminated in the urine. In humans, elimination is biphasic, with half-lives of 13 and 34 days (15,26).
13.20.5 Effects on laboratory animals and in vitro test systems

**Acute exposure**

Oral LD$_{50}$s ranging from 400 to 830 mg/kg of body weight have been reported for different forms of manganese (1, 5).

**Short-term exposure**

The central nervous system is the chief target for manganese. Doses ranging from 1 to 150 mg/kg of body weight per day produced a number of neurological effects in rats and mice, mainly involving alterations in neurotransmitter and enzyme levels in the brain. These changes were sometimes accompanied by clinical signs, such as incoordination and changes in activity level (1). Increased turnover of striatal catecholamines may be responsible for hyperactivity in early manganese intoxication (27).

**Long-term exposure**

Chronic ingestion of 1–2 mg of manganese per kg of body weight per day produced changes in appetite and reduction in haemoglobin synthesis in rabbits, pigs, and cattle (25). Transient effects on biogenic amine levels and activities of dopamine β-hydroxylase and monoamine oxidase in rat brain have been noted with long-term exposures to manganese (28–30). An increase in physical activity level and a transient increase in dopaminergic function were observed in rats given 40 mg/kg of body weight per day for 65 weeks (31). Weakness and rigidity were observed in monkeys given oral doses of 25 mg/kg of body weight per day for 18 months (32).

**Reproductive toxicity, embryotoxicity, and teratogenicity**

Several studies in rats and mice indicate that the ingestion of manganese can delay reproductive maturation in male animals. Testosterone levels were reduced in male rats given an oral dose of 13 mg of manganese per kg of body weight per day for 100–224 days (33), while delayed growth of the testes was observed in young rats ingesting 140 mg of manganese per kg of body weight per day for 90 days (34). These effects do not appear to be severe enough to affect sperm morphology or male reproductive function (33, 35, 36). In rats chronic parenteral administration of manganese produced marked degenerative changes in the seminiferous tubules, resulting in infertility (37).

**Mutagenicity and related end-points**

Manganese produced an increased frequency of mutations in *Salmonella typhimurium* strain TA1537, *Photobacterium fischeri*, and *Escherichia coli*, as well as in *Saccharomyces cerevisiae*, mouse lymphoma cells, and hamster embryo cells, in
both cases without metabolic activation. In *in vivo* assays, manganese increased both the frequency of mutations in *Drosophila melanogaster* and the number of chromosomal aberrations in rat bone marrow and spermatogonial cells (1).

**Carcinogenicity**

A 2-year oral study in rats and mice produced equivocal evidence of increased tumour incidence (35, 36). In male rats given oral manganese doses of 86, 290, or 930 mg/kg of body weight per day, the incidence of pancreatic cancer was slightly increased (4/50 for each dose, as compared with no tumours of this type in the control group) (35). Female mice given 810 mg/kg of body weight per day showed a small increase in pituitary adenomas, although the incidence of tumours of this type was within the range of historical control values (36).

Several studies in animals suggest that manganese may have an anticarcinogenic effect. It has been reported to inhibit the metabolic activation of aminoazodyes (38).

13.20.6 Effects on humans

Manganese is an essential element for many living organisms, including humans. Accordingly, adverse health effects can be caused by inadequate intake. Manganese-deficient animals exhibit impaired growth, skeletal abnormalities, reproductive deficits, ataxia of the newborn, and defects in lipid and carbohydrate metabolism (5, 6, 25). Although no specific manganese-deficiency syndrome has been described in humans, an association between manganese deficiency and disorders such as anaemia, bone changes in children, and lupus erythematosus has been suggested (39).

The neurological effects of inhaled manganese have been well documented in humans chronically exposed to elevated levels in the workplace. The syndrome known as "manganism" is characterized by weakness, anorexia, muscle pain, apathy, slow speech, monotonous tone of voice, emotionless "mask-like" facial expression, and slow clumsy movement of the limbs. In general, these effects are irreversible. The minimal exposure level producing neurological effects is not certain but is probably in the range 0.1–1 mg/m³ (1).

By the oral route, manganese is often regarded as one of the least toxic elements, although there is some controversy as to whether the neurological effects observed with inhalation exposures also occur with oral ones.

In 1941, in an epidemiological study in Japan, adverse effects were seen in humans consuming manganese dissolved in drinking-water, probably at a concentration close to 28 mg/litre (38). The manganese was derived from 400 dry-cell batteries buried near a drinking-water well. A total of 16 cases of poisoning were reported, the symptoms including lethargy, increased muscle tone, tremor, and mental disturbances. The most severe effects were seen in elderly people, but only minor ones in children. The concentrations of other metals, especially zinc,
were also excessive, and it was never unequivocally established whether manganese alone was responsible for the disease.

An epidemiological study was conducted in Greece to investigate the possible correlation between manganese exposure from water and neurological effects in elderly people (40). The levels of manganese were 3.6–14.6 µg/litre in the control area and 81–282 µg/litre and 1800–2300 µg/litre in the test areas. The authors concluded that progressive increases in the manganese concentration in drinking-water are associated with progressively higher prevalences of neurological signs of chronic manganese poisoning and higher manganese concentrations in the hair of older persons. However, no data were given on exposure from other sources such as food and dust, and little information was provided on nutritional status and other possible confounding variables.

In one area of Japan, a manganese concentration of 0.75 mg/litre in the drinking-water supply had no apparent adverse effects on the health of consumers (41). No signs of toxicity were observed in patients given 30 mg of manganese citrate (9 mg of manganese) per day for many months (11).

13.20.7 Provisional guideline value

The intake of manganese can be as high as 20 mg/day without apparent ill effects. With an intake of 12 mg/day, a 60-kg adult would receive 0.2 mg/kg of body weight per day. An uncertainty factor of 3 is applied to allow for the possible increased bioavailability of manganese from water, and 20% of the intake is allocated to water. This gives a value of 0.4 mg/litre.

Although no single study is suitable for use in calculating a guideline value, the weight of evidence from actual daily intake and from studies in laboratory animals given drinking-water in which neurotoxic and other effects were observed supports the view that a provisional health-based guideline value of 0.5 mg/litre should be adequate to protect public health.

It should be noted that manganese may be objectionable to consumers if it is deposited in water mains and causes water discoloration. Although concentrations below 0.1 mg/litre are usually acceptable to consumers, this may vary with local circumstances.

References


13. INORGANIC CONSTITUENTS AND PHYSICAL PARAMETERS


13.21 Mercury

13.21.1 General description

**Physicochemical properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical state</td>
<td>Dense, silvery-white metal; liquid at normal temperatures and pressures</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>0.16 Pa at 20 °C</td>
</tr>
<tr>
<td>Stability</td>
<td>Carbon–mercury bond in organic mercury compounds is chemically stable</td>
</tr>
</tbody>
</table>

**Major uses**

Mercury is used for the cathode in the electrolytic production of chlorine and caustic soda, in electrical appliances (lamps, arc rectifiers, mercury cells), in industrial and control instruments (switches, thermometers, barometers), in laboratory apparatus, in dental amalgams, and as a raw material for various mercury compounds. The latter are used as fungicides, antiseptics, preservatives, pharmaceuticals, electrodes, and reagents.

**Environmental fate**

The solubility of mercury compounds in water varies: elemental mercury vapour is insoluble, mercury(II) chloride is readily soluble, mercury(I) chloride much less soluble, and mercury sulfide has a very low solubility.

Methylation of inorganic mercury has been shown to occur in columns of fresh water and in seawater (1), and bacteria (*Pseudomonas* spp.) isolated from mucous material on the surface of fish and soil were able to methylate mercury under aerobic conditions. Some anaerobic bacteria that possess methane synthetase are also capable of mercury methylation (2). Once methylmercury \(^1\) is released from microbes, it enters the food chain as a consequence of rapid diffusion and tight binding to proteins in aquatic biota. The enzymology of CH\(_3\)Hg\(^+\) hydrolysis and mercury(II) ion reduction is now understood in some detail. Environmental levels of methylmercury depend on the balance between bacterial methylation and demethylation (3).

---

\(^1\) The generic term "methylmercury" is used throughout this text to refer to monomethylmercury compounds.
13.21.2 Analytical methods

Inorganic mercury is determined by flameless atomic absorption spectrometry (4). Cold vapour atomic absorption spectrometry and atomic fluorescence spectrometry have detection limits of 50 and 1 ng/litre, respectively.

Gas chromatography is commonly used for the determination of alkylmercury compounds. The neutron activation procedure is regarded as the most accurate and is generally used as the reference method (3).

13.21.3 Environmental levels and human exposure

Air

Mercury levels in air are in the range 2–10 ng/m³.

Water

Inorganic mercury

Levels of mercury in rainwater are in the range 5–100 ng/litre, but mean levels as low as 1 ng/litre have been reported (3). Naturally occurring levels of mercury in groundwater and surface water are less than 0.5 µg/litre, although local mineral deposits may produce higher levels in groundwater. In 16 groundwaters and 16 shallow wells surveyed in the USA, mercury levels exceeded the maximum contaminant level of 2 µg/litre set by the US Environmental Protection Agency for drinking-water (5). An increase in the mercury concentration up to 5.5 µg/litre was reported for wells in Izu Oshima Island (Japan), where volcanic activity is frequent (6). The concentration range for mercury in drinking-water is the same as in rain, with an average of about 25 ng/litre (3).

Organic mercury

In a contaminated lake system in Canada, methylmercury was found to constitute a varying proportion of total mercury, depending on the lake (3). There have been no reports of methylmercury in drinking-water.

Food

Food is the main source of mercury in non-occupationally exposed populations. Fish and fish products account for most of the organic mercury in food. The average daily intake of mercury from food is in the range 2–20 µg/day, but may be much higher in regions where ambient waters have become contaminated with mercury and where fish constitute a high proportion of the diet (7).

Estimated total exposure and relative contribution of drinking-water

On the assumption of an ambient air level of 10 ng/m³, the average daily intake of inorganic mercury by inhalation would amount to about 0.2 µg. If a level in
drinking-water of 0.5 µg/litre is assumed, the average daily intake of inorganic mercury from this source would amount to about 1 µg. The average daily intake of mercury from food is in the range 2–20 µg/day.

13.21.4 Kinetics and metabolism in laboratory animals and humans

**Inorganic mercury**

About 7–8% of ingested inorganic mercury in food is absorbed; absorption from water may be 15% or less, depending on the compound. About 80% of inhaled metallic mercury vapour is retained by the body, whereas liquid metallic mercury is poorly absorbed via the gastrointestinal tract. Inhaled aerosols of inorganic mercury are deposited in the respiratory tract and absorbed to an extent depending on particle size (8).

Inorganic mercury compounds are rapidly accumulated in the kidney, the main target organ for these compounds. The biological half-time is very long, probably years, in both animals and humans. Mercury salts are excreted via the kidney, liver, intestinal mucosa, sweat glands, and salivary glands, and milk; the most important routes are via the urine and faeces (8).

**Organic mercury**

Dimethylmercury is almost completely absorbed through the gastrointestinal tract; after absorption it rapidly appears in the blood where, in humans, 80–90% is bound to red cells. Demethylation of methylmercury to inorganic mercury occurs at a slow but significant rate. The greater intrinsic toxicity of methylmercury as compared with inorganic mercury is due to its lipid solubility, which enables it to cross biological membranes more easily, and especially to enter the brain, spinal cord, and peripheral nerves, and to cross the placenta (3).

Most methylmercury is excreted in the inorganic form. The site and mechanism of demethylation are still not well understood (3).

13.21.5 Effects on laboratory animals and in vitro test systems

**Inorganic mercury**

Short-term exposure

The toxic effects of inorganic mercury compounds are seen mainly in the kidney. Lesions in the proximal tubular cells were detected after a single intraperitoneal injection of 1 µmol of mercury(II) chloride per kg of body weight (0.2 mg/kg of body weight as mercury) in male rats. Accumulation of mercury in the kidneys, however, indicated that the absorption efficiency was much greater than that expected from the gastrointestinal tract (9).
When rats were given mercury(II) chloride (3 mg/kg of body weight) by gavage twice a week for 60 days, examination by immunofluorescence showed that deposits for IgG were present in the renal glomeruli. Morphological lesions of the ileum and colon were also observed, with abnormal deposits of IgA in the basement membranes of the intestinal glands and of IgG in the basement membranes of the lamina propria (10).

When rats were exposed to mercury(II) chloride (1 mg/kg of body weight per day) by intubation or subcutaneous injection for up to 11 weeks, the rate of body weight gain decreased after 20 days, and actual weight loss occurred after 65-70 days. There were also neuropathological effects, first detected after 2 weeks, namely peripheral vacuolization of cells in the dorsal root ganglia, followed by the development of multiple small lesions in the ganglia (11).

A single dose of 1 mg/kg of body weight of mercury(II) chloride or methylmercury(II) chloride, either orally or by subcutaneous injection, resulted in leakage of dye into the nervous parenchyma within 12 h, indicating that these compounds can increase the permeability of the blood-brain barrier (11).

Long-term exposure
Rats injected subcutaneously 3 times weekly for up to 8 months with doses of inorganic mercury ranging from 0.05 to 2.5 mg/kg of body weight per injection (0.02-1.07 mg/kg of body weight per day) developed renal damage. This was characterized by an initial production of antiglomerular basement membrane antibodies, followed by the appearance of immune complex deposits in the glomerular tufts and small renal arteries accompanied by proteinuria and hypalbuminaemia (12).

Reproductive toxicity, embryotoxicity, and teratogenicity
Controlled mating tests in which male mice were injected with single doses of mercury(II) chloride (1 mg of mercury per kg of body weight) showed a significant decrease in fertility as compared with controls (13). Normal fertility was restored after about 2 months.

Gradual changes in testicular tissues were noted in rats treated with mercury(II) chloride at doses of 0.05 or 0.1 mg/kg of body weight intraperitoneally over 90 days (14). There was a decrease in seminiferous tubule diameter, spermatogenic cell counts, and Leydig's cell nuclear diameter as compared with controls.

Of female hamsters given a total of 3-4 mg of mercuric chloride during the first estrous cycle, 60% did not ovulate by day 1 of the third cycle (15). Ovulation was inhibited in female hamsters injected with mercury(II) chloride at high doses (6.4 or 12.8 mg of mercury per kg of body weight) during day 1 of the estrous cycle (16). Female hamsters injected with 1 mg of mercury(II) chloride per day during one estrous cycle exhibited significantly higher levels of follicle-stimulating hormone in their pituitaries as compared with controls (17).
Pregnant Wistar rats were exposed intravenously to mercury(II) chloride on different days of gestation. At mid-gestation, the minimum effective teratogenic dose of mercury (0.79 mg/kg of body weight) was high in relation to the maternal LD$_{50}$, and the incidence of fetal malformations, mainly brain defects, was 23% in all live fetuses. In rats of different gestational ages, uptake of Hg$^{2+}$ by the fetuses at this dose level decreased sharply between days 12 and 13 (18).

**Organic mercury**

**Short-term exposure**

In rats fed methylmercury dicyandiamide 5 days per week for 59 days, extensive damage to the renal cortex occurred with extensive inflammatory reaction surrounding the tubules and some early fibrosis even at the lowest dose of 0.6 mg/kg of body weight per day (19). Tubular degeneration of the kidney was also evident after subcutaneous injection of 10 mg/kg of body weight per day into rats for 7 consecutive days (20). In contrast to the effects of high doses of methylmercury on rats, kidney damage was not reported in cats exposed to 0.45 mg/kg of body weight (21) or in monkeys exposed to either 0.05 mg/kg of body weight per day (22) or to doses resulting in blood levels of up to 4 µg of mercury per ml of blood (23).

In cats, convulsions occurred after 60–83 days of exposure to 0.45 mg of methylmercury per kg of body weight per day; they were preceded 4–11 days earlier by progressive behavioural changes. Kittens were fed commercially available tuna contaminated with 0.3–0.5 mg of methylmercury(II) chloride per kg for 11 months. The total mercury intake over the period averaged 6.3 mg per cat or about 19 µg/day. Neurological disturbances were observed in three kittens after 7–11 months (24).

Squirrel monkeys were exposed for periods of up to 35 days to repeated oral doses of methylmercury(II) nitrate mixed with food or by stomach tube. The threshold for both behavioural and central nervous system pathology occurred at blood mercury concentrations in the range 0.75–1.2 mg/litre (25).

**Long-term exposure**

In a study in which cats were fed methylmercury(II) chloride in a fish diet at doses of 0.003, 0.008, 0.020, 0.046, 0.074, or 0.176 mg/kg of body weight per day, 7 days a week for 2 years, detectable neurological impairment occurred in the group given 0.046 mg/kg of body weight per day after 60 weeks; this concentration was the lowest at which such impairment occurred. Pathological changes in the nervous system were restricted to the brain and dorsal root ganglia and were not seen at doses below 0.074 mg/kg of body weight per day (26).

Stumptail, pigtail, and squirrel monkeys were given methylmercury(II) chloride in food for periods in excess of 1000 days. This dosage regime was designed to maintain the blood mercury level at 1–4 mg/litre of blood. The critical effects seen were reduced sensitivity to visual stimuli at low luminescence and tremor on reaching for a small object. All monkeys with a blood concentration above 2
mg/litre developed symptoms with latent periods ranging from less than 20 to 200 days (23).

Cynomolgus monkeys were fed methylmercury from birth at doses of 0.05 mg/kg of body weight per day for 3–4 years. Blood concentrations of mercury peaked at 1.2–1.4 mg/litre, then declined after weaning to a steady level of 0.6–0.9 mg/litre. No overt signs of toxicity were noted but, when tested after 3–4 years, the monkeys exhibited impaired spatial vision under conditions of both high and low luminescence (22).

Reproductive toxicity, embryotoxicity, and teratogenicity
Mice were given single doses of 3.6, 5.3, 8, 12, 18, or 27 mg of methylmercury(II) chloride per kg of body weight at 9.5, 12.5, or 15.5 days post-fertilization (27). The trend among F₁ females towards an adverse effect of dose on litter size, although not statistically significant, was in the direction to be expected if methylmercury(II) chloride can affect oogenesis in females exposed during fetal life.

A single dose of 2, 3, or 4 mg of mercury(II) ethanoate (about 1.3–2.5 mg of mercury) was injected intravenously in three groups of female hamsters on day 8 of gestation (28). The exposed groups showed resorption frequencies of 12, 34, and 52%, respectively, as compared with 4% in the controls.

High doses of methylmercury given to pregnant rodents produced cleft palate (29, 30). Prenatal exposure of rats can produce renal functional abnormalities detectable in offspring at 42 days of age (31).

Female rats were injected with 0, 6, or 10 mg of methylmercury(II) chloride per kg of body weight on gestational days 6–9 (32). Dams given 10 mg/kg of body weight either failed to give birth or the young were stillborn. External morphology was normal in rats given either of the two lower doses. Methylmercury produced hydrocephalus, decreased thickness of the cerebral cortex in the parietal section, and increased thickness of the hippocampus in the occipital section; with these exceptions, the brains of mercury-treated rats showed normal development.

Hamsters were given either 10 mg of methylmercury per kg of body weight on gestational day 10 or 2 mg/kg on gestational days 10–15 (33, 34). In the neonatal cerebellar cortex, degenerative changes such as accumulation of lysosomes and areas of floccular cytoplasmic degradation were frequently observed in the neuroblastic granular layer as well as in more differentiated neural elements in the molecular and internal granular layers. Pyknotic nuclei were seen singly and in groups throughout the external granular layer of treated animals. In the adult cerebellum, focal areas of astroglialosis were observed in the molecular layer of treated animals.

Mutagenicity and related end-points
Animal and cell culture studies confirm that methylmercury damages chromosomes if given orally at a dose of 5 mg/kg of body weight to pregnant mice
(16, 35), intraperitoneally at 2 mg/kg of body weight daily for 3 weeks to adult
hamsters (36), and intraperitoneally at 10 mg/kg of body weight to ovulating
Syrian hamsters (37). Methylmercury at low concentrations (0.05–0.1 μmol/litre)
has been reported to interfere with gene expression in *in vitro* cultures of glioma
cells (38). Non-disjunction and sex-linked recessive lethal mutations were in­
duced in *Drosophila melanogaster* by treatment with methylmercury (39).

**Carcinogenicity**

Groups of mice were fed 15 or 30 mg of methylmercury per kg of diet for up to
78 weeks. The majority of the 30 mg/kg group died from neurotoxicity by week
26. Histopathological examination of kidney tissue from all animals surviving
after 53 weeks revealed renal tumours in 13 of 16 males in the 15 mg/kg group.
Of these, 11 were classified as adenocarcinomas and two as adenomas (40).

### 13.21.6 Effects on humans

**Inorganic mercury**

**Acute exposure**

Mercury will cause severe disruption of any tissue with which it comes into con­
tact in sufficient concentration, but the two main effects of mercury poisoning
are neurological and renal disturbances. The former is characteristic of poisoning
by methyl- and ethylmercury(II) salts, in which liver and renal damage are of
relatively little significance, the latter of poisoning by inorganic mercury.

In general, however, the ingestion of acute lethal toxic doses of any form of
mercury will result in the same terminal signs and symptoms, namely shock,
cardiovascular collapse, acute renal failure, and severe gastrointestinal damage.
Acute oral poisoning results primarily in haemorrhagic gastritis and colitis; the
ultimate damage is to the kidney. Clinical symptoms of acute intoxication
include pharyngitis, dysphagia, abdominal pain, nausea and vomiting, bloody
diarrhoea, and shock. Later, swelling of the salivary glands, stomatitis, loosening
of the teeth, nephritis, anuria, and hepatitis occur (41).

Ingestion of 500 mg of mercury(II) chloride causes severe poisoning and
sometimes death in humans (42). Acute effects result from the inhalation of air
containing mercury vapour at concentrations in the range 0.05–0.35 mg/m³
(43, 44). Exposure for a few hours to 1–3 mg/m³ may give rise to pulmonary
irritation and destruction of lung tissue and occasionally to central nervous
systems disorders (45).

Dermal exposure to alkyl mercurials may give rise to acute toxic dermatitis
and eczematous changes.

**Long-term exposure**

Many studies involving the observation of more than 1000 individuals indicate
that the classical signs and symptoms of elemental mercury vapour poisoning
(objective tremors, mental disturbances, and gingivitis) may be expected to
appear after chronic exposure to air mercury concentrations above 0.1 mg/m³ (8). Nonspecific neurological and physiological symptoms were also associated with lower exposure levels.

Considerable mercury exposure of children of workers at a thermometer plant has been reported (46). The median urine mercury level of 23 such children was 25 µg/litre as compared with 5 µg/litre in 39 controls. No signs of mercury intoxication were seen on clinical examination or reported by parents (3).

**Organic mercury**

The adverse health effects of occupational exposure to alkylmercury compounds constitute what is known as the Hunter-Russel syndrome (concentric constriction of the visual field, ataxia, dysarthria, etc.); this was seen in four workers exposed to methylmercury fungicide (47).

Methyl- and ethylmercury compounds have been the cause of the largest number of cases of mercury poisoning and of fatalities in the general population as a result of the consumption of contaminated fish or of bread prepared from cereals treated with alkylmercury fungicides. The earliest effects are nonspecific, e.g. paraesthesia, malaise, and blurred vision. These are followed by concentric constriction of the visual field, deafness, dysarthria, and ataxia. In the worst cases, the patient may go into coma and ultimately die. At high doses, methylmercury affects the peripheral nervous system in human subjects (48).

The two major epidemics of methylmercury poisoning in Japan, in Minamata Bay and in Niigata, both known as Minamata disease, were caused by the industrial release of methylmercury and other mercury compounds into Minamata Bay and into the Agano River, followed by accumulation of the mercury in edible fish. The maximum blood level of methylmercury without adverse health effects was estimated to be 0.33 µg/ml based on the epidemiological study of the Minamata disease endemic area (49). By 1971, a total of 269 cases of Minamata disease had been reported in Minamata and Niigata, of which 55 proved fatal. By March 1989, 2217 cases of Minamata disease had been officially recognized in Minamata and 911 cases in Niigata (50).

The largest recorded epidemic caused by the ingestion of contaminated bread prepared from wheat and other cereals treated with alkyl (methyl- or ethyl-) mercury fungicides took place in the winter of 1971–72 in Iraq, and resulted in the admission of over 6000 patients to hospital and over 500 deaths (57). Previous epidemics have occurred in Guatemala, Iraq, and Pakistan, and on a limited scale in other countries (3, 8, 52).

The Cree Indians of northern Quebec were also known to be exposed to methylmercury through the consumption of contaminated local fish. The relationship between measures of exposure and neurological abnormalities was studied in two communities. A positive association was found between neurological abnormalities and methylmercury exposure in both communities, but the relationship was statistically significant only in one of them (53, 54).
The first indication of possible congenital Minamata disease was the unusual occurrence of cerebral palsy-like conditions in nine infants in the endemic areas (population about 1700) during 21 months. These infants had severe cerebral involvement (palsy and mental retardation); mild or no symptoms of poisoning were seen in their mothers, although there is a possibility that slight symptoms might have been overlooked (3).

According to an epidemiological study of an outbreak in Iraq, the clinical picture was dose-dependent. In those who were exposed to high maternal blood levels of methylmercury, the picture was one of cerebral palsy indistinguishable from that resulting from other causes (microcephaly, hyper-reflexia, and gross motor and mental impairment, associated with blindness or deafness). Milder forms were not easy to diagnose during the first few months of life, but became clearer with time. The cases showed mainly psychomotor impairment and persistence of pathological reflexes (53, 55–57). The relationship between prenatal exposure to methylmercury and neurological and developmental abnormalities was also studied. Abnormality of the tendon reflex was positively associated with methylmercury exposure only in boys, without a dose–response relationship (58). Findings in the milder cases were quite similar to those associated with the minimal brain syndrome (3).

Marsh et al. (59) demonstrated a dose–response relationship between the deteriorated neurological score in children and the maximum mercury concentration during gestation in a single strand of maternal head hair.

13.21.7 Guideline value

Almost all mercury in uncontaminated drinking-water is thought to be in the form of Hg^{2+}. Thus, it is unlikely that there is any direct risk of the intake of organic mercury compounds, and especially of alkylmercurials, as a result of the ingestion of drinking-water. However, there is a real possibility that methylmercury will be converted into inorganic mercury.

In 1972, JECFA established a provisional tolerable weekly intake (PTWI) of 5 μg/kg of body weight of total mercury, of which no more than 3.3 μg/kg of body weight should be present as methylmercury (60). This PTWI was reaffirmed in 1978 (61). In 1988, JECFA reassessed methylmercury, as new data had become available; it confirmed the previously recommended PTWI for the general population, but noted that pregnant women and nursing mothers were likely to be at greater risk from the adverse effects of methylmercury. The available data were considered insufficient, however, to allow a specific methylmercury intake to be recommended for this population group (62, 63).

To be on the conservative side, the PTWI for methylmercury was used to derive a guideline value for inorganic mercury in drinking-water. As the main exposure is from food, 10% of the PTWI was allocated to drinking-water. The guideline value for total mercury is 0.001 mg/litre (rounded figure).
GUIDELINES FOR DRINKING-WATER QUALITY

References


### 13.22 Molybdenum

#### 13.22.1 General description

**Physicochemical properties** *(1, 2)*

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>2610 °C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>5560 °C</td>
</tr>
<tr>
<td>Density</td>
<td>10.2 g/cm³</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>0.133 kPa at 3102 °C</td>
</tr>
<tr>
<td>Water solubility</td>
<td>insoluble</td>
</tr>
</tbody>
</table>

**Organoleptic properties**

Ammonium molybdate imparts a slightly astringent taste to water at concentrations above about 10 mg of molybdenum per litre *(2)*.
Major uses
Molybdenum is used in the manufacture of special steels, in electrical contacts, spark plugs, X-ray tubes, filaments, screens, and grids for radio valves, and in the production of tungsten, glass-to-metal seals, nonferrous alloys, and pigments. Molybdenum disulfide has unique properties as a lubricant additive. Molybdenum compounds are used in agriculture either for the direct treatment of seeds or in the formulation of fertilizers to prevent molybdenum deficiency (1, 3, 4).

Environmental fate
Molybdenum disulfide is sparingly soluble in water but is readily oxidized to give more soluble molybdates, which are stable in water in the absence of a reducing agent (2).

13.22.2 Analytical methods
Molybdenum can be determined by graphite furnace atomic absorption spectrosopy with a detection limit of 0.25 µg/litre. Inductively coupled plasma atomic emission spectroscopy has a detection limit of 2 µg/litre (5).

13.22.3 Environmental levels and human exposure
Air
Human intake of airborne molybdenum is not likely to be a major exposure pathway (6).

Water
Molybdenum was present in 32.7% of surface water samples from 15 major river basins in the USA at concentrations ranging from 2 to 1500 µg/litre (mean 60 µg/litre) (7, 8). Levels in groundwater ranged from undetectable to 270 µg/litre in another survey in the USA (9).

In a survey of finished water supplies in the USA, concentrations ranged from undetectable to 68 µg/litre (median 1.4 µg/litre) (10). In another survey of 380 finished water samples from across the USA, 29.9% contained measurable concentrations of molybdenum, with a mean of 85.9 µg/litre and a range of 3-1024 µg/litre (8).

Levels of molybdenum in drinking-water do not usually exceed 10 µg/litre (11). However, in areas near molybdenum mining operations, the molybdenum concentration in finished water can be as high as 200 µg/litre. Tapwater concentrations as high as 580 µg/litre have been reported in Colorado (6).
Food

Legumes, grains, and organ meats are good food sources of molybdenum; fruits, root and stem vegetables, and muscle meat are relatively poor ones (12,13).

Estimated total exposure and relative contribution of drinking-water

Molybdenum intakes in the USA range from 240 µg/day for adult men to 100 µg/day for women. Average intake is higher in those on low incomes (13, 14). In most areas, molybdenum intake via drinking-water will not exceed 20 µg/day (11).

13.22.4 Kinetics and metabolism in laboratory animals and humans

The rate of gastrointestinal absorption of molybdenum is influenced by its chemical form and the animal species. Hexavalent molybdenum is readily absorbed following oral administration, the amount absorbed being higher in nonruminants than in ruminants (15-17). Tetravalent molybdenum is not readily absorbed (15). In humans, 30-70% of dietary molybdenum is absorbed from the gastrointestinal tract (18,19).

Following gastrointestinal absorption, molybdenum rapidly appears in the blood and most organs. Highest concentrations are found in the liver, kidneys, and bones (15, 16, 20). Molybdenum crosses the placental barrier (21). There is no apparent bioaccumulation of molybdenum in human tissues (20).

In rodents, molybdenum compounds are excreted largely in the urine, and only to a small extent in faeces (15,16). In ponies, cattle, and sheep, molybdenum excretion is generally divided between faeces and urine, owing to less complete gastrointestinal absorption (17, 22, 23). Molybdenum intake and excretion are balanced in most nonruminant species, including humans (20).

13.22.5 Effects on laboratory animals and in vitro test systems

Short-term exposure

Oral subchronic LD₅₀s for molybdenum(VI) oxide, calcium molybdate, and ammonium molybdate in rats were 125, 101, and 330 mg of molybdenum per kg of body weight per day, respectively (15). Death occurred over a period of 8-732 days.

In animals, molybdenum interacts in a complex manner with copper and sulfate by a mechanism which is as yet unknown. Animals on copper-deficient diets are generally more susceptible to molybdenum toxicity than those on copper-adequate diets. Dietary sulfate protects nonruminants against the symptoms of poisoning; if the animals are copper-deficient, however, it can intensify them (24, 26).
In a study in which Holtzman rats (4 per dose) were fed diets containing hydrogen molybdate at 75 or 300 mg/kg (7.5 or 30 mg of molybdenum per kg of body weight per day), molybdenum significantly inhibited growth and increased copper and molybdenum concentrations in liver. These effects were reduced or reversed by the addition of sulfate (25). An enlargement of the femorotibial joint and a thickening of the epiphysis of the femur and tibia were observed at both doses. This study suggests a LOAEL of 7.5 mg of molybdenum per kg of body weight per day, based on body weight loss and bone deformities.

Three weanling guinea-pigs were fed a low-copper basal diet with dietary additions of 0, 200, 500, 1000, or 2000 mg of molybdenum (8, 20, 40, or 80 mg/kg of body weight per day) for 8 weeks (27). An increase in molybdenum in the blood, liver, and kidneys was observed with increasing dietary molybdenum levels. An increase in copper was also observed in the blood and kidneys with increasing molybdenum intake; at the two highest doses, there was a decrease in liver copper concentrations.

Weanling Long-Evans rats receiving dietary sodium molybdate (50 or 80 mg of molybdenum per kg of body weight per day) over 5–8 weeks developed diarrhoea, while weight gain decreased and copper levels in the liver increased (28).

In ruminants, sulfate tends to increase the toxicity of molybdenum even in the absence of copper deficiency (26, 29, 30). Molybdenum concentrations of 10 mg/kg of body weight in the ruminant diet resulted in tissue copper depletion, potentiated by dietary sulfate (31).

A total of 12 male Holstein calves (3 per group) received ammonium molybdate at 0, 1, 10, or 50 mg of molybdenum per litre (average daily doses of < 0.01, 0.07, 0.7, or 3.7 mg of molybdenum per kg of body weight per day) in drinking water for 21 days (32). No effects on growth were observed, but nonceruloplasmic copper was significantly elevated and copper uptake from plasma into liver was less than the endogenous loss in calves receiving the highest dose. The author suggested that the minimum toxic concentration of molybdenum is between 10 and 50 mg/litre, so that the NOAEL would be 0.07 mg/kg of body weight per day.

The effects of dietary molybdenum (1.7 g/day) were tested in four Holstein cows that were on low copper intake (30). None of the animals showed overt signs of toxicity after 6 months. After the molybdenum intake was increased to 3.4 g/day (7 mg/kg of body weight per day), one cow developed severe diarrhoea and exhibited signs of lethargy, cessation of milk synthesis, and general emaciation. When the molybdenum dose was increased to 5.1 g/day (10 mg/kg of body weight per day), two of three cows exhibited diarrhoea and emaciation. The addition of 0.26% sulfate greatly increased the severity of molybdenum toxicity. Dietary molybdenum increased the content of copper in the kidney and brain but decreased it in the liver. The kidney and spleen concentrated molybdenum to a greater degree than the liver or other organs.
Reproductive toxicity, embryotoxicity, and teratogenicity

Five pairs of Charles River CD mice received 10 mg of molybdenum per litre (as molybdate) (about 1.5 mg of molybdenum per kg of body weight per day) in deionized drinking-water for up to 6 months (33). Excess fetal mortality was observed; there were 15 (of 238) dead pups in the F₁ generation and 7 (of 242) dead pups, five dead litters, and one maternal death in the F₂ generation. The experiment was discontinued after the F₃ generation because of the elevated incidence of deaths of offspring and parents and infertility.

Four pregnant Cheviot ewes given diets supplemented with 50 mg of molybdenum per day (as ammonium molybdate) gave birth to four lambs, three of which exhibited ataxia (34). Histological examination revealed degenerative changes in the cytoarchitecture of the cerebral cortex and demyelination of the cortex and spinal cord, lesions similar to those described by other investigators as “swayback”.

The effects of dietary molybdenum on reproductive ability and pup growth during lactation were studied in Long-Evans rats fed diets containing 0.1, 2, 8, or 14 mg of molybdenum per kg of body weight per day and either 5 or 20 mg of copper per kg for 13 weeks (35). The reduced number of litters at the two highest molybdenum concentrations was attributed to the apparent infertility of males in the groups concerned as a result of varying degrees of degeneration of the seminiferous tubules. Lactating mothers at the two highest doses lost less weight during lactation than females in the lower-dose groups, and there were indications that pups from mothers exposed to the highest dose of molybdenum gained less weight at weaning than other pups; these effects were probably due to reductions in milk production associated with high maternal dietary intake of molybdenum. The NOAEL was 2 mg/kg of body weight per day.

Molybdenum administered orally by capsule for 129 days to two male Holstein calves at doses between 4.1 and 7.8 mg/kg of body weight per day caused a gradual disappearance of the spermatogenic and interstitial tissue. The LOAEL was 4.1 mg/kg of body weight per day (36). Female sheep fed a diet low in copper (1 mg/kg) and high in both molybdenum (25 mg/kg) and sulfate (0.53%) exhibited signs of reproductive failure (37).

Mutagenicity and related end-points

Ammonium molybdate was mutagenic in two of three Escherichia coli strains. Molybdenum(V) chloride was negative and ammonium molybdate strongly positive in the Bacillus subtilis rec-assay using DNA repair-competent H17 and repair-deficient M45 strains (38). Ammonium and sodium molybdates were neither mutagenic nor recombinogenic in the Saccharomyces cerevisiae reverse mutation and gene conversion assays (39).
Carcinogenicity

Although a significantly increased incidence of lung adenomas was observed in strain A mice injected intraperitoneally with molybdenum(VI) oxide (40), this study has no direct relevance to molybdenum intake via drinking-water. Recent studies suggest that molybdenum may act to prevent certain forms of cancer induced by N-nitroso compounds, e.g. oesophageal, forestomach, and mammary gland cancer, in laboratory animals (41, 42).

13.22.6 Effects on humans

Molybdenum is considered to be an essential trace element in both animals and humans. Safe and adequate intake levels have been suggested for various segments of the population, namely 0.015–0.04 mg/day for infants, 0.025–0.15 mg/day for children aged 1–10, and 0.075–0.25 mg/day for all individuals above the age of 10 (43).

An infant with inborn deficiency of the molybdenoenzymes sulfite oxidase and xanthine dehydrogenase exhibited abnormal distribution of urinary metabolites, neurological disorders, dislocated ocular lenses, and failure to thrive (44). A Crohn disease patient receiving total parenteral nutrition developed tachycardia, tachypnoea, severe headaches, night blindness, nausea, vomiting, central scotomas, generalized oedema, lethargy, disorientation, and coma; these symptoms were attributed to dietary molybdenum deficiency resulting in impaired function of the two molybdenoenzymes (45).

Urinary levels of molybdenum and copper and serum levels of uric acid and ceruloplasmin appeared to be affected by molybdenum levels in drinking-water over a 2-year period (12). The low-molybdenum group consisted of 42 individuals from Denver, Colorado (USA), where the molybdenum concentration in drinking-water ranged from 1 to 50 µg/litre. The high-molybdenum group consisted of 13 college students from Golden, Colorado, where the drinking-water molybdenum concentrations were equal to or greater than 200 µg/litre. Plasma molybdenum levels were within the normal range among subjects in the low-molybdenum group, and no adverse health effects were observed in these subjects. Higher daily urinary molybdenum was associated with higher molybdenum intake; the mean urinary molybdenum for the Denver subjects was 87 µg/day compared with 187 µg/day for those from Golden. Higher mean serum ceruloplasmin (401 v. 30 mg per 100 ml) and lower mean serum uric acid (4.4 v. 5.3 mg per 100 ml) were also associated with the higher molybdenum intake. Because no adverse effects were seen in either group, this study suggested a NOAEL for molybdenum in drinking-water of 200 µg/litre.

Evidence to support the suggestion that the molybdenum intake may have influenced serum ceruloplasmin was provided by a follow-up study of 13 students in Golden, Colorado, 2 years after the initial study. During this time, the average concentration of molybdenum in the Golden water supply decreased to
40 μg/litre (12). At this lower level of molybdenum in the drinking-water, serum molybdenum was nearly identical to the mean for the Denver residents. Serum ceruloplasmin was within the normal range of 20–35 μg/dl. Although serum uric acid levels increased, this was believed to be the result of alcohol consumption. There were no significant differences in urinary copper levels.

An epidemiological study involving 557 subjects in India indicated that a form of lower-limb osteoporosis may be associated with the high molybdenum content of the cereals consumed by the population (46).

The results of a cross-sectional study of 400 persons in two settlements of a molybdenum-rich province of the former Soviet Union suggested that the high incidence (18–31%) of a gout-like disease was associated with high intake of molybdenum (10–15 mg/day). The disease was characterized by joint pains in the legs and hands, enlargement of the liver, disorders of the gastrointestinal tract, liver, and kidney, increased blood levels of molybdenum and uric acid, increased xanthine oxidase activity, decreased blood levels of copper, and increased urinary copper. An increased synthesis of the molybdenoenzyme xanthine oxidase resulting from high dietary molybdenum levels was proposed as the mechanism for this disorder (47).

A cross-sectional study was conducted with 25 workers at a molybdenum smelter in Denver, Colorado, exposed to molybdenum in dust (predominantly molybdenum (VI) oxide and other soluble oxides). The calculated minimum daily body burden was 0.15 mg/kg of body weight per day. High levels of molybdenum were present in the blood of 15 workers (up to 300 μg/litre) and in the urine of 12 of 14 workers (up to 11 mg/litre) (48). Mean serum ceruloplasmin and uric acid were higher for workers than controls. According to answers to medical questionnaires, six workers had upper respiratory infections in the 2 weeks prior to the questionnaire, and 15 reported joint pains, back pains, headaches, or skin or hair changes.

13.22.7 Guideline value

No data are available on the carcinogenicity of molybdenum by the oral route. In a 2-year study of humans exposed via drinking-water, the NOAEL was found to be 0.2 mg/litre (12), but there are some concerns about the quality of this study. Although an uncertainty factor of 10 would normally be applied to reflect intraspecies variation, it is recognized that molybdenum is an essential element, and a factor of 3 is therefore considered to be adequate. This gives a guideline value of 0.07 mg/litre (rounded figure), which is in the same range as that derived on the basis of the results of toxicological studies in animals and is consistent with the essential daily requirement for molybdenum.
References


### 13.23 Nickel

#### 13.23.1 General description

**Identity**

Nickel occurs as a mixture of five natural stable isotopes, with relative atomic masses of 58, 60, 61, 62, and 64.

**Physicochemical properties**

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>1453 °C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>2732 °C</td>
</tr>
<tr>
<td>Density (g/cm³)</td>
<td>8.90 at 25 °C</td>
</tr>
</tbody>
</table>

**Major uses**

Nickel is used in a large number of alloys, including stainless steel, in batteries, chemicals, and catalysts, and in the electrolytic coating of items such as chromium-plated taps and fittings used for tapwater.
Environmental fate

The nickel ion combines with oxygen-, nitrogen-, and sulfur-containing compounds and forms an extensive series of complexes in solution, including hydroxides, carbonates, carboxylic acids, phosphates, amines, and thiols (1). In aqueous solution, nickel occurs mostly as the green hexa-aquanickel(II) ion, \( \text{Ni(H}_2\text{O)}_6^{2+} \) (2). The nickel ion content of groundwater may increase as a result of the oxidation of natural nickel-containing ferrousulfide deposits. Oxidation can occur if the groundwater table is lowered or if nitrate is leached from the soil.

13.23.2 Analytical methods

Nickel can be determined by atomic absorption spectrophotometry; the detection limit is 50 ng/litre (3).

13.23.3 Environmental levels and human exposure

Air

Inhalation of airborne nickel may result in a total pulmonary exposure of 0.5 \( \mu \text{g/day} \). The pulmonary exposure of a person smoking 20 cigarettes may be 4 \( \mu \text{g/day} \) (4).

Water

Nickel concentrations in drinking-water around the world are normally below 20 \( \mu \text{g/litre} \), although levels up to several hundred micrograms per litre in groundwater and drinking-water have been reported (5, 6). Nickel concentrations in drinking-water may be increased if raw waters are polluted by natural or industrial nickel deposits or if leaching from nickel–chromium plated taps and fittings occurs. Levels up to 1000 \( \mu \text{g/litre} \) have been reported in first-run water that had remained in the tap overnight (4).

Food

The natural nickel content of various food items varies from 0 to 10 mg/kg. Nickel concentrations of more than 1 mg/kg have been found in cocoa, chocolate, soya beans, soy products, other dried legumes, nuts, oatmeal, and buckwheat. Nickel may be leached from kitchen utensils by acidic boiling water (4).

Estimated total exposure and relative contribution of drinking-water

The average daily intake of nickel from food is between 100 and 300 \( \mu \text{g} \) and probably lower than 150 \( \mu \text{g} \). A diet containing large amounts of food items in which nickel is present at concentrations above 1 mg/kg may result in a daily in-
take of 900 µg (4). Intake from food exceeds that from drinking-water (about 40 µg/day) and air (<5 µg/day).

13.23.4 Kinetics and metabolism in laboratory animals and humans

In humans, absorption of soluble nickel from drinking-water may be 40 times higher than that of nickel from food (7). Intestinal absorption may be increased several-fold by chelating agents such as disulfiram (6). Nickel penetrates the skin very slowly (7). It appears to be distributed to all organs, primary accumulation taking place in the kidneys, lungs, and liver. The formation of lipophilic nickel complexes can alter the distribution, and lead to much higher deposition in the brain than under normal conditions (6). Nickel is able to pass through the human placenta (4). It is excreted mainly through the urine. The estimated mean elimination half-time for serum is about 60 h (8).

13.23.5 Effects on laboratory animals and in vitro test systems

**Acute exposure**

Oral LD$_{50}$s of nickel in mice or rats are in the range 67–139 mg/kg of body weight (1).

**Long-term exposure**

In a 2-year study, dogs were fed nickel chloride in the diet at doses of approximately 3, 29, or 70 mg of nickel per kg of body weight per day. Depressed body weight gain, altered organ-to-body-weight ratios, and histopathological effects in the lungs were observed at the highest dose. The NOAEL was 29 mg of nickel per kg of body weight per day (9).

In a 2-year study with Wistar rats fed nickel chloride in the diet at doses of approximately 5, 50, or 125 mg of nickel per kg of body weight per day, depressed body weight gain and altered organ-to-body-weight ratios were observed at the two highest doses, but there were no effects on haematology or histopathology. The NOAEL was 5 mg of nickel per kg of body weight per day (9).

**Reproductive toxicity, embryotoxicity, and teratogenicity**

In a three-generation study in rats given 12.5, 25, or 50 mg of nickel per kg of body weight per day in drinking water, a higher incidence of stillbirths was observed in all groups in the first generation; decreased body weights of weanlings were seen in all generations at the highest dose (9).
**Mutagenicity and related end-points**

The nickel ion has been observed to enter cells and bind to DNA and RNA. Nickel has given positive results in tests with *Salmonella typhimurium*, *Corynebacterium*, and *Escherichia coli*. DNA damage has been observed in mammalian cells after *in vivo* exposure of Sprague-Dawley rats and *in vitro* exposure of Chinese hamster ovary (CHO) cells. Inhibition of DNA synthesis and induction of DNA repair have been shown in CHO cells. At nickel levels similar to those experienced by heavily exposed workers, sister chromatid exchange in peripheral blood lymphocytes, cell transformation *in vitro* in hamster cells and in human bronchial epithelial cells, and chromosomal aberrations in hamster cells were observed (10).

**Carcinogenicity**

IARC has evaluated the data on pulmonary exposure to soluble nickel compounds and has concluded that the evidence for a carcinogenic effect in animals is limited. Several experimental investigations have demonstrated that a number of nickel compounds are carcinogenic after administration via various parenteral routes (e.g. inhalation, intramuscular injection, intrarenal injection). Together, these studies suggest that some nickel compounds, especially nickel sulfide, possess carcinogenic potential (11).

13.23.6 Effects on humans

Acute nickel intoxications are rare, and most reported cases are the result of industrial exposure to nickel carbonyl. Of 32 electroplating workers who inadvertently drank water heavily contaminated with nickel sulfate and chloride (1.63 g of nickel per litre), 20 developed symptoms (e.g. nausea, vomiting, abdominal discomfort, diarrhoea, giddiness, lassitude, headache, cough, shortness of breath) that typically lasted for a few hours but persisted for 1–2 days in seven cases. The nickel doses that caused symptoms were estimated to be in the range 7.1–35.7 mg/kg of body weight. Laboratory tests showed elevated levels of blood reticulocytes, urine albumin, and serum bilirubin. All the workers recovered rapidly without evident sequelae (8). Similar symptoms were observed in 23 patients at plasma nickel concentrations of approximately 3 mg/litre following exposure to nickel-containing water during haemodialysis. The nickel was leached from a nickel-plated, stainless-steel water-heating tank. In another accident, a 2-year-old girl died after swallowing 2.2–3.3 g of nickel as sulfate crystals (10).

Several epidemiological studies have suggested a risk of nasal, sinus, and lung cancer in workers in the nickel-producing industry by inhalation of soluble nickel at concentrations in excess of 1 mg/m³ and of less soluble forms at concentrations greater than 10 mg/m³ (12). IARC has recently re-evaluated the epidemiological data on respiratory exposure and found that sufficient human data are available to conclude that inhaled nickel sulfate is carcinogenic to humans (11).
Nickel is also a common skin allergen. The prevalence of nickel sensitivity is about 8–14.5% for adult women and about 1% for men. About 50% of nickel-sensitive women develop hand eczema, which in severe cases may cause incapacitation. Although only continued dermal exposures to nickel can lead to sensitization, subsequent dermal application or oral intake of extremely low doses of nickel may provoke eczema in sensitized individuals. Single oral doses of 36–80 μg of nickel per kg of body weight in lactose capsules have been shown to exacerbate hand eczema. In a study in which natural dietary nickel was ingested daily at a dose level of 8.3 μg/kg of body weight for 5 days, a worsening of vesicular hand eczema was observed in 10 out of 12 patients (13).

13.23.7 Guideline value

The guideline value is based on a dietary study in rats that showed a NOAEL of 5 mg/kg of body weight per day for altered organ-to-body-weight ratios (9). A TDI of 5 μg/kg of body weight was derived using an uncertainty factor of 1000, made up of 100 for inter- and intraspecies variation and an additional factor of 10 to compensate for the lack of adequate studies on long-term toxicity and reproductive effects, the lack of data on carcinogenicity by the oral route (although both the soluble and the sparingly soluble compounds of nickel are now considered to be human carcinogens in relation to pulmonary exposure), and a much higher intestinal absorption when taken on an empty stomach in drinking-water than when taken together with food.

With an allocation of 10% of the TDI to drinking-water, the health-based guideline value is 0.02 mg/litre (rounded figure), which should provide sufficient protection for nickel-sensitive individuals.

References


13.24 Nitrate and nitrite

13.24.1 General description

Identity

Nitrate and nitrite are naturally occurring ions that are part of the nitrogen cycle. The nitrate ion (NO$_3^-$) is the stable form of combined nitrogen for oxygenated systems; although chemically unreactive, it can be reduced by microbial action. The nitrite ion (NO$_2^-$) contains nitrogen in a relatively unstable oxidation state; chemical and biological processes can further reduce nitrite to various compounds or oxidize it to nitrate (1).